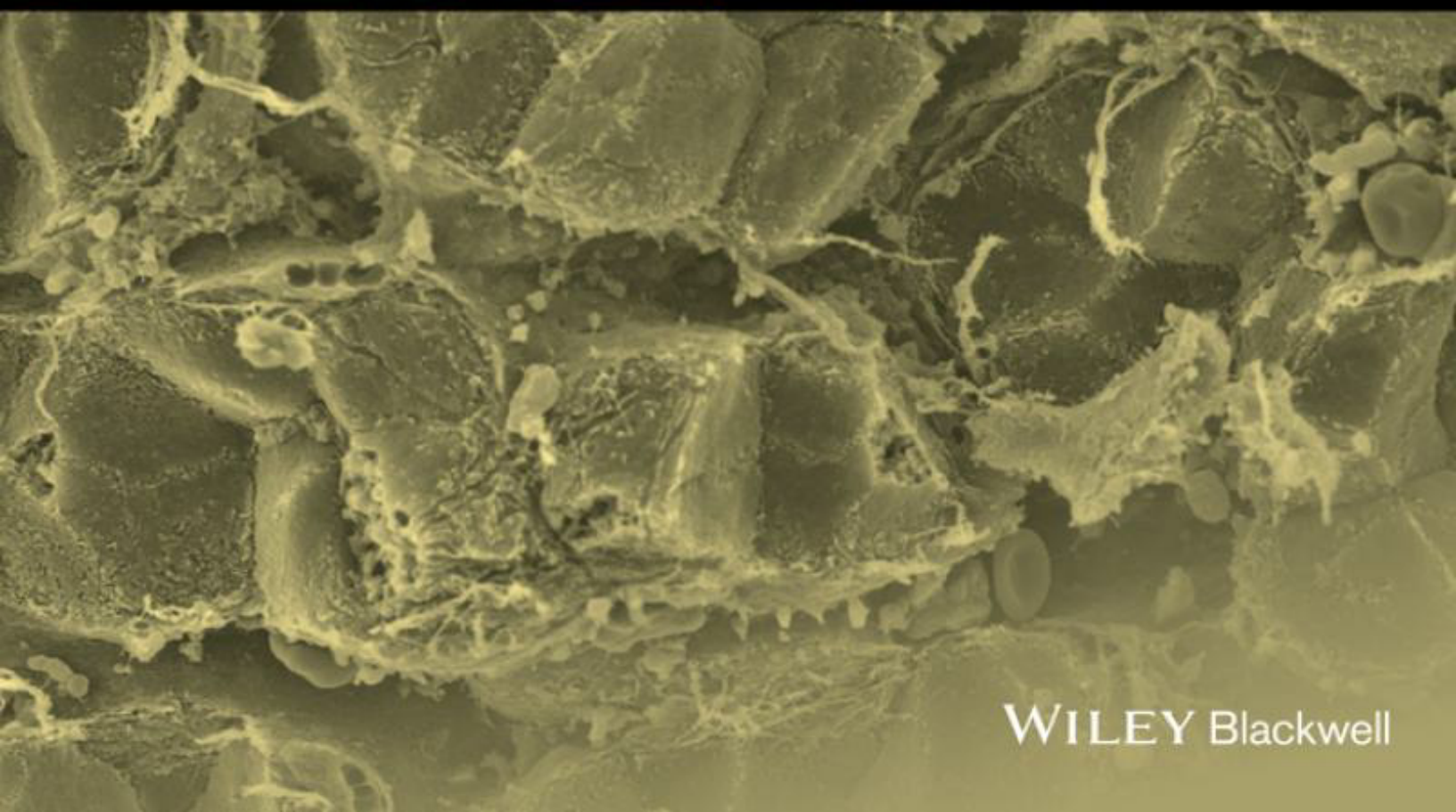
DISEASES OF THE LIVER AND BILIARY SYSTEM **IN CHILDREN**

FOURTH EDITION

Edited by **Deirdre A. Kelly**



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Diseases of the Liver and Biliary System in Children

Dedication

To my grandchildren, Finlay and Nina Parker, and all the children whom I have had the privilege to care for.

Diseases of the Liver and Biliary System in Children

Edited by

Deirdre A. Kelly

The Liver Unit
Birmingham Children's Hospital NHS Foundation Trust
and
University of Birmingham
Birmingham, UK

Fourth Edition

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Foreword to the First Edition

Although the Ancient Egyptians believed that the liver had mystic powers of healing, and Hippocrates gave a full description of hepatic encephalopathy, modern hepatology has only taken off in the last 50 years. Accelerated progress has followed discovery of the hepatitis viruses, now a virtual alphabet from A to E and beyond. Hepatobiliary imaging and endoscopy have added to the progress. Developments have depended not only on specialist hepatologists, but on developments in other related disciplines of medicine – particularly virology, immunology, biochemistry, and now, molecular medicine. A huge literature is available describing liver disease in adults, but pediatrics has lagged behind.

This book covers all the essentials of pediatric hepatology and is therefore particularly timely. The material covered is wide, from such aspects as the psychology of parents of children on transplant waiting lists to the genetic disturbances of bilirubin and bile salt transport in the neonate. The chapter authors have been well chosen. They are international authorities, active both clinically and in research. They write lucidly from personal experience.

Many helpful algorithms and tables are included. The references at the end of each chapter have been carefully selected and are up-to-date… This book should be available in every pediatric department. It should be at hand at all times to offer practical advice on any childhood liver disease. General pediatricians will certainly benefit. It would be a suitable gift to reward a trainee.

This book fills a real gap in our knowledge of liver disease. It will be a well-deserved success.

Professor Dame Sheila Sherlock
1918–2001

Preface

The diagnosis and management of pediatric liver disease has been transformed since the first edition of this book in 1999.

The rapid developments in cellular and molecular genetic techniques have identified new genes, discovered the causes of rare diseases, taught us much about pathophysiology, and revealed new targets for therapy.

National and international networks utilizing clinical biobanks and databases have helped us consolidate our knowledge and treatment of cholestatic liver disease, biliary atresia, and acute liver failure.

The medical management of pediatric liver disease owes much to the development of new drugs, particularly antiviral therapy, which has changed the outcome for many children and significantly improved the quality of their lives.

The successful development of transplantation, now extended to multiorgan transplantation, has dramatically improved the outcome of infants and children with liver or metabolic disease so that many have become adults, completed their education, and contribute equally to society. This means that it is important for adult hepatologists not only to become familiar with pediatric diseases new to them, but also to learn how best to manage young people with a lifetime of chronic illness.

The investigation and management of most pediatric liver disease should be based in specialist or transplant units so that patients benefit from centralized expertise. Nevertheless, it is essential for general pediatricians to recognize the early presentation of liver disease, know when to refer to a specialized unit, and be aware of the range of new therapies and their complications, especially transplantation.

The fourth edition of this book summarizes the advances of the last few years, and provides a practical approach to the diagnosis and management of pediatric liver diseases, highlighting the importance of multidisciplinary team working and holistic management of the child and family.

The remit has been extended to include information on structure and function, immunology, and genetics with an emphasis on basic mechanisms of disease. New chapters describe the effects of liver and kidney disease, combined liver and kidney transplantation, the management of anesthesia and intensive care for children with liver disease, and a summary of what the adult hepatologist needs to know.

The book should interest the adult gastroenterologist and hepatologist, the general pediatrician, and pediatric trainee as well as provide guidance to nurses and allied health professionals.

Deirdre A. Kelly

Acknowledgments

The investigation and management of pediatric liver disease requires skill, compassion and a dedicated multidisciplinary approach. I am indebted to my colleagues in the Liver Unit, in Birmingham Children's Hospital NHS Foundation Trust and elsewhere in the world for their expert knowledge and help with this book, which we hope will aid the management of children with liver disease everywhere.

I am particularly grateful to Angela Green for her help in coordinating the work for this book.

Deirdre A. Kelly

SECTION 1

Understanding the Liver

CHAPTER 1

Structure, Function, and Repair of the Liver

Christoph Leiskau and Ulrich Baumann
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Key points

- The subdivision of the liver into segments by the Couinaud system, with an independent arterial, portal venous, and biliary supply has important implications for liver (transplant) surgery.
- Blood flows in the portal vein and hepatic artery are closely linked, with obstruction of one vessel leading to compensatory flow rates in the other (hepatic arterial buffer response).
- 50% of hepatic oxygen supply is provided by the portal vein so the liver can maintain function for weeks without arterial blood supply. Bile duct epithelial necrosis is the first sign of an inadequate oxygen supply.
- Hepatocytes are polar cells with a basal domain contacting the fenestrated sinusoids for blood supply and an apical domain where bile is drained into the bile canaliculus.
- Proliferation and development of the intrahepatic biliary system continue until around 15 years of age, which must be taken into account when interpreting liver histology in children.
- Several hepatic enzymes are not fully expressed in newborn children, with immaturity of UDP-glucoronyl-transferase being an important factor in physiological jaundice of the newborn.
- Alpha-fetoprotein levels may exceed levels of 100,000 ng/mL in the healthy newborn, only reaching normal levels at 1 year of age, and is not necessarily a sign of malignancy.
- After trauma or partial hepatectomy, the liver regenerates by proliferation of mature hepatocytes within weeks.

The liver has fascinated mankind ever since medicine existed. Our knowledge, however, about the anatomy, structure, and function of the liver has changed dramatically over the last 1800 years. Ancient medicine was aware of the liver's central role in nutrition, and for Galen it was a "principal instrument" of the body. In Greek mythology, Prometheus – the friend of mankind who was chained to a rock by the god Zeus as punishment for giving humans the use of fire – suffered daily as an eagle devoured his liver, only for it to restore itself overnight. This association with Prometheus and the capacity of the liver to regenerate has been quoted many times in textbooks, editorials, and reviews.

Patients and families find it difficult to understand the role of the liver and the implications of liver failure, and this has to be taken into consideration when counseling children and their families. In order to gain an understanding of liver disease, it is necessary to study the basics of the development, anatomy, and function of the liver and its responses to injury.

Structure

Development

Overview

Embryonal organ development derives from the three germ layers: endoderm, mesoderm, and ectoderm. Human liver development begins during the third week of gestation from the ventral foregut endoderm cells (the future duodenum), by differentiation into hepatoblasts triggered by cytokine fibroblast growth factor (FGF) and bone morphogenetic protein (BMP). The hepatoblasts are precursor cells of hepatocytes and cholangiocytes, they express organ-specific proteins α-fetoprotein (AFP) and albumin as well as different transcription factors, and they give rise to the liver bud or hepatic diverticulum (in the fourth gestational week). The liver bud grows into the septum transversum and the cardiac mesoderm under the influence of GATA binding protein 6 (GATA6) and its target protein hematopoietically expressed homeobox (HHEX). These structures provide connective

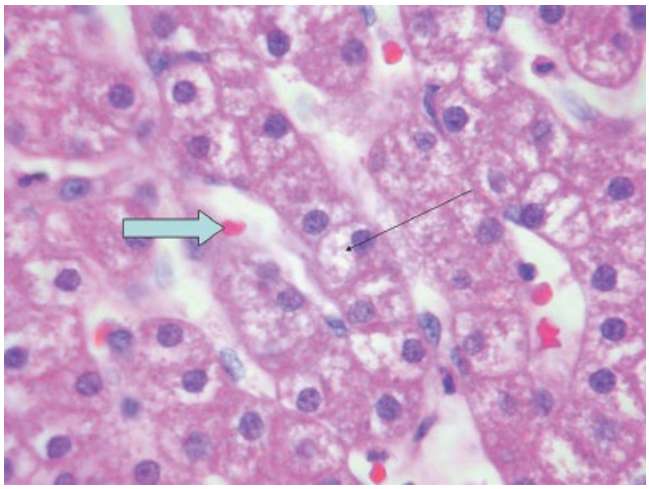
Diseases of the Liver and Biliary System in Children, Fourth Edition. Edited by Deirdre A. Kelly.

tissues to the developing liver and appropriate gene expression, which is regulated in a time-specific manner by liver-enriched transcription factors such as hepatocyte nuclear factor 6 (HNF6), required for normal development in the endoderm and mesoderm [1]. This process is termed "mesoderm inductive signaling" [2], and ß-catenin and wingless-related integration site (WNT) signaling play a crucial role in this pathway.

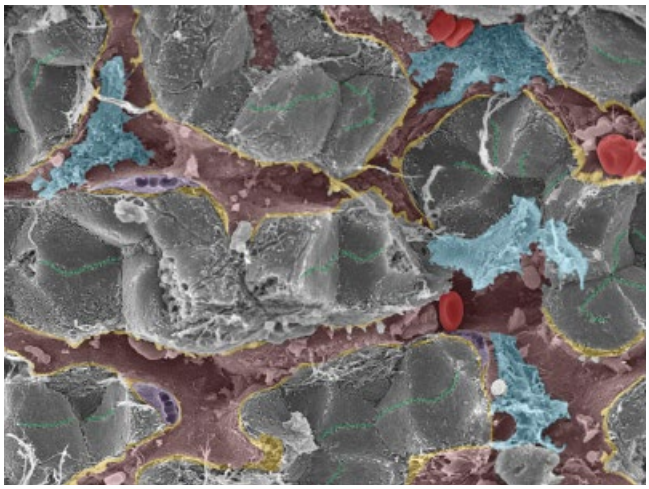
In this environment, cells from the liver bud form thick plates of hepatoblasts surrounding sinusoids fed from vitelline vessels derived from the wall of the yolk sac. The hepatic laminae initially consist of 5–7 cell layers, but by 5 months after birth the plates are two cells thick. The adult pattern of plates being one cell thick (Figure 1.1A) is not seen until at least 5 years of age [3]. The liver reaches a peak of relative size at the ninth gestational week, accounting for 10% of fetal weight, with the fetal liver being an important hematopoietic organ. In the healthy neonate, it represents up to about 5% of the body's weight; during adolescence, this decreases to the final adult proportion of 2% of body weight, or a weight of around 1400 g in the female and 1800 g in the male.

Vascular development

The liver grows under the influence of its blood supply. Initially, blood is provided by the symmetrical vitelline veins, which ultimately join to form the portal vein. From the fifth week of gestation, the vitelline veins join the umbilical veins, and the liver is supplied with placental blood rich in oxygen and nutrients. In this time, the liver grows rapidly and reaches its peak size around the ninth gestational week. From the sixth week, the hematopoietic function of the liver is developed and the liver is the major hematopoietic organ until the fifth month of gestation, when the bone marrow takes over. The right umbilical vein then disappears, leaving the left umbilical vein as the principal supplier. Blood in the left umbilical vein takes one of three routes – supplying sinusoids on the left side of liver; supplying sinusoids on the right half of the liver via retrograde flow through a connection with the left branch of the portal vein; or supplying the inferior vena cava via the ductus venosus. Ultrasound studies in fetuses near term have shown that the left lobe receives almost exclusively nutrient-rich umbilical vein blood, whilst the right lobe only receives 50% of its supply from the umbilical vein, with the remaining 50% coming from the nutrient-poor portal vein. The left lobe is therefore significantly better perfused in utero, and is better able to withstand hypoxic insults. At birth, the left umbilical vein becomes the ligamentum teres and is replaced by the portal vein as the afferent venous vessel, and the ductus venosus becomes the ligamentum venosum. Hepatic artery branches appear later in development, emerging alongside the portal veins (Figure 1.2), first near the hilum and then toward the periphery deriving from the ductal plate. This spatial and temporal sequence mirrors that seen in the developing bile ducts. The artery appears before the definitive



(A)



(B)

Figure 1.1 Mature hepatic plates and sinusoids. (A) Mature hepatic plates and sinusoids are easily identified on light microscopy. The small black arrow shows a hepatocyte in a plate one cell thick, while the large blue arrow shows an erythrocyte in a sinusoid. (H&E, original magnification × 400.) (B) Mature hepatic plates and sinusoids: overview in a scanning electron microscopy. Sinusoids are colored in pale red, with erythrocytes (bright red) and Kupffer cells (bright blue) seen on the sinusoidal endothelium within the sinusoids. Stellate cells (purple, with fat droplets from vitamin A storage) are seen below the sinusoidal endothelium in the space of Disse. Bile canaliculi are colored in green. (From Baumann *et al.* [5].)

bile duct and is formed from portal constituents, specifically myofibroblasts.

Biliary development

The extra- and intrahepatic biliary systems develop from the endoderm as two independent subunits, merging at the end of the developmental process. The extrahepatic bile ducts and gallbladder develop from the pars cystica, the lower part of the hepatic diverticulum and its elongated stalk, as the duodenum withdraws from the septum transversum. The stalk develops behind the duodenum, the proximal part

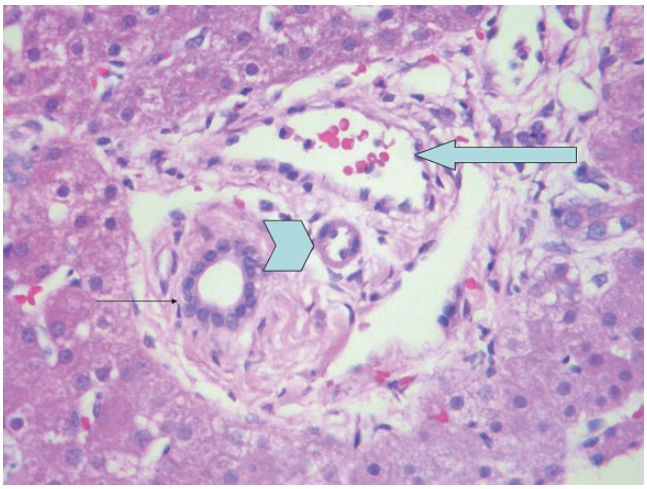


Figure 1.2 Normal portal tract. The normal portal tract consists of the hepatic artery (blue arrowhead), the portal vein branch (blue arrow), and bile duct (small black arrow). (H&E, original magnification × 200.)

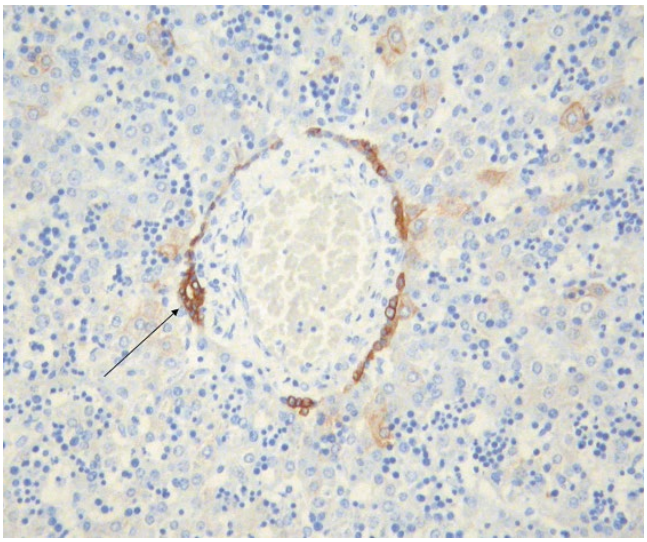


Figure 1.3 Ductal plate. The oval-shaped ductal plate highlighted in this 17-week fetus on cytokeratin immunohistochemistry (AE1/AE3) is undergoing the process of remodeling. A tubular structure has formed within the ductal plate (arrow), which will subsequently become incorporated into the developing portal tract to occupy a position as seen in Figure 1.2. (Original magnification × 200.)

becoming the ductus hepaticus, while the distal part is transformed to the ductus choledochus with its aperture in the duodenum.

Formation of the intrahepatic bile duct system begins around the eighth week of gestation. The periportal hepatoblasts become smaller and express cytokeratins (intermediate cytoskeletal components). This single-layer "sleeve" of epithelial cells surrounding the portal vein branch, with its associated mesenchyme, is the ductal plate (Figure 1.3) [4]. Some parts of the ductal plate are duplicated to a discontinuous second layer of cells around the first, resulting in a double layer around variable stretches of the portal perimeter. Within this double layer, slit-like lumens appear.

The early liver cells are bipotential, capable of differentiating into biliary epithelial cells or mature hepatocytes, a process that is started around the ninth gestational week and continues until after birth. The hepatoblasts destined to form ducts express biliary-type cytokeratins (CK19), identifiable by immunohistochemistry; while those differentiating into mature hepatocytes express different cytokeratins. The proximity to the portal vein endothelium and mesenchyme induces the differentiation toward biliary epithelium by substitution of hepatoblasts by cholangiocytes. The portal mesenchyme is important in inducing this differentiation as ductal plates do not form around the central veins. From 12 weeks' gestation onward, the ductal plate is remodeled into individualized bile ducts. Both ductal plate development and its subsequent remodeling begin in the largest portal areas near the hilum and proceed outward toward the smaller portal tracts. During the migratory stage the tubular structures that have formed in the double-layered ductal plate become surrounded by portal mesenchyme and separated from the parenchyma. Connections are retained between the newly forming duct in the portal tract and the ductal plate and to the canaliculi (canals of Hering). The condition for a controlled development of the ducts is called "planar cell polarity" [6].

New studies suggest that remodeling does not happen by apoptosis, but by transformation of the ductal plate into periportal hepatocytes and hepatic progenitor cells [7]. Failure of the precise scheme of spatial and temporal remodeling leads to persistence of the ductal plate, known as "ductal plate malformation," which can affect any caliber of portal tract. Periportal cells may retain the ability to differentiate toward bile duct epithelium, for instance the ductules that appear at the portal tract margins in biliary diseases. It is not clear whether these ductules originate from metaplasia of mature hepatocytes or biliary epithelial cells, or from progenitor cells located in the canals of Hering, possibly of bone marrow origin [8].

The integral membrane proteins of the JAGGED/NOTCH pathway have a crucial role in biliary development – a lack of Jagged1 (JAG1) results in malformation of the bile ducts, and mutations in the respective genes cause Alagille syndrome with biliary hypoplasia [9].

Extrahepatic biliary system development takes place before the intrahepatic bile duct formation begins. The extrahepatic bile ducts develop from the ventral foregut endoderm in proximity to the liver bud and the pancreatic bud. In contrast to the intrahepatic cholangiocytes, the extrahepatic biliary cells develop directly from the endoderm. Pancreatic and duodenal homeobox 1 (PDX1), hepatocyte nuclear factor 6 (HNF6), and Forkhead box protein F1 (FoxF1) play a role in the development, and the absence of the relevant genes leads to malformation.

The proliferation and development of the intrahepatic biliary system is not complete by 40 weeks of gestation, and bile duct genesis continues postpartum. The number of bile ducts per portal tract continues to increase and only reaches the adult ratio of a 1 : 1 pairing of hepatic arteries and bile ducts per portal tract at about 15 years of age.

Functional development of the liver and physiological adaptations at birth

The liver is the main site of hematopoiesis during gestation, starting at the sixth week, with a peak by the end of the first trimester, until the bone marrow becomes the main site at the end of the second trimester. New studies have shown that immature hematopoietic stem cells colonize the fetal liver and mature extravascularly, adjacent to the hepatocytes. It is normal to see evidence of residual hemopoiesis in the neonatal liver for some weeks after birth [10], but it is particularly prominent in neonatal hepatitis. Hemosiderin (as hemopoiesis decreases) and copper-associated protein accumulate in the liver and are deposited in periportal hepatocytes. Both are normal constituents of the neonatal liver and are not indicative of disease.

In utero, the placenta carries out most of the metabolic and detoxifying functions that normally take place in the liver, so hepatic enzymes such as glutamate dehydrogenase (GLDH), aspartate aminotransferase (ASAT), phosphoenolpyruvate carboxykinase (PEPCK), alanine aminotransferase (ALAT), and aldehyde dehydrogenase (ALDH) are rapidly induced at birth. Many conjugation reactions are mature by 2 weeks, but some uridine diphosphate (UDP)-glucuronyltransferase genes are not fully expressed for 2 years. The cytochrome P450 group and peroxisomal enzymes in the newborn function early (as in CYP 3A7) but others are delayed (e.g., CYP 1A2 and 3A4), so if the infant is unwell, acute phase proteins may have a long half-life because the immature liver is unable to clear them [10].

α-Fetoprotein, one of the main fetal serum proteins, is synthesized by fetal hepatocytes from 25–30 days after conception, and by the yolk sac and intestinal epithelium. Levels peak by the end of the first trimester but may exceed 100,000 ng/mL in healthy term newborns and only reaching normal adult levels around the first birthday. Albumin levels are close to adult levels at birth, but coagulation proteins and the activities of coagulation factors are low, increasing the risk of bleeding and hence the need for vitamin K administration at birth.

Bile acid synthesis starts at the fifth gestational week so that bile is secreted by the beginning of the second trimester. However, the change from placental to enteral nutrition at birth stimulates bile flow, bile acid secretion, and the enterohepatic circulation [3]. Gallbladder contraction is highly dependent on the maturity of the infant. γ-Glutamyltransferase, located at the canalicular surface of the hepatocytes, is slightly elevated in the serum in the first few months of life.

The immaturity of bile formation and the immaturity of the UDP-glucuronyltransferase enzymes lead to the development of physiological jaundice in neonates, while prematurity, hypoxia, sepsis, drug administration, or total parenteral nutrition may lead to cholestasis or hepatitis.

Term newborns have hepatic glycogen stores up to three times those of the adult liver, but these are quickly depleted, making the infants prone to hypoglycemia unless fed frequently before hepatic gluconeogenesis is induced.

The transition from umbilical venous to portal blood supply means that new substrates and bacteria are carried to the immature neonatal liver by the portal vein, exposing infants to infection.

Mature macroanatomy

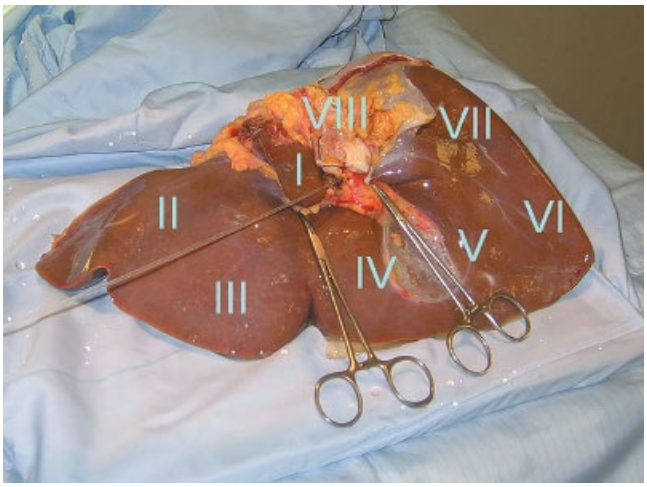
The liver occupies most of the right upper quadrant of the abdomen under the diaphragm, nearly completely protected by the ribcage. Physical examination demarcates the borders of a normal liver in the midclavicular line, from the fifth intercostal space to just below the costal margin. In infants, a liver palpable below the right costal margin is normal. A normal liver span on percussion and palpation can be estimated as:

- <1 year: 4–5 cm.
- 1–5 years: 6–7 cm.
- 5–12 years: 8–9 cm.

A prominent left lobe that is palpable in the epigastrium may be normal in infants, but in older children is suggestive of pathology.

The macroscopic division of the liver into the right, left, quadrate, and caudate lobes does not correspond to the segmental division into eight (or nine, if segment IV is subdivided into IVa and IVb) segments (Figure 1.4). The right and left lobes of the liver are defined by the principal plane, or "Cantlie line" (named after the anatomist Sir James Cantlie, who was the first to accurately describe the division of the liver) [11]. The right and left halves of the liver are further subdivided into two sectors by the right and left fissures, which roughly correspond to the positions of the right and left hepatic veins [12]. The shape of the left lateral segment (segments II and III) varies greatly between a thin, "flatfish" lobe and a short, thick lobe (particularly segment III) or "blowfish" shape. This has particular relevance in monosegmental liver transplantation.

More important than the topographic description of macroscopically visible lobes is the segmental organization of the liver by the Couinaud system, which provides the basis for all major liver surgery, including liver transplantation. The caudate lobe is segment I, and the remainder of the segments are labeled according to their clockwise position. Each segment has its own independent vascular (apart from the liver veins) and biliary supply, which is surrounded by a fibrous sheath, an extension of the Glisson capsule.



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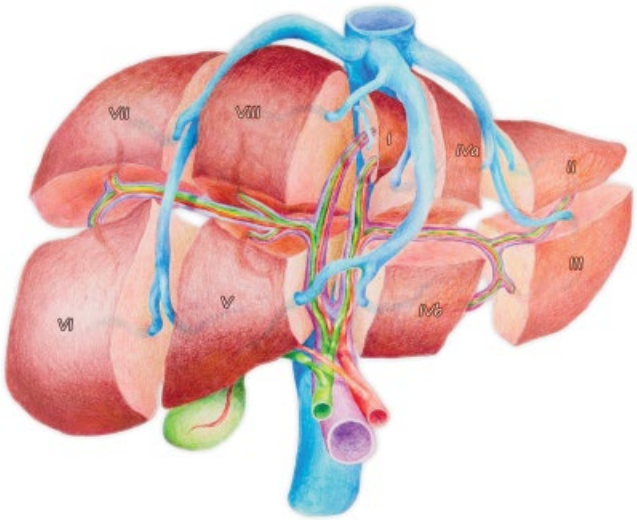


(B)

Figure 1.4 Segmental anatomy of the liver. (A) Dorsoposterior view of a normal adult liver. All segments can be seen only from this perspective. (B) Schematic view of the anterior aspect of a normal liver. The blood supply of segment IV is retrograde, which is of relevance in split liver techniques in liver transplantation. Segments II and III are also used for reduction hepatectomies and live-related donor transplantation. (From Baumann *et al.* [5].)

Partial hepatectomies for tumor surgery or liver transplantation follow these segmental borders, achieving hemostasis in the residual liver instead of the traditional lobar macroanatomy [13].

Portal venous anatomy

The portal vein is valveless and is a unique construction in the human body, being the third type of blood vessel supplying the liver. It drains blood from the splanchnic area and normally commences behind the neck of the pancreas as a cranial continuation of confluence of the superior mesenteric vein and the splenic vein, with a wide range of normal variants. The portal vein has two distinct muscle layers: a relatively thin, inner layer consisting of circular, smooth muscle cells, as in the normal media of a vein, and an outer layer of longitudinal muscle with abundant vasa vasorum – architecture resembling that of the gastrointestinal tract.

The portal vein branches extrahepatically at the hilum into a right and left portal vein; the latter supplies the caudate and quadrate lobes before it enters the parenchyma. The venous return from the gallbladder drains into the right branch of the portal vein. Each segment of the liver is supplied by its own branch of the portal vein. Anomalies of the portal vein are rare, but those most frequently seen are an abnormal position anterior to the head of the pancreas (typically associated with syndromic biliary atresia) and an abnormal communication with the inferior vena cava, resulting in a congenital portocaval shunt (Abernethy syndrome).

Hepatic artery anatomy

The arterial supply to the liver and biliary tree is notorious for variation in its origin and course relative to the surrounding anatomy, due to the complex embryological development of the celiac and superior mesenteric arteries. The hepatic artery usually originates from the celiac axis and divides into a right and left branch after the gastroduodenal artery has left the arteria hepatica communis (about 60% of cases). In about 25% of individuals, the right hepatic artery or an accessory artery arises from the superior mesenteric artery. In another quarter of individuals, the left lobe of the liver may be partially or completely supplied by an artery arising from the left gastric artery. Other less common anomalies are a very short common hepatic artery with long right and left arteries, with the gastroduodenal artery arising from the right hepatic artery or even arising separately from the celiac trunk.

The blood supply to the bile ducts is mainly arterial, although new studies have shown a portal venous contribution [14], and may be divided anatomically into hilar, supraduodenal, and pancreatic sections. The blood supply to the mid-portion of the common duct is axial, with a 3 o'clock and 9 o'clock positioned artery running alongside the duct, receiving an average of eight contributions from all of the surrounding named vessels. There is a 60% contribution from the gastroduodenal artery and 40% from the right hepatic artery. An additional supply to the supraduodenal duct is a consistent retroportal artery, arising from the celiac axis or superior mesenteric artery close to their origin from the aorta. These all form a plexus of vessels surrounding the bile ducts that extends into the liver. The ducts at the hilum receive blood from the right and left hepatic arteries and multiple small vessels that enter the caudate lobe. These vessels may be arranged in an arcade pattern, suggesting good collateral supply, or in a tree-like fashion from either the left or right hepatic arteries.

It is also important to note the frequency of segment IV arterial supply either from the right, proper, or left hepatic

artery, which has important implications for split liver transplantation. From corrosion-cast studies, it is obvious that a very important role for the hepatic arteries is the nourishment of the biliary system, and impairment of this blood supply will lead to ischemic consequences, with necrosis or stricture, while the liver parenchyma can survive by the oxygen provided by the portal vein supply.

Hepatic vein anatomy

The hepatic venous anatomy is relatively simple as there are three main hepatic veins, which lie above the portal structures within the liver, draining into the inferior vena cava (IVC). They divide the liver into sectors along an oblique plane; the middle hepatic vein separates the liver into right and left, while the left and the right hepatic veins divide the respective lobes into posterolateral and anteromedial sectors. The caudate lobe also has bilateral drainage with a relatively clear median plane, with direct venous channels into the IVC – these are more on the left, as this part of the caudate lobe is the larger and more consistent. The right hepatic vein may not be dominant, and much of the right posterior sector may drain into the IVC as a large accessory, caudally placed vein.

There are multiple other "dorsal" hepatic veins that drain directly into the IVC, which are thin-walled and fragile and require delicate ligation during right hepatectomy. The middle hepatic vein usually drains into the left hepatic vein within the liver substance, resulting in a common confluence, and receives branches from the right and left liver to a variable extent – mainly from segments V, IVb, and VIII. This venous drainage area becomes crucially important in live-donor right liver transplants, as adequate drainage must be ensured for the donor (segment IV) as well as the graft (segments V and VIII) (Figure 1.4).

Biliary anatomy

The biliary system consists of both intra- and extrahepatic parts in which the interlobular or terminal bile ducts belong to the portal triad and have a diameter of <100 µm. The terminal bile ducts are accompanied by arterial vessels, which supply oxygenated blood to the bile ducts and also play a role in the immediate reabsorption of organic compounds from primary bile into the general circulation. Bile is then drained into the septal, segmental, and right or left hepatic ducts. The left hepatic duct drains segments II, III, and IV, and the right hepatic duct drains segments V, VI, VII, and VIII. Segment I, the caudate lobe, has its own biliary drainage. Variations of this are common, and in 78% of individuals the caudate lobe drains into both the left and right hepatic ducts. The left hepatic duct lies predominantly outside the liver parenchyma, and this can be used to advantage in dealing with more distal bile duct strictures. The right and left hepatic ducts join to form the common hepatic duct.

An important and common anomaly is for the right sectional (sectoral) duct to cross to the left and drain into the left hepatic duct. There is considerable variation in ductal anomalies. In about 70% of cases, there is a clear right–left confluence, and in 12% there is a trifurcation of the ducts at the porta hepatis [15], but many patterns of drainage are discernible. The right hepatic posterior and anterior sectoral ducts may drain separately at different levels or may join the left duct, as mentioned. A right posterior sectoral duct may join the hepatic duct as low as the insertion of the cystic duct or may even drain into the gallbladder.

The cystic duct drains the gallbladder and joins the hepatic duct in most cases at an acute angle on the right side. However, the level and type of insertion is variable and may be anterior or on the left, with a spiral or parallel configuration around the duct, and sometimes the cystic duct is joined with the right hepatic duct. The term "hepatocystic triangle" describes the inferolateral base, with the cystic duct and hepatic duct medially and the inferior surface of the liver superiorly. The length and diameter of the cystic duct also vary greatly – from 4 to 65 mm in length and from 3 to 9 mm in diameter.

The gallbladder lies on the anterior undersurface in the median plane between the two liver lobes. It is wrapped in the extension of Glisson's capsule and may be embedded within the liver substance to a variable degree, or may even have a mesentery of its own.

The common bile duct, with a mean diameter of 6 mm and a length of 4–6 cm in adults, passes distally behind the duodenum and sometimes through the pancreas to reach its destination in the mid second part of the duodenum, the papilla duodeni major or papilla vateri, surrounded by sphincter muscle. At its terminal portion, it is joined by the pancreatic duct, with a short common channel in most cases. However, not infrequently, there may be pancreaticobiliary malunion with a long common channel, which is associated with choledochal dilation and cystic change due to pancreatic juice reflux (see the section on choledochal cysts in Chapter 25).

Malformation of the intra- and extrahepatic bile ducts is the major reason for chronic liver disease and liver transplantation in childhood.

Lymphatics

The lymphatic system of the liver consists of a deep and superficial part. Hepatic lymph is generated in the space of Disse, which is continuous with the lymph vessels. In the deep system, lymphatic vessels originate in the connective tissue spaces within the portal tracts and follow the arterial and portal vein branches toward the hepatic hilum. Superficially, lymphatics in the hepatic capsule drain to vessels either at the hilum or around the hepatic veins and IVC and eventually into the thoracic duct [16].

Microanatomy

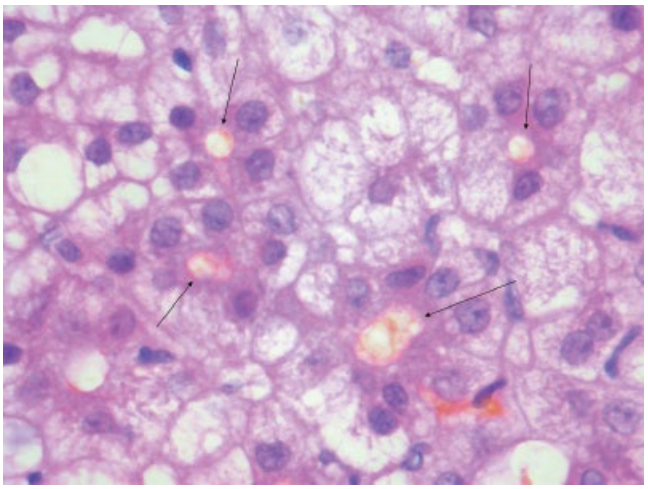
Microanatomy is intimately related to function and is best considered by linking individual cellular constituents and their local relationships with function. Blood from the hepatic artery and portal vein needs to come into intimate contact with hepatocytes to allow the metabolism of dietary molecules and detoxification of compounds, and to distribute the diverse proteins synthesized by the liver. In order for the liver to fulfill its exocrine function, bile secreted into intercellular canaliculi has to find its way to the biliary duct system and ultimately to the intestine. These functions require a complex interaction between individual cells, as well as regulation of blood supply and innervation. The way in which groups of cells are organized into "functional units" has been the subject of much debate and is discussed further here.

Cellular constituents of the liver

The liver parenchyma consists of a number of different cell types. About 80% are hepatocytes, 10% are sinusoidal endothelial cells, 5% are lymphocytes, and 4% are Kupffer cells (hepatic macrophages), while biliary epithelial cells account for 1–3%.

Hepatocytes, arranged in branched and anastomosing cords, have a diameter of 25 μm with the nucleus in the center. In keeping with their diverse functions, the cytoplasm is rich in organelles, up to 1000 mitochondria in a single cell, with endoplasmic reticulum and a Golgi complex for protein production. Particulate glycogen forms much of the "background" of the cell. The hepatocytes have different surfaces or "domains" where they abut other hepatocytes, with which they communicate via gap junctions (lateral domain). The basal domain is where the hepatocyte contacts blood in the sinusoid, and the apical domain forms the connection to the bile canaliculus. The latter two domains are covered with microvilli, providing an enlarged surface area. The sinusoids are lined by a specialized endothelium, which has fenestrae (apertures) to facilitate the transfer of molecules and particles. The sinusoidal endothelium lacks a basement membrane, further facilitating exchange between the blood and hepatocyte.

Canaliculi only become visible on light microscopy in cholestatic disease (Figure 1.5). The canaliculus is demarcated from the sinusoids and the intercellular space by tight junctions. The 1–2 μm wide bile canaliculi constitute the outermost reaches of the biliary tree. They are interconnected and form a network of intercellular channels, which receive the bile secreted from hepatocytes. Actin and myosin filaments of the hepatocyte propel the bile into the canals of Hering (ductules or cholangioles) with the help of aquaporine-dependent and adenosine triphosphatas (ATPase)-dependent transporters, which are lined with a mixture of biliary epithelium and hepatocytes. They have a diameter of less than 15 μm and are located at the periphery of a portal triad.



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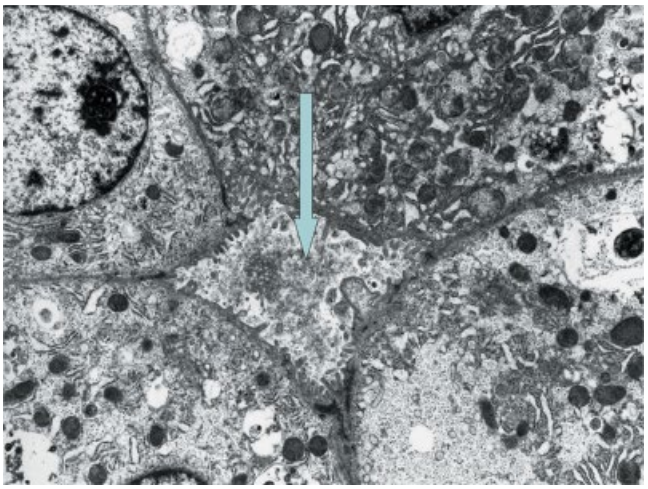
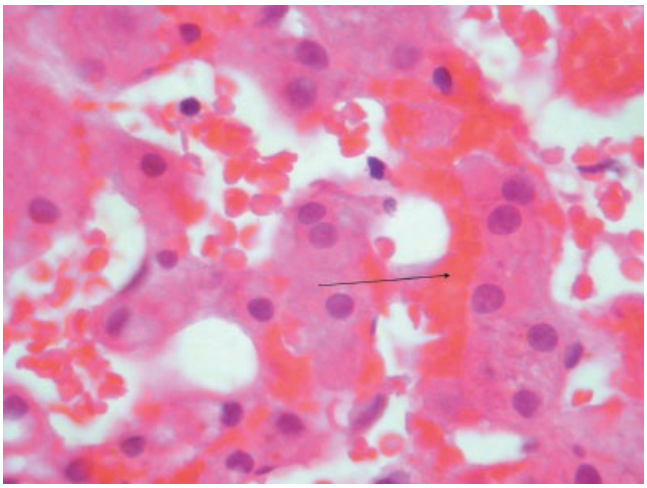


(B)

Figure 1.5 Bile canaliculi formed by apical sides of hepatocytes in cholestatic liver disease. (A) Canaliculi in a child with neonatal cholestasis. The canaliculi are not visible in light microscopy in a normal liver. In this child with neonatal hepatitis, they are distended by bile plugs, making them prominent (arrows). (H&E, original magnification × 400.) (B) Electron microscopy of a canaliculus. The arrow shows granular bile in a canaliculus in a child on parenteral nutrition. There are microvilli lining the edge of the canaliculus.

Between the endothelial cells and the basal aspect of the hepatocytes lies the space of Disse (Figure 1.6). This is not normally visible with light microscopy, but can be seen if there is hepatic venous obstruction, and is easily seen on scanned electron microscopy (Figure 1.6B). The space of Disse is the source of lymph production and contains extracellular matrix components, including type IV collagen, laminin, and proteoglycans. This matrix interacts via adhesion molecules with the hepatocytes, modulates the cell phenotype and serves as a reservoir for cell growth factors, cytokines, and albumin, which are released by matrix degradation.



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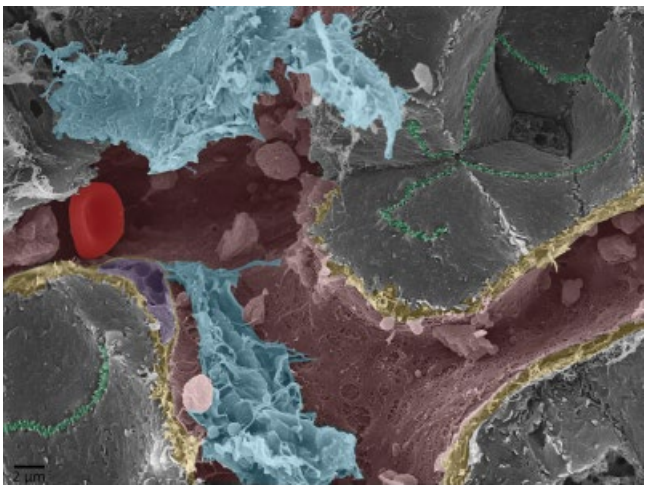


(B)

Figure 1.6 The space of Disse. (A) Liver histology in a child with Budd–Chiari syndrome. The space of Disse is not normally visible in light microscopy, but in this image blood has been forced into the space of Disse (arrow) and renders it visible. (H&E, original magnification × 400.) (B) Magnification of a scanning electron microscopy image for insights into the space of Disse (yellow) with an inlying Stellate cell (purple). Sinusoids (pale red) with two Kupffer cells (bright blue) and one erythrocyte (red) can be seen. Note the fenestration of sinusoidal endothelium. Polar hepatocytes with basolateral contact to the space of Disse and apical formation of bile canaliculi with microvilli (green) are also visible. (From Baumann *et al.* [5].)

Hepatic stellate cells (previously known as Ito cells) are found in the space of Disse and produce extracellular matrix, cytokines, and growth factors, store vitamin A and lipid, and have fine extensions surrounding the sinusoids, possibly related to the control of vascular tone. When activated by liver injury, they transform into myofibroblasts and have an important role in fibrosis by secreting collagen into the space of Disse and hence obstructing oxygen exchange [17].

Kupffer cells are the central part of the phagocytic system of the liver. They are mostly found on the luminal side of the endothelial wall of the sinusoids, but they migrate to areas of injury or infection. In addition to phagocytic function, they are an important source of cytokine secretion. They have different phenotypes: the M1 phenotype is proinflammatory and the M2 phenotype supports healing and suppression of inflammation [18].

Functional anatomy/regulation of blood supply

The dual blood supply to the liver, by the hepatic artery and portal vein, is unique in the body. In resting conditions, the liver receives 800–1200 mL blood per minute accounting for a quarter of the cardiac output. About 25% of this hepatic inflow is oxygen-rich blood arriving via the hepatic artery; the remaining 75% is partially deoxygenated nutrient-loaded blood from the intestine, pancreas, gallbladder and spleen, supplied by the portal vein. Arterial and portal blood merges freely at the level of the sinusoids. Total blood flow into the liver varies considerably and is reduced during sympathetic stimulation or sleep. In contrast, portal blood flow increases following a meal; it is stimulated by a protein-rich feed, only moderately stimulated by carbohydrates, and there is little effect following lipids. The arterial blood supply is not determined by oxygen demand as half of the oxygen required is provided by the portal vein. Portal and arterial flow are closely related, and an experimental reduction of portal flow results in arterial hyperemia. This phenomenon is also known as the hepatic arterial buffer response (HABR) and becomes apparent in liver transplantation, when thrombosis of either the hepatic artery or the portal vein leads to compensatory flow rates in the other vessel.

About 20–25% of the normal liver consists of blood, 40% of which is situated in the large vessels and 60% in the sinusoids. As this is 10–15% of the body's total blood volume, the liver serves as a reservoir with capacitance function. Liver blood volume can increase by hepatic venous pressure and may be tripled to about 60% in states of severe outflow obstruction. In hemorrhagic shock, in sympathetic stimulation, and in vascular dehydration, the liver can replace systemic volume rapidly.

Portal vein perfusion pressure is determined by the splanchnic arterioles and intrahepatic resistance and is approximately 6–10 mmHg. Arterial perfusion pressures depend on systemic perfusion pressures. The sinusoidal perfusion pressure is regulated by a number of factors in the afferent and efferent vessels, including muscular sphincter, autonomic nervous innervation, and paracrine function; it ranges between 2 and 4 mmHg.

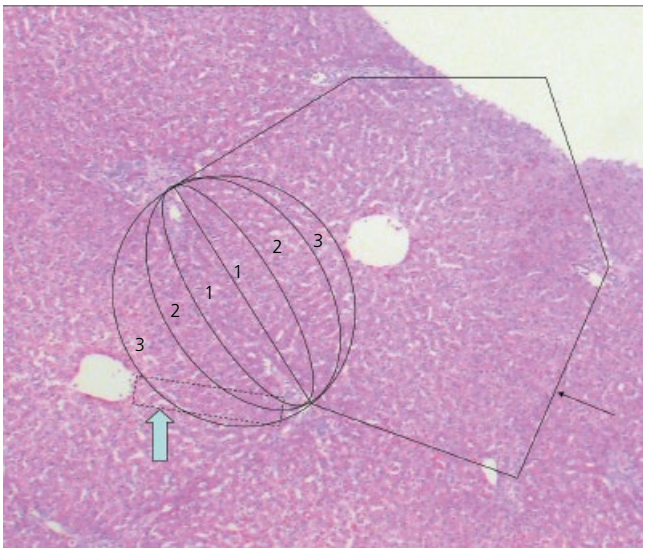
The distribution of blood flow in the sinusoids is determined by variation in the size of the Kupffer and endothelial cells, which swell and shrink to control the patency of the sinusoidal lumen, while the stellate cells impair oxygen exchange by collagen synthesis in fibrosis.

Functional versus anatomical units

In the absence of explicit connective tissue septa delineating structural units, different models have been used to define the smallest functional unit in the liver (Figure 1.7):

- The *classic lobule (central venous lobule)*, hexagonal in shape, was described in 1833 [19]. It has a hepatic vein branch ("central vein") at its center. Blood arriving in the portal tracts at the periphery of the hexagon feeds sinusoids around the whole of their circumference and hence different adjacent classic lobules, rather than all draining into the interior of the hexagon. It therefore has limited application as a functional primary unit.
- The *primary lobule*, described by Matsumoto *et al.* [20] uses the portal vein branches at the edges of adjacent central venous lobules to act as the center of the functional unit, giving rise to tortuous and branching three-dimensional units surrounding portal vein branches; it includes the classic lobule as a secondary structure [3, 15]. This model is based on actual vascular reconstruction (rather than the gelatin infusions used in the acinar concept) and is gaining widespread acceptance. Descriptive histology in the lobular model hence includes such terms as "centrilobular" hepatocytes (those around the central vein).
- The work of Rappaport *et al.* in 1954 defined the functional unit as an *acinus*, consisting of parts of two adjacent lobules [21]. The axis of the acinus is formed by the terminal branch of the portal vein, not visible in routine light microscopy. The three zones of the acinar concept corresponding to different levels of oxygen supply are illustrated in Figure 1.7. It should be noted that the three acinar zones do not equate to the regions described in the lobular concept. Acinar zone 3 is located around the center of the classic lobule, but not exclusively "perivenular," and extends in an arc-like fashion from one portal tract to another. The acinar concept proved popular for pathologists as necrosis occurs first in the least well-oxygenated hepatocytes in a portal–central distribution, which corresponds to the most peripheral acinar regions (zone 3 in Figure 1.7), and not in the perivenular region.

However the functional unit is defined, the function of the hepatocytes, sinusoidal endothelium, Kupffer cells, and extracellular matrix composition varies between regions. "Periportal," "perivenular," and "midzonal" serve as useful descriptors for considering functional differences or gradients. Gene expression also shows a functional or a compartmental zonation [15]. The phenotypic variation may be determined by the declining gradient in oxygen concentration, the decreasing glucagon : insulin ratio, or other autocrine signals such as phosphoenolpyruvate carboxykinase (PCK) and carbamoylphosphate synthase (CPS). There are also compartment zonations, meaning that hepatocytes in a specific region express certain genes. Periportal hepatocytes are responsible for oxidative energy metabolism,



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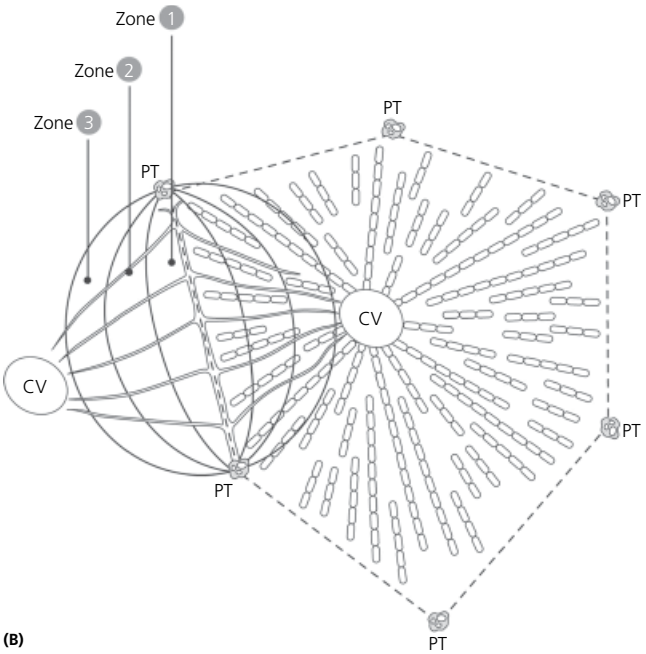

(B)

Figure 1.7 Normal liver tissue. (A) Light microscopy of normal liver tissue. The small arrow points to the approximate outline of a classic hepatic lobule, centered around a central vein. In schematic diagrams this is often illustrated as a regular hexagon, with portal tracts at four points and "nodal points of mall" at the other two. This is rarely reproducible in practice, leading to the slightly irregular hexagon shown. The elliptical structure denotes postulated acinar zones 1, 2, and 3, centered around a terminal portal venule (not visible). This occupies portions of two adjacent classic lobules. The dotted rectangle shows the location of portal central bridging necrosis, which is observed in the clinical situation and which made the acinar concept popular from a pathological point of view. (H&E, original magnification × 40.) (B) Schematic view of the same anatomical and functional units of the liver. CV, central vein; PT, portal tract.

such as gluconeogenesis, β-oxidation, and amino acid catabolism, bile formation, and cholesterol synthesis. Perivenous hepatocytes are involved in detoxification, glucose uptake for glycogen synthesis, glycolysis, liponeogenesis, and ketogenesis. Periportal Kupffer cells are more active in phagocytosis than the centrilobular cells, which produce cytokines.

Innervation

The liver is innervated by afferent and efferent nerves of the autonomic nervous system, through sympathetic nerve fibers from the celiac ganglia and some parasympathetic input from the vagus nerve. Sympathetic nerve bundles accompany the large vessels and supply a dense perivascular plexus around the hilar blood vessels into the sinusoids, where nerves course in the space of Disse and surround isolated hepatocytes and stellate cells. Parasympathetic nerve fibers accompany the hepatic inflow system with ganglion cells close to the liver, forming a plexus around the hepatic artery and portal vein, but there is little cholinergic innervation beyond the portal tract.

The gap junctions may also provide direct electrical coupling between cells, bypassing the need for nervous innervation. Cholinergic stimuli increase metabolic activity, whereas adrenergic stimuli increase glucose mobilization into the blood. The realization that hepatic function is effective even in the denervated graft following liver transplantation has challenged longstanding views about the role of the autonomic nervous system in regulating metabolic activity in the liver.

Recent studies have shown that the glucose disposal effect of insulin and its negative effect on hepatic gluconeogenesis is significantly impaired by parasympathetic denervation. Furthermore, regulation of hepatic and muscle glucose uptake by portal vein glucose load does not function in a denervated liver. α-Adrenergic innervation is involved in hepatocyte replication and hepatic progenitor cells are activated by the vagal nerve [22].

Function

The liver is the central organ for metabolic homeostasis. Its main functions are:

- Regulation of uptake and processing of nutrients from the intestinal tract.
- Synthesis and biotransformation of proteins, carbohydrates, and lipids.
- Excretion of bile and elimination of hydrophobic compounds.
- Regulation of energy metabolism.
- Endocrine functions and mediation of normal growth and development.
- Immunological function.
- Drug metabolism.
- Regulation of fluid balance.

Uptake and processing (synthesis, storage, and degradation) of proteins, carbohydrates, and lipids

Proteins

The liver accounts for 15% of total body protein production, and the majority of these proteins are secreted as plasma proteins such as albumin (responsible for transport, keeping up osmotic pressure), other transport proteins such as ceruloplasmin, complement, protease inhibitors, and – clinically very important – coagulation and fibrinolytic proteins. Proteins are synthesized from dietary amino acids, and alanine and glutamine from muscle after transcription of protein-decoding genes into mRNA. Following translation and modification, proteins are secreted from the sinusoidal aspect of the hepatocytes into the circulation. Protein production is regulated by gene expression, protein synthesis, nutritional status, and hormone secretion. There is a higher production rate in acute illnesses – the acute phase response, in which C-reactive protein is the most commonly measured sign. Proteins are not stored in the liver, but amino acids are recycled to synthesize new molecules. The liver also plays a role in protein and glycoprotein degradation. Amino acid degradation takes place in the liver, generating the highly toxic metabolite ammonia, which crosses the blood–brain barrier readily and is associated with hepatic encephalopathy (see Chapters 9 and 18). The urea cycle, which is active in the liver, is largely responsible for its removal, and urea cycle defects present with severe encephalopathy (see Chapter 9).

Carbohydrates

The liver has a major role in maintaining blood glucose. Glucose, fructose, and galactose are taken up by the hepatocytes from portal blood. Glucose – in the fed state – is converted to glucose-6-phosphate by glucokinase and used as a precursor for glycogen synthesis by glycogen synthase and pyruvate via glycolysis, or else used in triglyceride production. Important hormones are insulin, which induces glycogen synthase and reduces gluconeogenesis and glucose output, FGF 15/19, which also stimulates glycogen synthesis, and glucagon, which stimulates gluconeogenesis and increases glucose output. Glucose is either released from glycogen by glycogenolysis mainly in short-term fasting periods or is synthesized from amino acids or substrates such as lactate or glycerol (gluconeogenesis) in long fasting periods, regulated by the metabolic state and numerous transcription factors.

In conditions of stress or fasting, insulin and FGF 15/19 are downregulated, and therefore glucose uptake is reduced and glucose production is increased from glycogenolysis. Hypoglycemia is a sensitive test of liver function and is a sign of severe hepatic necrosis, indicating loss of liver function (see Chapters 9, 18, and 21). For the same reason, many infants with severe liver disease are unable to maintain their blood sugar levels during prolonged fasts.

Lipids

Lipid metabolism and lipogenesis are regulated by a complex interaction of hormones, such as insulin, and transcription factors but also by the metabolic state and circadian rhythm.

The liver is essential for cholesterol and lipoprotein metabolism. Dietary fat is absorbed in the small intestine by enterocytes and carried by chylomicrons, lipoprotein transport particles, from the intestine to the circulation and delivered as triglycerides to the peripheral tissues. The resulting cholesterol-rich chylomicron releases non-esterified fatty acids which are taken up by the liver. The liver also synthesizes fatty acids from glucose in times of dietary excess, and these are subsequently stored as triglycerides, which are the principal source of energy, in lipid droplets in hepatocytes, or secreted as very-low-density lipoprotein (VLDL) particles. Fatty acids that are not converted to triglycerides or used in the synthesis of other molecules are used as energy supply by ß-oxidation, and generate ketone bodies in the mitochondria, or in the case of very-long-chain fatty acids in the peroxisomes. Microvesicular steatosis in hepatocytes is a sign of mitochondrial or peroxisomal disease or drug toxicity (see Chapter 12).

Cholesterol is a component of all cell membranes and is essential for the production of steroid hormones and bile acids. The liver synthesizes cholesterol and fatty acids de novo from carbohydrates. Cholesterol homeostasis is controlled by uptake from lipoproteins and chylomicrons, which increase hepatic cholesterol, and by the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA), which synthesizes cholesterol de novo. The amount synthesized in the liver is around 25% of the total amount synthesized and twice that absorbed from the diet. In the liver, cholesterol is either "free" or stored as cholesterol ester. The degradation of cholesterol also takes place in the liver through the oxidation to bile acids and biliary excretion of cholesterol. Cholesterol may crystallize in the gallbladder and forms part of most gallstones. A number of cholestatic liver diseases (e.g., biliary atresia or Alagille syndrome) lead to elevated plasma cholesterol due to deficient biliary excretion and catabolism.

VLDLs are the main lipoproteins secreted by the liver and carry triglyceride and cholesterol to other tissues, where they are converted to low-density lipoproteins (LDLs) and then to free fatty acids. High-density lipoproteins (HDLs) carry cholesterol from the peripheral tissues back to the liver. Fatty liver occurs when the synthesis of triglycerides exceeds the liver's capacity for export or internal metabolism [23].

Bile and bile acids

The production and excretion of bile is an elemental function of the liver, ensuring the elimination of unwanted internal and external metabolites and lipid absorption. Bile is produced in hepatocytes (75%) and cholangiocytes (25%), is further modified in the bile ducts, and is concentrated in the gallbladder. In adults, about 600 mL of isotonic watery bile with a pH of 7.8 is produced daily in order to facilitate the excretion of many compounds, including drugs, toxins, and waste products as well as cholesterol and bilirubin, and to provide bile salts to the intestine for the emulsification and absorption of dietary lipids and fat-soluble vitamins. Bile formation is an osmotic process driven by the excretion of organic metabolites (mainly bile acids) and the influx of electrolytes and water. It is traditionally divided into "bile salt dependent" (the relationship of canalicular bile flow to bile salt excretion) and "bile salt independent" (the active secretion of electrolytes and other solutes, mainly glutathione).

The main components of bile besides water are bile acids (12%), phospholipids (4%), cholesterol (0.7%), and conjugated bilirubin (0.1%). Lecithin increases the solubility of cholesterol in bile by micelle formation exponentially to allow a 10-fold concentration of bile acids and cholesterol after concentration by the gallbladder. The main electrolyte in the bile is sodium at a concentration of 280 mmol/L; other electrolytes and bicarbonate are less concentrated, or unchanged. The primary bile acids – cholic acid and chenodeoxycholic acid – are synthesized from cholesterol by 7α-hydroxylase and subsequently conjugated with taurine and glycine to facilitate secretion.

Primary bile salts are transformed by intestinal bacteria into secondary bile salts – cholic acid into deoxycholic acid and chenodeoxycholic acid into lithocholic acid and subsequently to ursodeoxycholic acid (UDCA). They are reabsorbed in the ileum and returned to the liver via the portal vein and are the major stimulus for bile secretion. In normal conditions, UDCA represents only 3% of the bile salt pool. It is more hydrophilic than the other bile salts and is used therapeutically to stimulate bile secretion; it may prevent the hepatocytes from damage caused by hydrophobic bile salts. In chronic liver disease, this balance is shifted to a predominant production of chenodeoxycholic acid, which lowers the bile pH.

Hepatic bile formation and the biliary excretory function are closely related. In adults, the enterohepatic circulation of bile salts occurs 4–12 times in 24 h, enabling the body to retain most of the 5–6 g in the body bile salt pool, as the total stock of bile salts is not sufficient for fat absorption. Neonates have about half the bile salt pool of an adult, and ileal bile salt reabsorption is lower. Their intestinal bile acid concentration may be low, leading to poor micelle formation and reduced uptake of fat-soluble vitamins and dietary lipid in comparison with older children and adults. Although this is rarely a cause of malnutrition and/or steatorrhea, it needs to be considered in cholestatic conditions when early supplementation of fat-soluble vitamins is indicated. Bile acid uptake from portal blood is physiologically lower in neonates in comparison with older children, and elevated levels of bile acids may be mistaken for cholestatic liver disease.

Intrahepatic and extrahepatic bile salt transport

The transport processes for bile salts are complex and quite efficient, as 95% of the excreted bile salts are recycled from the enterohepatic circulation. The polarized hepatocytes

absorb substrates from the portal vein blood in the sinusoids, such as bile salts, phospholipids, and metabolites of toxic substances, and transport them across the cell to the canalicular membrane to secrete into bile. The uptake of conjugated bile salts (e.g., taurocholate) by hepatocytes at the basolateral plasma membrane is mediated by different (ATP, sodium, and OH dependent) transporters, primarily the sodium-dependent bile acid importer NTCP (Na^+ taurocholate cotransporting polypeptide) through an active transport process driven by a sodium gradient. The uptake of unconjugated bile salts at the sinusoidal membrane is sodium independent and mediated by the organic anion transporting polypeptide (OATP), defects of which lead to Rotor syndrome. This transporter also transports steroids such as progesterone and ciclosporin. After uptake into hepatocytes, the intracellular transport across the cell is mediated by binding to cytosolic proteins, ligandins, and Y9 proteins or fatty acid-binding proteins. Some free intracellular bile salts reach the canalicular plasma membrane by diffusion [24].

Excretion of bile salts across the canalicular plasma membrane is the rate-limiting step in the transport of bile salts from blood into bile, as it happens actively against a c. 1000-fold concentration gradient. It is facilitated by the ATP-dependent "bile salt-excreting pump" (BSEP) [25]. A defect in this transporter caused by a mutation of the decoding gene *ABCB11* is responsible for the genetic condition known as progressive familial intrahepatic cholestasis (PFIC) type 2 and milder forms such as intrahepatic cholestasis of pregnancy (ICP). In contrast to monoanionic bile salts, divalent sulfated and glucuronidated bile salts are excreted into bile by the multidrug resistance-associated protein 2 (Mrp2) and multidrug resistance p-glycoprotein type 1 (MDR-1). Failure to express Mrp2 at the canalicular membrane results in conjugated hyperbilirubinemia and forms the basis of hereditary Dubin–Johnson syndrome. Canalicular phospholipid secretion is mediated by a different transporter protein, MDR-3, which is important in preventing bile salt-induced toxic damage to the biliary epithelium. Failure to express this transporter results in PFIC type 3 and biliary cirrhosis (see Chapter 8). PFIC-1 is an aminophospholipid translocator in the canalicular membrane of hepatocytes that is also found on the apical membrane of enterocytes. It is responsible for the transport of phosphatidylserine and phosphatidylethanolamine. A genetic defect in the expression or function of this transporter causes PFIC type 1 or benign recurrent intrahepatic cholestasis type 1 (BRIC 1; see Chapter 8).

Excretion of bilirubin

As well as its role in facilitating bile salt homeostasis, the biliary system also serves as the primary pathway for eliminating bilirubin, excess cholesterol, and hydrophobic xenobiotics. Bilirubin is a toxic degradation product, 80% of which is derived from the breakdown of erythrocytes; the remainder stems from heme-containing myoglobin, cytochromes, or failed erythropoiesis. Mononuclear phagocytic cells oxidize heme to form biliverdin, which is then reduced to unconjugated bilirubin. This unconjugated bilirubin is usually albumin-bound, but – when exceeding the binding capacity of the albumin – it can diffuse across the blood–brain barrier and cause kernicterus in neonates.

The albumin-bound bilirubin is transported to the hepatic sinusoids, is actively transported into the hepatocytes via the basolateral membrane with the contribution of OATP-1B, and bound to ligandin in the cytoplasm is transported to the endoplasmatic reticulum.

The enzyme UDP glucuronyltransferase (UGT1A1) catalyzes the conjugation of bilirubin with one or two molecules of glucuronic acid in the endoplasmic reticulum, and the conjugated or direct bilirubin is excreted with the help of Mrp2 as hydrophilic bilirubin glucuronides via the canalicular membrane. Some may be secreted to the sinusoids and, following intestinal excretion, bacterial β-glucuronidases degrade most of these bilirubin glucuronides to colorless urobilinogen, which is reduced to stercobilin, accounting for the brownish color of feces. About 20% of urobilinogen is reabsorbed in the ileum and colon and returned to the liver via the portal vein and excreted into the urinary tract.

UGT1A1 belongs to the UGT family of conjugating enzymes, which are expressed in a wide range of tissues and which catalyze glucuronidation of various substrates, including steroid hormones, carcinogens, and drugs. UGT1A1 activity in the first days after birth is below 10% of adult activity, contributing to physiological neonatal jaundice. Mutations in the *UGT1A1* gene either reduce the affinity of UGT1A1 toward bilirubin or reduce enzyme activity. Complete absence of UGT1A1 activity causes Crigler–Najjar syndrome type 1 presenting with severe hyperbilirubinemia, and a significant reduction of activity causes Crigler–Najjar syndrome type 2. A very mild reduction of UGT1A1 activity by missense mutation or reduced expression of the enzyme is present in 6% of the general population, causing Gilbert syndrome with its characteristic mild elevation of unconjugated bilirubin (see Chapter 8).

Regulation of energy metabolism

The energy metabolism of the body is integrated by the liver through glucose metabolism and fatty acid oxidation. The liver has a central role in maintaining blood glucose homeostasis at constant levels. The liver is responsible for the disposal of around two-thirds of the oral glucose load, divided into uptake and downregulation of glucose release, so absorbed glucose is taken up into peripheral tissue. Hepatic and extrahepatic uptake of glucose are controlled by the load of glucose in the portal vein blood. The glucostat function of the liver is achieved by controlling the storage and release of glucose from glycogen, followed by glycolysis and gluconeo-

genesis in long episodes of fasting. The glycogen content of a liver of a 10 kg child is around 20–25 g, increasing to about 100 g (55–72 mg glycogen per gram of liver tissue) in an adult. Studies have shown that increased glycogen content is associated with impaired hepatic insulin signaling and glycogenesis. As the normal resting glucose requirement is between 4 and 6 mg/kg/min, the glycogen stores last for less than a day of fasting, after which gluconeogenesis is activated. In prolonged fasting, total body glucose requirements decrease from 160 to 40 g glucose/day after 5–6 weeks of starvation. The healthy body can tolerate this, because fatty acid oxidation becomes the main source of fuel for respiration. Glucose uptake into the hepatocyte is insulin independent, via glycogen phosphorylase and glycogen synthase, and has a direct regulatory effect on glycogen synthesis and the storage and release of glucagon and adrenalin.

The liver acquires fatty acids from the blood, from chylomicron remnants, and by de novo synthesis. Its triglyceride content correlates with visceral fat content. The conversion of excess glucose to fatty acids only takes place when hepatic glycogen stores are complete. Fatty acids are esterified to triglycerides and exported from the liver as VLDLs. Triglycerides in VLDLs from the liver and from the intestinal absorption of lipids are hydrolyzed by lipoprotein lipase and taken up in the peripheral tissues, where fatty acids are metabolized for energy or stored [23].

Endocrine function

The liver plays an active role in endocrine regulation. In response to pituitary growth hormone activation, the liver produces the majority of the circulating mitosis-inducing (mitogenic) polypeptide hormones insulin-like growth factor 1 and 2 (IGF-1 and IGF-2). These have anabolic and metabolic effects, regulate the proliferation of various cells, and are crucial in growth and development leading to failure to thrive in chronic liver disease. IGF-1 may therefore be used as a marker of hepatocellular dysfunction. The specific endocrine effect of IGFs and other hormones, such as steroid hormones, is modulated by different binding proteins (IGF-binding proteins 1–6, sex hormone-binding globulin, or thyroid-binding globulin) that are synthesized in the liver. These binding proteins transport the hormones, regulate their metabolic clearance, and directly modulate hormone interactions with specific receptors. Angiotensinogen (important in the renin–angiotensin–aldosterone system for blood pressure regulation) and thrombopoietin (stimulating megakaryopoiesis) are also synthesized by the liver. Thyroxine (T_4) is converted into the metabolically active form of T_3 in the liver by iodothyronine deiodinase, which accounts for the low T_3 syndrome in patients with decompensated cirrhosis. Adrenocortical dysfunction is known to frequently occur in liver disease [26]. Hormonal dysfunction in liver disease may develop from a reduced clearance of hormones (e.g., gynecomastia in men), from portosystemic shunting, dysregulated synthesis of binding proteins, or impaired endorgan sensitivity to the hormone (i.e., insulin resistance in cirrhosis).

Immunological function

Lymphocytes enter the liver through the sinusoids and the space of Disse. The liver contains approximately 10^{10} lymphocytes, both of the adaptive immune system (predominantly T cells and a smaller amount of B cells), which require previous exposure to antigen for efficacy, and of the innate immune system (natural killer (NK) cells). The liver also contains NK T cells that express both T-cell and NK-cell markers, which play a role in the clearance function of the liver in filtering gut-derived endotoxins and microorganisms. Activated cluster of differentiation 8 (CD8) T cells (effector cells) induce and maintain the immune reaction to hepatotropic pathogens. In liver injury, inflammatory and anti-inflammatory response is mediated by hepatic NK cells.

The liver contains three types of antigen-presenting cells: Kupffer cells, liver sinusoidal endothelial cells (LSECs) and dendritic cells. Kupffer cells are macrophages derived from monocytes that are important in the first line of clearing toxins, viruses, and bacteria from the portal vein blood and initiate the immune response by cytokine release (tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), IL-10) and antigen presentation (they are conspicuous in acute hepatitis). Following liver transplantation, donor Kupffer cells are rapidly (within days) replaced by recipient Kupffer cells infiltrating the liver. LSECs – as their name suggests – are lined around sinusoids, whereas dendritic cells in the liver usually surround central veins and portal tracts; both are efficient in antigen processing and presenting (see Chapter 3) [27].

Drug metabolism (xenobiotic metabolism)

The liver is the prime site for drug metabolism in the body, as most of the xenobiotics are absorbed by the gastrointestinal tract and reach the liver via the portal vein. Most drugs are transported into the hepatocytes by ATP-dependent solute carriers and are metabolized in the smooth endoplasmatic reticulum of the hepatocytes. During the first phase, enzymes (mainly of the cytochrome P450 family) change the xenobiotic structure mostly by oxidation, and rarely by reduction or other reactions. Reactive oxygen species that are toxic to the cell are generated during this process and require a range of antioxidant mechanisms (molecules (e.g., glutathione and vitamin E) and enzymes (e.g., superoxide dismutase)) to render them inert. Thus, CYP3A4, the most important member of the P450 group, is expressed in zone 3. The metabolized drug, which may itself be toxic, enters the second phase of metabolism: the conjugation with hydrophilic compounds (e.g., glucuronic acid or glutathione) to increase the water solubility and the detoxification of toxic molecules produced in the first phase of drug metabolism.

Once rendered hydrophilic, the drug metabolite is either further metabolized or directly excreted via the kidneys or the bile. There are individual differences in drug metabolism, and the enzymes responsible for drug metabolism may be either induced or inhibited by other drugs or chemicals. Severe liver failure reduces the ability to metabolize drugs, so that drug effects are prolonged (e.g., sedatives or anesthetic agents), or there may be an accumulation of toxic metabolites, which complicates hepatic encephalopathy (see Chapter 12).

Liver function in maintaining fluid balance

The liver can retain and release a significant volume of whole blood and/or plasma and hence influences the circulating blood volume. Although the direct interaction between the liver and kidney is not fully understood, impaired liver function leads to a reduced ability to excrete sodium and water leading to hepatorenal syndrome with renal failure. A number of factors are involved, which include: hyperaldosteronism and/or increased tubular sensitivity to aldosteronism, and increased renal sympathetic nerve activity. The hepatorenal syndrome is caused by reduced renal perfusion, renal vasoconstriction, cardiac dysfunction, and release of cytokines. Portal hypertension may precipitate the hepatorenal reflex to activation of the renin–angiotensin–aldosterone system and the release of antidiuretic hormone [28]. Splanchnic vasodilation is probably an initial adverse event that leads to renal vasoconstriction, followed by a reduction of renal blood flow and of the glomerular filtration rate. Sodium retention is the first sign of renal dysfunction, followed by water retention, leading to dilutional hyponatremia in plasma. Plasma volume expansion due to sodium and water retention, together with sinusoidal hypertension (portal pressure gradient of >12%), is a key factor in the pathogenesis of cirrhotic ascites (see Chapter 21).

Liver growth and regeneration

The expected lifespan of a hepatocyte is about 200–500 days. Even though the liver is more stable in adulthood, the liver cell mass remains highly flexible and varies throughout life, depending on metabolic demands such as disease or pregnancy. In normal children and adults, hepatic regeneration occurs by replication of mature hepatocytes, cholangiocytes, and endothelial cells and the liver can recover from mild injury completely. This process can be upregulated – for instance, following trauma or partial hepatectomy – and the liver can be reconstituted by proliferation of mature hepatocytes within days and weeks.

Liver regeneration is also reliant on hepatic progenitor cells. Pluripotent, so-called oval cells, that are located in the canals of Hering, are stimulated by injury and differentiate into hepatocytes and/or cholangiocytes. It is not known whether the recruitment of hepatic progenitor cells from bone marrow stem cells is a significant part of liver regeneration.

The finding of the hemopoietic stem cell marker and proto-oncogene c-*kit* in certain biliary cells from diseased pediatric liver was one of the first steps in demonstrating the presence of stem cells in humans [29]. Theise *et al.* [8] demonstrated the presence of Y chromosome-positive hepatocytes and biliary epithelium in female recipients of a therapeutic bone marrow transplant from male donors, confirming the ability of human bone marrow-derived stem cells to differentiate into the hepatic cell lineages.

Liver regeneration varies with circadian rhythms and metabolic requirements. Increased metabolic demands and pro-inflammatory cytokines are essential. Cytokines (TNF-α, interferon-γ, IL-6) and growth factors (hepatocyte growth factor) initiate liver regeneration by activating hepatocyte replication and hepatic progenitor cells. The degradation of extracellular matrix by metalloproteinases to release growth factors is an essential step in hepatocyte proliferation; on the other hand, the loss of certain metalloproteinase inhibitors leads to liver failure.

Hepatocyte transplantation and hepatic stem cell therapy have been studied widely over the last years but have not (yet) proven to be equivalent to liver transplantation.

Liver fibrosis

The outcome of most chronic disease processes in the liver is fibrosis. In hepatic fibrogenesis, the activation of stellate cells by injured hepatocytes, biliary cells, or Kupffer cell secretion leads to the conversion of quiescent vitamin A- and fat-storing cells into proliferative, contractile, and fibrogenic myofibroblasts. These cells produce an excess of type I and III collagen, which replaces the normal extracellular matrix in the portal tracts and lobules and leads to fibrous septal tracts and obstruction of the space of Disse. Liver fibrosis is a partially reversible process, even though complete restitution remains debatable. Different metalloproteinases that cleave collagens are mainly involved in matrix degradation of the liver, although neutrophils, macrophages, and stellate cells also contribute to this process. Tissue inhibitors of matrix metalloproteinases (TIMPs) are the key regulators in determining the reversal of fibrosis. Sustained TIMP-1 expression inhibits protease activity for matrix degradation and blocks apoptosis of activated stellate cells. Furthermore, soluble mediators such as transforming growth factor β (TGF-ß), platelet-derived growth factor (PDGF), and fibroblast growth factors may regulate fibrogenesis by influencing stellate cells and are the subject of further studies of pharmacological intervention [30].

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CHAPTER 2

Liver Pathology in Children

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Key points

- Patterns of injury (e.g., biliary, hepatitic, steatotic/metabolic, vascular), rather than specific diseases, can be seen in liver biopsies.
- Meaningful interpretation of biopsies can only happen alongside a global assessment of the clinical scenario and other investigations.
- Biopsies should be examined methodically and then interpreted in the light of the clinical setting; this is facilitated by experience.

In the context of "understanding the liver," a knowledge of what disease processes look like in the liver is helpful for understanding disease presentation and natural history. In the interests of providing a practical guide this chapter will focus on liver pathology as seen in liver biopsies in a diagnostic setting. Tumor pathology will not be included here.

What should you expect from your pathologist?

The same pattern of injury can be seen with a wide variety of very different disease processes. Liver biopsies are small and the liver is large. These two facts mean that expecting a one line and definitive diagnosis from your pathologist is not realistic. Liver biopsy in children is not without risk, so careful consideration should be given to whether biopsy interpretation will influence management or prognosis [1]. Genetic markers may be available for some diseases and non-invasive options for the assessment of fibrosis may be preferable, such as "elasticity" measurements or serum markers of fibrosis [2, 3]. Liver tissue may be helpful for reasons other than histopathology; biochemical estimations of copper, for example. All of these factors may influence the decision to biopsy.

In cases where biopsy is indicated, the more clinical details provided, the better the pathologist will be to focus the differential diagnosis [4]. This process is facilitated by multidisciplinary meetings and access to an electronic patient record.

In order to have a meaningful discussion it is helpful to understand the pathological diagnostic process and some of the terms used. This chapter provides a systematic explanation of biopsy interpretation briefly referencing commonly encountered pathology in the settings described in the rest of this book (the infant and older child, acute and chronic conditions, metabolic, surgical, and transplant) – indicated in italics.

Approach to biopsy interpretation

Adequacy

Once the decision has been made to perform a biopsy every effort should be made to provide an adequate sample. Small biopsies will be less representative of the whole liver and may underestimate disease severity. The question of what constitutes an adequate biopsy has been debated but 15–25 mm biopsies containing at least 11 portal tracts are usually adequate.

Architecture

Architectural disturbance relates to the distortion of normal structures giving rise to abnormal spatial relationships between portal tracts and central veins – "vascular relationships." Fibrosis is the main cause of architectural distortion, but it is not the only one.

Necrosis

Acute damage to the liver may lead to hepatocyte necrosis and the collapse of liver cell plates. This process often begins around central veins. The next step is bridging necrosis (between portal tracts and central veins) and then pan-acinar necrosis.

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Bridging may leave nodules of hepatocytes, which looks like cirrhosis to the unwary. In pan-acinar necrosis, portal tracts and central veins are seen in abnormally close proximity to one another. Cases of acute liver injury leading to *fulminant hepatic failure* have this pattern of injury. Once the liver has become necrotic the pathologist is usually not in a position to offer a cause. *Drug/toxin-related injury*, *infections "non A non B*," and *acute presentations of autoimmune hepatitis* are offered as differential diagnoses. Viral inclusions should be sought. If there is any surviving parenchyma, severe steatosis might suggest a *metabolic* cause. This pattern is seen in the neonate in *neonatal hemochromatosis* or *gestational alloimmune liver disease*, accompanied by iron deposition in hepatocytes and biliary epithelium [5].

Sometimes acute damage occurs on a background of chronic liver disease. This happens in acute presentations of *Wilson disease*. Good-quality connective tissue stains are vital in making the distinction between acute and chronic damage. It is not always as easy as might be imagined. Reticulin outlines areas of collapse but is also present in fibrosis. Collagen stains are darker in areas of mature/longstanding fibrosis but they will show some paler staining in collapsed areas. Critically, elastin is present in longstanding fibrosis, which can be usefully stained with orcein.

Vascular flow-related changes

Abnormal blood flow through the liver secondary to compromise of either portal or hepatic venous flow, in any caliber vessel, can produce architectural changes in the liver under an umbrella term of *non-cirrhotic portal hypertension*. Changes can be subtle. Outflow obstruction as seen in *Budd–Chiari syndrome* causes dilation of, typically empty, sinusoids. The red blood cells are pushed out into the space of Disse. Small vessel veno-occlusive lesions might be seen. In *nodular regenerative hyperplasia* there is a subtle pattern of nodularity outlined on a reticulin stain. The nodules in this case are outlined by atrophic liver cell plates, rather than by either fibrosis or necrosis. The pathologist can recognize a "vascular" pattern of injury but is not often able to precisely localize where the vascular lesion lies.

Fibrosis

The end result of most diseases affecting the liver is scarring. Fibrosis often begins around the portal tracts. Fibrous bands might then develop, forming bridges between the portal tracts and/or central veins. The next stage is the formation of complete nodules entirely surrounded by fibrous tissue, which is called cirrhosis. In end-stage biliary disease and in *congenital hepatic fibrosis* the nodules can have a lobulated "jigsaw-like" appearance. It should be noted that fibrosis can affect the liver in a patchy manner – what is seen in the biopsy might not reflect the liver as a whole. This is true of many biliary diseases and especially *cystic fibrosis*. In *fatty liver disease* fibrosis begins around the central veins and has a pericellular pattern. Perisinusoidal fibrosis can occur in vascular disease. The stage of fibrosis is assessed by using scales such as the METAVIR (stages I–IV) and Ishak (stages I–V) scores and is most useful for assessing the prognosis of liver disease or response to therapy in clinical trials.

Portal tracts

Bile ducts and ductules

The bile duct proper is adjacent to, and a similar size to, the hepatic artery branch. The duct is missing in *Alagille syndrome* and in cases of non-syndromic bile duct paucity. It should be noted that bile ducts are often difficult to see in neonatal biopsies, so examination of several levels is helpful. The duct can be lost secondary to inflammatory damage in *chronic cellular rejection* and in *primary sclerosing cholangitis* (PSC). Periductal, "onion skin" nodular fibrosis is seen around ducts of medium caliber in PSC; large ducts are dilated and ulcerated. Causes of secondary sclerosing cholangitis, cryptosporidia for example, should be excluded [6].

The bile duct proper should be separately assessed from ductules, which appear at the portal tract margins. The archetypal marginal ductular reaction is seen in large duct obstruction. *Biliary atresia* is an example of large duct obstruction [7]. The ductules are typically plugged with bile. In neonatal biopsies a bile duct is still seen (Figure 2.1). In end-stage biliary atresia seen at transplantation, ducts can be missing due to ongoing large duct obstruction. Ductules can also be seen in other conditions, not necessarily implying a disease primarily of the biliary tree, for example in *sepsis*, *α1-antitrypsin deficiency*, and *intestinal failure-associated liver disease* [8]. In *cystic fibrosis*, eosinophilic secretion is typically seen in the ductules associated with neutrophil polymorphs.

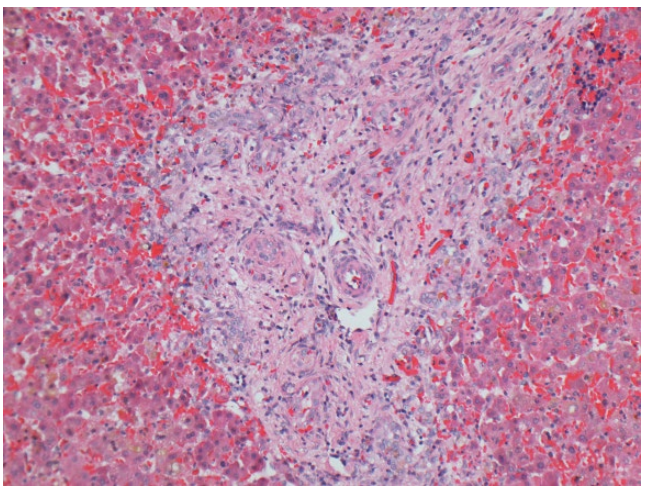


Figure 2.1 Biliary portal tract. This is from a case of biliary atresia. Peripheral portal tracts show the manifestations of large duct obstruction. Ductules are visible at the margin of the portal tract, and some contain bile plugs. Centrally, a paired arty and bile duct are seen. (H&E, original magnification × 200.)

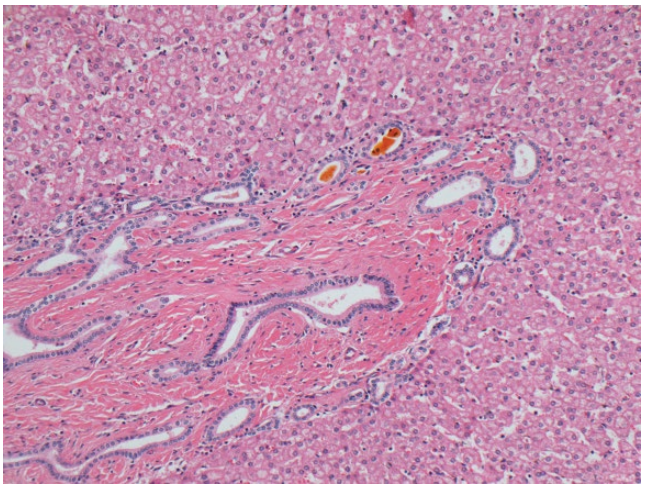


Figure 2.2 Ductal plate malformation. Contrast the appearances of the ductules here with those in Figure 2.1. Note how strikingly normal the adjacent parenchyma is. (H&E, original magnification × 200.)

Neutrophils should always be expected in association with ductules and regarded separately from other portal inflammatory cells. Ductules are seen as a response to necrosis. In *ductal plate malformation*, where remodeling of the embryonic precursors to the bile ducts has been stalled, abundant, oddly shaped, slit-like, or cystically dilated, basophilic ductule-like structures are seen. These can contain secretion (Figure 2.2).

Inflammation

A few lymphocytes are normal in portal tracts. When inflammation is significant, its severity should be semiquantitatively assessed and attention paid to the cell types present. Eosinophils may be an indicator of a *drug* reaction but are commonly seen in the infiltrates associated with *autoimmune hepatitis* (AIH) and *acute cellular rejection* (ACR). In AIH, the infiltrate is usually dominated by plasma cells and interface hepatitis is present. The interface is the edge of the portal tract where the portal connective tissue contacts the parenchyma. Inflammatory cells spill out from the portal tract to infiltrate around individual hepatocytes or small clusters of cells, the latter gives rise to "rosettes." Apoptotic hepatocytes may be seen close to the interface. Interface hepatitis is also a feature of the chronic hepatitis seen with *hepatitis B and C* [9]. The presence of interface hepatitis is regarded as an indicator of progressive disease and a driver of fibrosis.

Portal vessels

Endothelial inflammation of portal vein branches is one of the triad of features seen in ACR (along with a portal infiltrate and bile duct damage). It can be seen in any cause of chronic hepatitis. An absence of portal veins, or veins that are dilated and accompanied by additional marginal venules bulging out into the parenchyma, are seen with vascular disease, often along with the architectural changes described above. Hepatic artery branches are lost in late *chronic rejection* but in general lesions of hepatic arteries are rare. Frequent unaccompanied (by a bile duct and other portal structures) vessels should arouse suspicion of hepatocellular mass lesions.

Parenchyma

Inflammation

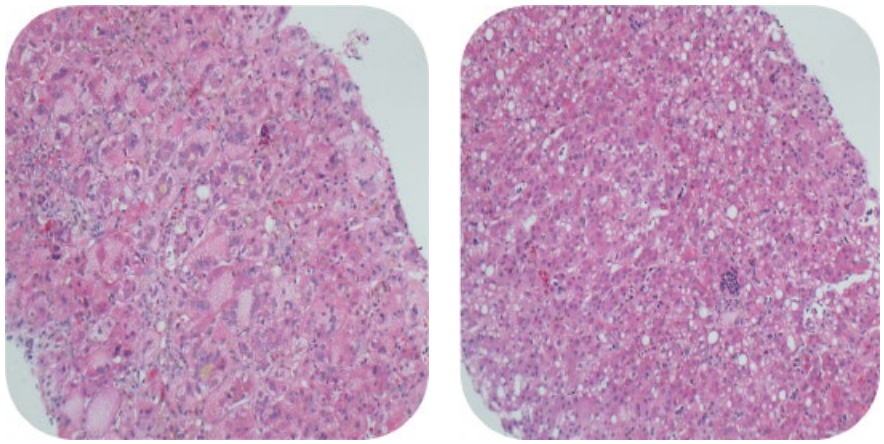
The normal orderly arrangement of hepatocytes in plates separated by sinusoids is disturbed in *acute hepatitis*, where "parenchymal disarray" is described. Apoptotic cells/acidophil or Councilman bodies are often present and small clusters of inflammatory cells can be seen – called spotty inflammation. Canalicular cholestasis may be conspicuous. Acute hepatitis is recognizable as a pattern of injury but can rarely be attributed to a specific cause. In the neonate, giant cell change, secondary to the formation of multinucleate hepatocytes, is often a marked feature. Numerous foci of extramedullary hematopoiesis are seen. This pattern is termed *neonatal hepatitis* (Figure 2.3a); this is a pattern rather than a diagnosis. It may be caused by a variety of infectious and metabolic insults, the cause often remains elusive, and there is spontaneous resolution. In chronic hepatitis, the parenchymal inflammatory changes are less florid and portal/interface inflammation becomes dominant, accompanied by progressive fibrosis.

Cholestasis

Canalicular cholestasis is part of acute hepatitis but sometimes is the only feature in the parenchyma, when the term "bland cholestasis" may be used. This pattern is seen in *drug reactions* and, in the neonate with a low γ-glutamyl transferase, in *progressive familial intrahepatic cholestasis 1* (PFIC 1; *FIC1* disease). In *PFIC 2* (bile salt export pump deficiency), the pattern is neonatal hepatitis-like. Parenchymal cholestasis is a late finding in biliary tract disease. Portal features – of ductular proliferation ± bile duct damage, and copper-associated protein deposition – are the earlier manifestations. Longstanding cholestasis leads to ballooning or "feathery" degeneration of periseptal hepatocytes; these cells may contain Mallory–Denk bodies.

Steatosis

Large droplet, macrovesicular steatosis is seen in *non-alcoholic fatty liver disease* (NAFLD). The hepatic manifestation of the metabolic syndrome (obesity and type II diabetes), NAFLD encompasses a spectrum of disease ranging from simple steatosis, regarded as reversible, to *steatohepatitis*, which signifies potentially progressive liver disease. In adult, and in most childhood, cases the fat accumulates around the central vein. It is accompanied by ballooned hepatocytes containing Mallory–Denk bodies



(A) (B)

Figure 2.3 Both of these biopsies were taken from jaundiced neonates at 1 month of age. (A) A pattern of neonatal hepatitis is seen. Multinucleate hepatocytes "giant cells" cholestasis and extramedullary hematopoiesis are present. (B) Steatosis dominates the picture here, which is highly suggestive of a metabolic disorder. (H&E, original magnification × 200.)

(these can be highlighted with immunohistochemistry for ubiquitin or cytokeratin 8/18) and pericellular fibrosis in perivenular regions. A second potential pattern can be seen in childhood, however, where the fibrosis, and sometimes the fat deposition itself, is periportal. This has been called *type II steatohepatitis*.

When hepatocytes assume a foamy appearance secondary to the accumulation of very small fat droplets (microvesicular pattern), metabolic liver disease should be considered – *mitochondrial disorders* or *Reye syndrome* for example. Macrovesicular steatosis accompanied by extreme canalicular cholestasis – where the hepatocytes can assume a "pseudoglandular" appearance – is seen in galactosemia and tyrosinemia, although these conditions are rarely biopsied now because the diagnosis can be made through other means (Figure 2.3b) [10].

Storage material

Storage disorders are rarely seen in biopsy practice because genetic analysis is now the usual method of diagnosis. Storage disorders may involve hepatocytes, Kupffer cells, or both. Ultrastructural examination can be helpful. In the neonate with a picture of neonatal hepatitis, additional splenomegaly raises the possibility of *Niemann–Pick type C disease*, where myelin figures may be seen on electron microscopic examination although storage cells are not seen on light microscopy at this age. *Cholesterol ester storage disease* features scanty, tan-colored macrophages that are periodic acid–Schiff (PAS) positive on a background of microvesicular steatosis. Pericellular fibrosis may be a clue to the presence of storage cells (Figure 2.4) [11, 12].

Sinusoidal lesions

The sinusoids are sometimes the primary site of accumulation of inflammatory cells such as in *Epstein–Barr virus* infection [13]. In addition, abnormal blood flow through the liver alters the appearance of the sinusoids as described in the architecture section.

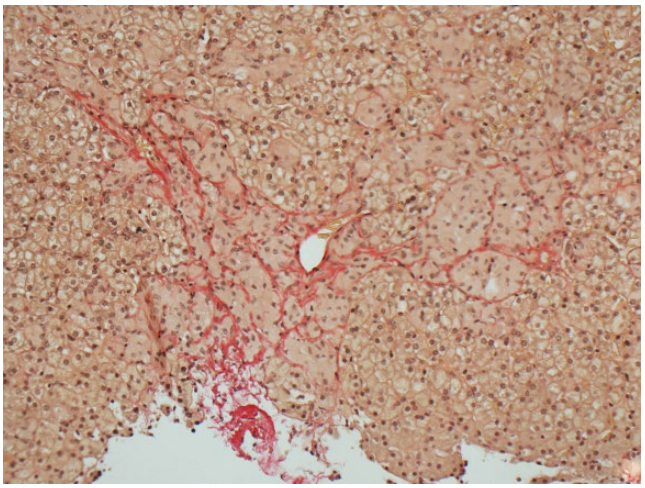


Figure 2.4 Storage cells in Gaucher disease are surrounded by slender fibrous septa. (HVG stain, original magnification × 200.)

Special stains

Good connective tissue stains are essential for the assessment of liver architecture. Collagen can be stained with van Gieson or trichrome techniques; these stains will show paler staining in areas of collapse. Reticulin will outline plate architecture and is positive in both fibrotic and collapsed areas of the liver. Orcein stains elastin fibers in mature fibrosis. It also stains copper-associated protein (CAP). CAP is a normal, physiological, finding in the neonate, but in older children it is a valuable indicator of biliary tract disease. It will accumulate in *PSC*, for example, well before ductules, sclerosing duct lesions, or visible parenchymal cholestasis are prominent

and facilitates early diagnosis. Orcein also stains the ground glass hepatocellular inclusions of hepatitis B surface antigen.

Iron is stained with a Perls stain. In iron overload, when a child has received multiple transfusions for example, the iron will accumulate in Kupffer cells/macrophages in the sinusoids and portal tracts. In *hemochromatosis* the iron accumulation is in the hepatocytes and sometimes also in biliary epithelium. Iron accumulates in any cause of fulminant hepatic failure, so to establish a diagnosis of *neonatal hemochromatosis* iron should be demonstrated in extrahepatic parenchymal cells (not macrophages) in minor labial salivary glands, for example.

PAS stains glycogen and hepatocellular inclusions, which may be variably sensitive to diastase (DPAS). DPAS-positive globules are typical of *α1-antitrypsin (A1AT) deficiency*, and immunohistochemistry can confirm their nature. It should be noted that DPAS-positive material often accumulates in macrophages and should not be interpreted as globules and also that globules have not had time to accumulate in the neonate to allow detection. α1-Antitrypsin deficiency should be considered in any neonatal biopsy with either a biliary pattern or a neonatal hepatitis pattern.

In *intestinal failure-associated liver disease* it is not uncommon to see kidney bean-shaped hepatocellular inclusions, often with a ground glass appearance and a "halo," which are PAS positive and diastase sensitive, representing altered glycogen. These inclusions have also been described in the setting of patients receiving multiple *drugs*/polypharmacy.

Immunohistochemistry with biliary cytokeratins (7 and/or 19) can be helpful for confirming bile duct paucity and ductular transformation. Cells with the morphology of hepatocytes, but showing an expression of biliary cytokeratins, can be seen in biliary diseases in periportal regions. All immunohistochemical stains are best applied according to the clinical situation, with awareness that available tissue is often limited, rather than as routine.

Conclusions after assessment and patterns of liver disease

After making the observations as outlined above, it is then necessary to interpret the findings. Has the liver acute or chronic damage? How severe is the disease process? This includes assessing the extent of necrosis: single cells, groups of adjacent cells/confluence, bridging or pan-acinar necrosis?

In chronic liver disease it is important to assess the degree of fibrosis. This is referred to as the fibrotic "stage." Fibrosis often begins around portal tracts although in *NAFLD* it begins around the central veins. It progresses to bridging fibrosis, between portal tracts. If complete nodules are surrounded by mature fibrosis, then cirrhosis has developed.

In inflammatory diseases the severity of inflammation, the "grade," should be described. It is possible to use numeric scoring systems (such as the Ishak and METAVIR scores) for recording the presence and severity of features present in a biopsy. It is the author's preference not to use these in routine practice but rather to use descriptive terms. The reasons for this are that scoring systems have been developed for specific diseases and should only be applied to that disease and when that is the only disease present. Reflex application of scoring systems hinders a proper assessment of the biopsy; subtle indicators of additional, perhaps unsuspected, disease may be missed if you are focussed only on the components of a scoring system. Scoring systems can lead all biopsies being erroneously labeled "chronic hepatitis." They also do not describe linear variables: in scoring systems "4" is not twice as bad as "2."

Table 2.1 Patterns of injury.

Pattern	Key histological features	Common childhood diagnoses
Biliary	Early, easily missed, copper-associated protein. Ductules at portal tract margins. Late, parenchymal cholestasis and periportal feathery degeneration. May, or may not, see lesions of the bile duct proper	Large duct obstruction – biliary atresia and choledochal cyst. Sclerosing cholangitis, primary or secondary. Later stages of PFIC
Bile duct loss without ductules	Bile duct, a similar size to and adjacent to the artery, is damaged or lost	Alagille syndrome. Chronic rejection. GVHD
Oddly shaped ductules	Normal parenchyma	Ductal plate malformation/fibrocystic diseases
Bland cholestasis	Absence of portal biliary features	Drug reactions. PFIC or BRIC. (Drug reactions can mimic any pattern of liver injury)
Hepatitic	Acute hepatitis dominated by parenchymal inflammation, chronic by portal. May see interface hepatitis	Viral infections. Autoimmune hepatitis
Metabolic	Steatosis	Non-alcoholic fatty liver disease. Wilson disease. Cholesterol ester storage disease, and some types of glycogen storage disease. Mitochondrial disorders, tyrosinsemia and galactosemia
Vascular	Abnormalities of portal vein branches. Nodular regenerative hyperplasia. Dilated sinusoids	Portal venous compromise. Vascular outflow obstruction

BRIC, benign recurrent intrahepatic cholestasis; GVHD, graft-versus-host disease; PFIC, progressive familial intrahepatic cholestasis.

Alongside the assessment of the duration of damage and its severity, patterns of injury emerge. These are summarized in Table 2.1.

Although less of a problem in childhood than in adulthood, it is possible that more than one process is damaging the liver and is visible to the pathologist. If this is the case, there should be an attempt to determine the dominant process. At the end of the process of observation and assessment of emergent histological patterns, the pathologist must correlate their findings with the clinical presentation in order to formulate a clinically useful report that will guide clinical management.

It is important to remember that multiple different insults can cause the same pattern of damage in the liver, which has a limited repertoire of responses. A biopsy is only 1/50,000 of the liver as a whole, so sampling error can be significant. It is important to think of these factors when the pathological report does not fit the clinical picture.

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CHAPTER 3

Liver Immunology and Its Application in Diseases

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Key points

- The liver is a tolerogenic lymphoid organ comprised of innate and adaptive immune cells with antigen-presenting cells.
- The balance of effector and regulatory immune cells dictates the outcome of hepatic inflammation, either towards resolution or chronic active hepatitis.
- The hepatic microenvironment, biliary epithelial cells, and fibrous stromal framework influences functions, survival, differentiation, and apoptosis of immune cells.
- Kupffer cells are the vascular firewall of the liver, protecting against an invasion of pathogenic organism to the systemic circulation from the gut, and recently discovered immune cell subsets such as mucosal-associated invariant T cells and innate lymphoid cells appear to play crucial roles in mucosa immunology as a biliary firewall.
- Emerging immune-based therapies are beneficial for both autoimmune and hepatocellular carcinoma patients.

The liver as a lymphoid organ

The liver behaves as an immunological lymphoid organ similar to the thymus, lymph nodes, and spleen [1]. Resident professional antigen-presenting cells (APCs) such as dendritic cells (DCs) and other non-professional APCs such as Kupffer cells (KCs), hepatic sinusoidal endothelial cells (HSECs), and hepatocytes convey information regarding microbial infection in the periphery to the lymphocytes. In the liver, the lymphocyte population is largely resident in the portal tract and sometimes, in conditions such as primary biliary cirrhosis (PBC) and hepatitis C-associated liver disease/infection, there is a formation of tertiary lymphoid follicles. The large intravascular bed of macrophage-like KCs that lines the liver sinusoids serves as a vital frontline hepatic firewall scanning, presenting, and clearing pathogens. Persistent activation of liver APCs by microbial and food antigens induces a state of immune unresponsiveness. The liver is also a "graveyard" of immune cells due to apoptosis of activated lymphocyte populations. Owing to its position, the liver constantly receives antigen-loaded blood products from the gut and plays an essential role in oral tolerance, the phenomenon of tolerance induced by portal vein infusion of antigenic cells [2].

Hepatic tolerance

The liver is a unique organ in which induction of tolerance is favored over induction of immunity. This is necessary due to its constant exposure to harmful and harmless antigens, especially from the gut. The administration of antigens via the portal vein was found to induce immune tolerance [3]. A seminal study by Sir Roy Calne *et al.* in the 1960s found that while allogeneic kidney transplants were poorly received, dual transplantation of the donor's liver together with one of their kidneys improved the engraftment of the donor kidney [4]. Moreover, it has since been described that allogeneic liver transplantation could be established and maintained even without immunosuppression [5]. One model accounting for such hepatic tolerogenicity is donor cell chimerism, in which donor-derived leukocytes, including liver-resident APCs, migrate to central lymphoid organs and persist for a long period [6].

Hepatic tolerance is achieved by a combination of both immune cells and parenchymal cells. The constant exposure to gut-derived bacteria triggers a downregulation of Toll-like receptor on the sinusoidal endothelium. Liver-resident DCs have distinct properties that promote tolerance rather than an immune response. These tolerogenic DCs secrete

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anti-inflammatory cytokines interleukin 10 (IL-10) and transforming growth factor β (TGF-β) to dampen the immune response and can promote T-cell "hyporesponsiveness". The intrinsic tolerogenic capacity of HSECs and KCs also contributes to hepatic tolerance. Regulatory T cells (Treg), which were previously known as suppressor T cells, play a crucial role in maintaining the tolerogenic atmosphere [7].

Immune response by the liver

Despite the mechanisms of hepatic tolerance, the liver is able to mount an effective immune response to invading pathogens or when there is insult or loss of peripheral self-tolerance in immune-mediated liver injury such as autoimmune hepatitis. The balance between immunity and tolerance is established by competition for primary activation of effector T cells between the liver and secondary lymphoid tissues. For example, naive $CD8^+$ T cells activated within liver-draining lymph nodes are capable of mediating hepatitis, while cells undergoing primary activation within the liver exhibited defective cytotoxic function and do not mediate hepatocellular injury [8]. Pathogenic antigens or insults can invade the liver from the gut via the portal vein or via the common bile duct (e.g., in ascending cholangitis) or they can be carried from the hepatic artery in the case of septicemia. The hepatic immune response will depend on the nature of the injury: adaptive immune cells are dominant in chronic injury resulting from, for example, hepatitis B and C, alcoholic and non-alcoholic steatohepatitis, or autoimmune hepatitis. In acute injury due to, for example, Epstein–Barr virus and cytomegalovirus, acute hepatitis A and E, and drug-induced liver diseases, an innate immune cell infiltrate appears important, with eosinophil or neutrophils and natural killer (NK) cells being the predominant immune cells. In general, the balance of effector and regulatory T cells determines the outcome of inflammation, either resolution or chronic active hepatitis (Figure 3.1).

Hepatic antigen-presenting cells

Antigen-presenting cells in the liver consist of both professional and non-professional types. *Hepatic dendritic cells* are known as professional APCs. First described by Steinman and Cohn in 1973 [9], the DC proved to be the essential link between the innate and adaptive immune responses. Acting as sentinels of the immune system, DCs are well equipped at internalizing foreign antigen in hepatic environments and presenting them to T cells at the local draining celiac lymph nodes. Two main types of liver DC are described: myeloid DCs and plasmacytoid DCs [10]. Hepatic DCs play a key role in the host response to blood-borne pathogens, and in the pathogenesis of infectious and autoimmune liver diseases. They can also be tolerogenic under certain cytokine milieu (e.g., IL-10, TGF-β).

Dendritic cells are uniquely potent in their ability to capture, process, and present antigens to T cells. By culturing DCs with tumor-associated antigens or different cellular products, immunogenic or tolerogenic DCs can be obtained for treatment of hepatocellular carcinoma [11]. When antigen-pulsed DCs are administered, patients given antigen-loaded DCs exhibit an augmentation of antitumor immunity.

Kupffer cells, named after Karl Kupffer, a 19th century German who first described endocytic activity in the hepatic sinusoid [12], are the largest group of fixed tissue-resident macrophages in the body [13]. KCs are derived from

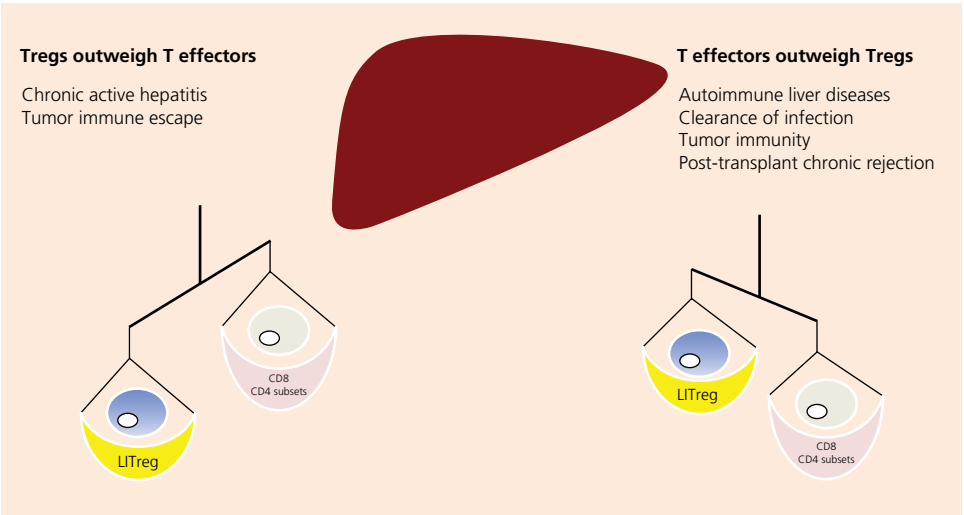

Figure 3.1 Regulatory T cells (Tregs) and T effector cell balance in liver diseases. Tregs control the proliferation and cytokine secretion of effector T cells to maintain the peripheral immune tolerance. The balance of Treg and T effector cells (CD4 = Th1 and Th17; CD8 = cytotoxic T cells and Tc17) dictates the outcome of: (i) inflammation (either resolution or chronic active hepatitis, such as autoimmune liver diseases and viral hepatitis and post-transplant rejection); and (ii) tumor immune response (either tumor immunity or tumor immune escape).

circulating monocytes. Their intimate association with the luminal surface of the low-flow hepatic sinusoidal endothelium [14, 15] facilitates their close contact with passing lymphocytes. They act as sentries, constantly surveying blood entering the liver from the gut, and are the main phagocytic cells of the hepatic immune system [16]. Their localization in the periportal hepatic sinusoids means they are strategically positioned to clear the endotoxins, antigens, and microorganisms entering in the portal venous circulation. Their important role in tolerance has been suggested in rat cardiac allograft transplantation [17]. Prostaglandin E2 from KCs can inhibit T-cell activation by DCs. Kupffer cells challenged with Gram-negative bacteria can not only release IL-10 [18], but also reduce major histocompatibility complex class II (MHC-II) and co-stimulatory molecules expression by HSECs. IL-10 produced by KCs may also downregulate liver sinusoidal endothelial cell expression of cell adhesion molecules thereby inhibiting the recruitment of antigen-specific T cells [19]. This physiological hyporesponsive action of KCs to stimulation by gut-derived bacteria is called portal venous tolerance. KCs are also important in clearing microbes that breach the gut mucosal barrier and circulate to the liver. This is evidenced by delayed clearance of circulating microbes in experimental animals treated with clodronate liposomes, which deplete KCs.

Innate immune cells of the liver

The innate cells include monocytes, macrophages, mast cells, neutrophils, and NK cells. The primary roles of these cells include cytotoxicity, recruitment of other immune cells, and phagocytosis with presentation of pathogenic antigens to other immune cells. Pattern-recognition receptors (PRRs) assist in the discrimination of self and non-self. PRRs recognize pathogen-associated molecular patterns (PAMPs), which are evolutionarily conserved molecules exhibited by exogenous pathogens, ranging from lipopolysaccaharide to viral nucleic acids. PRRs also recognize damage-associated molecular patterns, which are released from cells after injury or necrosis allowing immune recognition of potentially harmful endogenous cells. Activation of PRRs triggers the innate response through a reactive release of cytotoxic agents, phagocytosis, and recruitment of other immune cells. In contrast to the adaptive response, the innate response has rapid activation kinetics and is not generally associated with the induction of memory function.

Natural killer cells

The liver's lymphocyte population is selectively enriched in NK cells, which play critical roles in first-line immune defense against invading pathogens; 20–30% of liver resident lymphocytes are NK cells and they provide immune surveillance against viral infection (hepatitis B and C) and cancer. Antiviral immunity of NK cells in hepatitis B is via their death ligands killing hepatocytes. Although this clears the virus and provides antiviral immunity it leads to hepatocyte damage. NK cells can also limit the progression of liver fibrosis by killing activated stellate cells via TRAIL-TRAIL receptors playing a beneficial role in preventing the progression of liver fibrosis.

Innate lymphoid cells

Innate lymphoid cells (ILCs) are non-CD3 and have a high expression of the IL-7 receptor CD127. At present, there are three types of ILC described: ILC1, ILC2, and ILC3. Their cytokine expression profiles resemble those of Th1, Th2, and Th17 cells. The frequency of the ILC1 subset was found to be much higher in the inflamed intestines of people with Crohn disease, which indicated a role for these interferon γ (IFN-γ) producing ILC1 cells in the pathogenesis of gut mucosal inflammation [20]. A recent study also indicated that IL-33 mediates biliary epithelial proliferation [21]. Phenotyping and functional studies of ILC subsets in the human liver are currently in progress to investigate their roles in the liver diseases.

Adaptive immune cells of the liver

The fundamental components of the vertebrate adaptive immune system are B and T lymphocytes, which specifically target their cognate antigens. Both hepatic B and T lymphocytes have the capacity to produce memory cells, which specifically recognize the pathogen in a repeated infection, resulting in a faster immune response. B cells produce antibodies, known as immunoglobulins, that control pathogens by neutralization of an antigen, opsonization, complement-dependent cytotoxicity, or antibody-dependent cell-mediated cytotoxicity. The majority of CD4 T cells are helper T cells, which recognize antigens presented by MHC-II, whereas CD8 T cells bind to MHC-I and predominantly act in a cytotoxic manner. Every nucleated cell in the body expresses MHC-I, which allows any cell to communicate with the immune system, while MHC-II is mainly present on dendritic cells.

T cells are a major component of cell-mediated immunity. There are two main classes of T cells: effector T cells, which are necessary in the elimination of the pathogen, and regulatory T cells (Treg), which enhance immune tolerance to non-harmful foreign bodies.

Effector T cells

Conventional T effector cells

T lymphocytes express a T-cell receptor (TCR) capable of recognizing an array of pathogens through pathogenic antigen presentation by polymorphic MHCs. The TCR of conventional T cells is composed of α- and β-glycoprotein chains. Mossman and Coffman first described Th1 and Th2 effector T cells in the early 1980s [22]. The lineages defined have

since been expanded to include Th17 (IL-17 secreting CD4 subsets), Th9 (IL-9 secreting CD4 subsets), and Th22 [23]. These lymphocyte lineages are defined by certain chemokine receptors, transcription factors, and cytokines. Chemokine receptors CCR5 and CXCR3, transcription factor Tbet, and cytokine IFN-γ define the Th1 lineage, which contributes towards cell-mediated immunity, virus destruction, and other intracellular pathogen destruction. Th2 cells are defined by CCR4 and the expression of GATA-3, and they secrete IL-4, IL-5, IL-10, and IL-13. They mainly target extracellular bacteria and fungi but are also connected to inflammatory and autoimmune disease. Th17 cells are characterized by CCR6 expression along with transcription factor RORc and a cytokine profile of IL-17, IL-22, IFN-γ, granulocyte–macrophage colony-stimulating factor (GMCSF), and CCL20 and play a role in regeneration and autoimmunity. Hyperimmunoglobulin E syndrome (HIES) is a primary immune deficiency characterized by abnormal and devastating susceptibility to a narrow spectrum of infections, most commonly *Staphylococcus aureus* and *Candida albicans*. Recent investigations have identified mutations in *STAT3*, the main signaling molecule of Th17 cells in patients with HIES. A deficiency of Th17 cells, which are important in host defense against the common pathogens *S. aureus* and *C. albicans*, was observed in these patients [24]. Th17 cells play a major role in biliary pathology, thus phase II studies are in progress to assess the use of ustekinumab in patients with biliary diseases (ClinicalTrials.gov). So far, there is no report by our group or others on Th9 cells in the human liver.

Unconventional T cells

Unconventional T cellshave a restricted TCR repertoire and these cells in the liver consist of mucosal-associated invariant T (MAIT) cells and gamma delta (γδ) T cells.

MAIT cells are a T-cell subset that has two unique properties compared with conventional T cells: they express an evolutionarily conserved, semi-invariant T-cell receptor, Vα7.2, and they are restricted by MHC-I-related protein, MR1 [23, 25]. MAIT cells are characterized by a high expression of CD161 and are capable of recognizing bacterial vitamin B metabolites. In the human liver, they are predominantly CD8 T cells and crucial in biliary immune surveillance [26]. MAIT cells are abundant at mucosal surfaces where protection against bacteria is crucial, especially at the bronchial mucosa in the respiratory tract and the lamina propria in the gastrointestinal system.

γδ T cells are a subset of unconventional T cells that express γ-chain and δ-chain TCR heterodimers, as opposed to the α- and β-chain dimer exhibited by the TCR of conventional αβ T cells. They have been associated with immune surveillance, immunoregulation, antigen presentation, and rapid cytotoxic response kinetics to target pathogenic antigen. The liver plays a key role in the maintenance of immune homeostasis and contains a high number of innate response-associated immune cells. The role of γδ T cells in the liver is not well established. γδ T cells function in many cases as innate-like cells, and hence are thought to bridge the gap between the innate and adaptive immune responses [27]. γδ T cells have also been reported to contribute to liver autoimmunity, such as in autoimmune hepatitis and primary sclerosing cholangitis. Significant proportions of these cells produced proinflammatory cytokines including IFN-γ and tumor necrosis factor α (TNF-α). TCR composition of intrahepatic γδ cells is different from that of γδ cells in circulating blood. Dissecting the roles of the γδ T-cell subsets is essential as the liver harbours the majority of these cells and as such they have considerable therapeutic potential.

Regulatory T cells

Regulatory T cells in hepatic immune regulation

Sakaguchi and colleagues first described regulatory T cells in late 1990s [28]. Tregs are derived from the thymus as a novel subset of $CD4^+$ T cells, expressing high levels of CD25 (the α-chain of the IL-2 receptor) and low levels of IL-7 receptor (CD127). Thus, Tregs are defined as CD4, $CD25^{high}$, $CD127^{low}$. Tregs express the lineage-defining transcription factor, FOXP3. Mutations in *FOXP3*, which is the master regulator of Treg development and function, can lead to severe immunopathology with multiorgan lymphoproliferative autoimmune disease in humans known as IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked). The main function of Tregs is to control both innate and adaptive effector cells, thereby maintaining a peripheral immune tolerance. Many investigators including our group are conducting intensive basic studies on human blood and intrahepatic Tregs in the context of recruitment to the liver and interaction with other immune cells and parenchyma and epithelial cells (Figures 3.2 and 3.3) [29]. A few early phase translational studies are also in progress as there is considerable potential for Treg-based cell therapy in a variety of autoimmune-related liver diseases and liver transplantation (see Figure 3.6) [30].

B cells and autoimmunity

B cells play a major role in liver pathology in autoimmune-related liver diseases as a common feature of all autoimmune liver diseases is the presence of self-reactive autoantibodies. B cells perform various immunological functions that include the production of antibodies, presentation of antigens, secretion of multiple cytokines, and regulation of immune responses via IL-10. Autoimmune hepatitis is characterized by the presence of antinuclear antibodies or anti-smooth muscle antibody as well as hyper-immunoglobulin

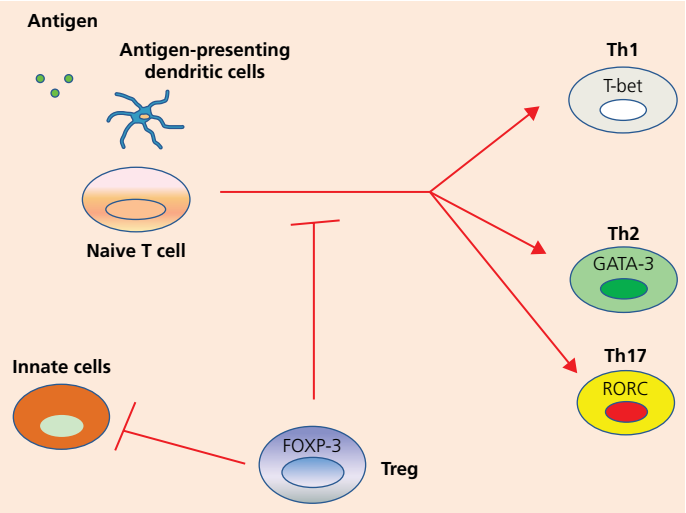

Figure 3.2 Regulatory T cells (Tregs) control effector cells. The differentiation of naive T cells into different T-cell lineages (Th1, Th2, Th17) depends on the nature of the antigen to which they are exposed by the antigen-presenting dendritic cells. Th1, Th2, and Th17 cells express key transcription factors T-bet, GATA-3, and RORC. Tregs control the proliferation and cytokine production of these adaptive immune cells and innate cells. (Adapted from Oo *et al.* 2010 [32].)

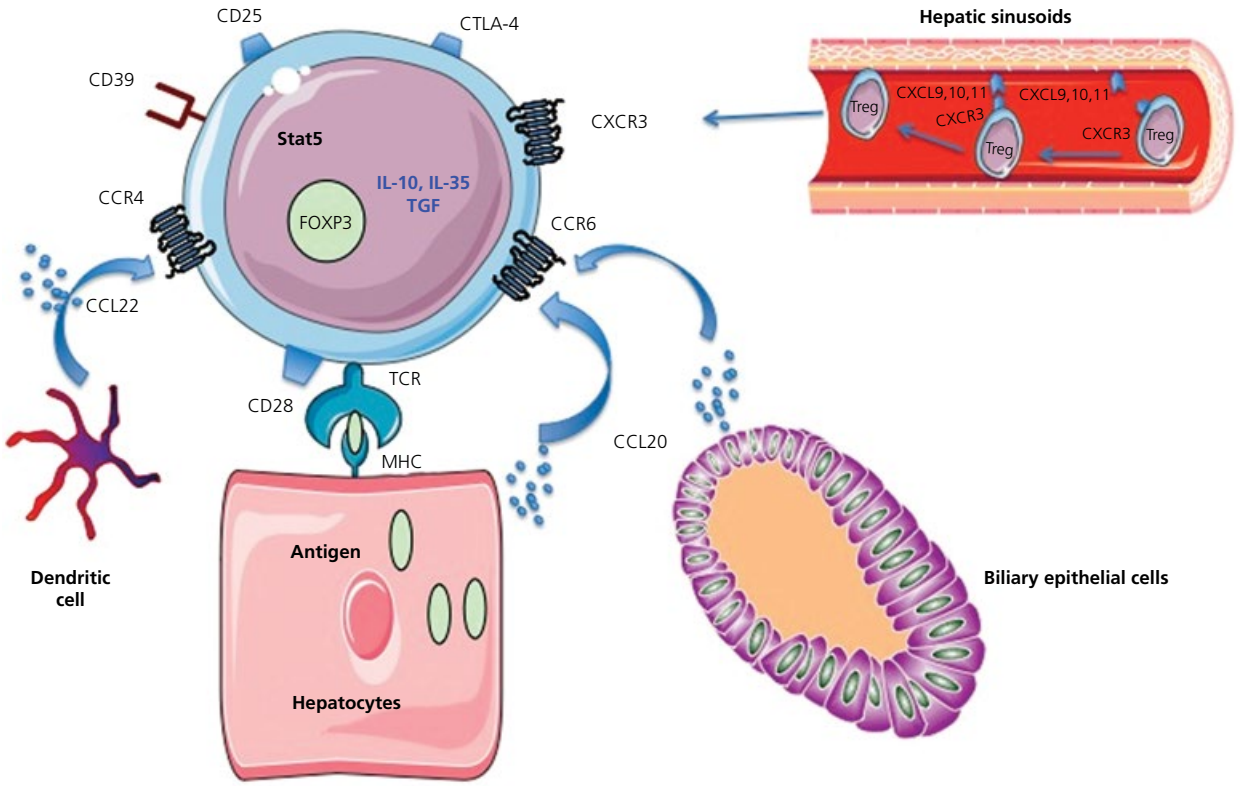

Figure 3.3 Intrahepatic regulatory T cells are crucial cells in maintaining liver tolerance. They express high-affinity IL-2 receptor CD25, liver homing chemokine receptor CXCR3 (the ligands for CXCR3 receptors are CXCL9, -10, and -11 and these ligands are secreted from the hepatic sinusoidal endothelium), biliary homing chemokine receptor CCR6 (ligand CCL20 secreted from the biliary epithelium), and CCR4 for localization with dendritic cells (ligand CCL22 secreted from the dendritic cells). Intrahepatic Tregs are also equipped with functional suppression markers such as CTLA-4 and CD39, and secrete immunosuppressive cytokines IL-10 and TGF-β. (Adapted from Oo *et al.* 2010 [32].)

G (IgG) globulinemia. Primary biliary cholangitis is also characterized by the presence of antimitochondrial antibodies (AMA) and hyper-IgM globulinemia. Biliary epithelial cells can transcytose IgA, thus forming IgA–pyruvate dehydrogenase complex E2 (PDC-E2) immune complexes, which accumulate in the bile duct lumen causing caspase activation leading to apoptosis of the bile duct epithelium. In addition, Treg dysfunction and CTLA-4 polymorphisms have also been implicated in the development of autoreactive AMA-producing B cells and hence in the pathogenesis of PBC. In primary sclerosing cholangitis,autoantibodies such as antineutrophil cytoplasm antibody and anticardiolipin antibodies are also expressed.

B cells are associated with the development of fibrosis in liver disease. In the carbon tetrachloride (CCL_4) model of liver injury, B-cell-depleted mice had an almost eight-fold reduction in collagen deposition compared with wild-type mice. B cells are professional APCs and so can interact and modulate T-cell responses. B cells bind to a specific antigen epitope via their B-cell receptor (BCR) and present antigen to naive T cells. They also produce an array of different cytokines that can regulate the immune response. For example, regulatory B cells can secrete immunosuppressive IL-10. Thus B cells are crucial immune cells of the adaptive immune system and provide immunoregulatory roles in limiting autoimmunity, as well as effector roles in mounting effective immune responses. Thus pre-clinical therapies targeting B cells and BAFF (B-cell-activating factor belonging to the TNF family) are currently in progress.

Hepatic sinusoidal endothelial cells

Hepatic sinusoidal endothelial cells, which line the liver sinusoids, were first reported by Wisse [31]. HSECs are fenestrated and lack a basement lamina, which allows direct contact between the hepatocytes. It provides a low-flow vascular bed thus allowing immune cells in blood recruitment and cell-to-cell interactions. The recruitment of lymphocytes into the liver occurs in four stages: initial capture, rolling, adhesion, and finally transmigration. These stages are controlled by the expression of chemokines and cell adhesion molecules on the surface of the hepatic sinusoidal endothelium (Figure 3.4) [32]. Once the leukocytes have migrated across the endothelium they cross the space of Disse. HSECs are also non-professional APCs.

Chemokines

Chemokines are a family of structurally related proteins, 8–12 kDa heparin-binding cytokines, which bind to specific G-protein-coupled receptors expressed by leukocytes. More than 50 chemokines have been identified, along with 20 chemokine receptors, and the chemokines attributed to hepatic immunology are described in Table 3.1. Chemokines act as postcodes, which recruit certain lymphocytes to specific places. For example, lymphocytes expressing the CXCR3 chemokine receptor will migrate to the inflamed liver where CXCR3 ligands CXCL9, -10, and, -11 are expressed by endothelial cells. Thus the interaction between chemokines and their receptors dictate the lymphocyte subsets homing to certain tissues.

Hepatic stellate cells

Hepatic stellate cells (HSCs) are the stromal frameworks of the human liver and are a rich source of retinoic acids. HSCs support post-endothelial migration of immune cells to the site of inflammation and aid in the survival of immune cells. Post-endothelial migration to the site of inflammation is generally mediated by CD44 ligation with hyaluronic acids in the matrix stroma, and integrins on immune cells interacting with cell adhesion molecules such as the intercellular cell adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) on the hepatic fibroblastic stroma. HSCs play an important role in hepatic fibrogenesis [35]. The activation of HSCs is a crucial event in liver fibrosis. KCs play a major role in HSC activation by promoting upregulation of smooth muscle actin and the release of retinoic acid by HSCs. The death of parenchymal cells, such as hepatocytes, can generate apoptotic bodies, which can drive HSC fibrogenesis. Cytokines such as IL-4 and IL-13 also lead to proliferation and collagen production by HSCs. TGF-β is a potent fibrogenic cytokine secreted by HSCs, KCs, and platelets and it leads to collagen gene expression from HSCs during fibrosis. In fatty liver diseases, leptin secreted by adipocytes can also stimulate collagen production from HSCs and act as a profibrogenic hormone.

In response to liver injury, HSCs switch their quiescent phenotype to a myofibroblast-like phenotype [36]. The fate of activated HSCs is dependent on the apoptotic and survival signals that they receive. The apoptosis of HSCs lead to the resolution of fibrosis. Pre-clinical therapies directing HSC apoptosis are promising as a treatment for liver fibrosis and cirrhosis. Potential antifibrotic agents include vitamin E and pentoxifylline, which inhibit HSC activation; bortezomib and adiponectin, which induce HSC apoptosis; and losartan or prostaglandin E2, which are both antiproliferative and inhibit the contractility of HSCs.

Biliary epithelial cells

Cholangiocytes or biliary epithelial cells (BECs) account for 3–5% of liver cells and are significant in the autoimmune liver pathologies of PBC and primary sclerosing cholangitis. BECs have a mucosal surface with mechanisms to prevent infection and inflammation. Their environment is usually sterile, however connection to the intestinal tract may harbor a source of pathogens. Secretory IgA is produced and present in the bile; it can bind and neutralize bacterial toxins and is also able to prevent bacterial adhesion to the mucosal membrane. In addition, the antimicrobial peptides, defensins,

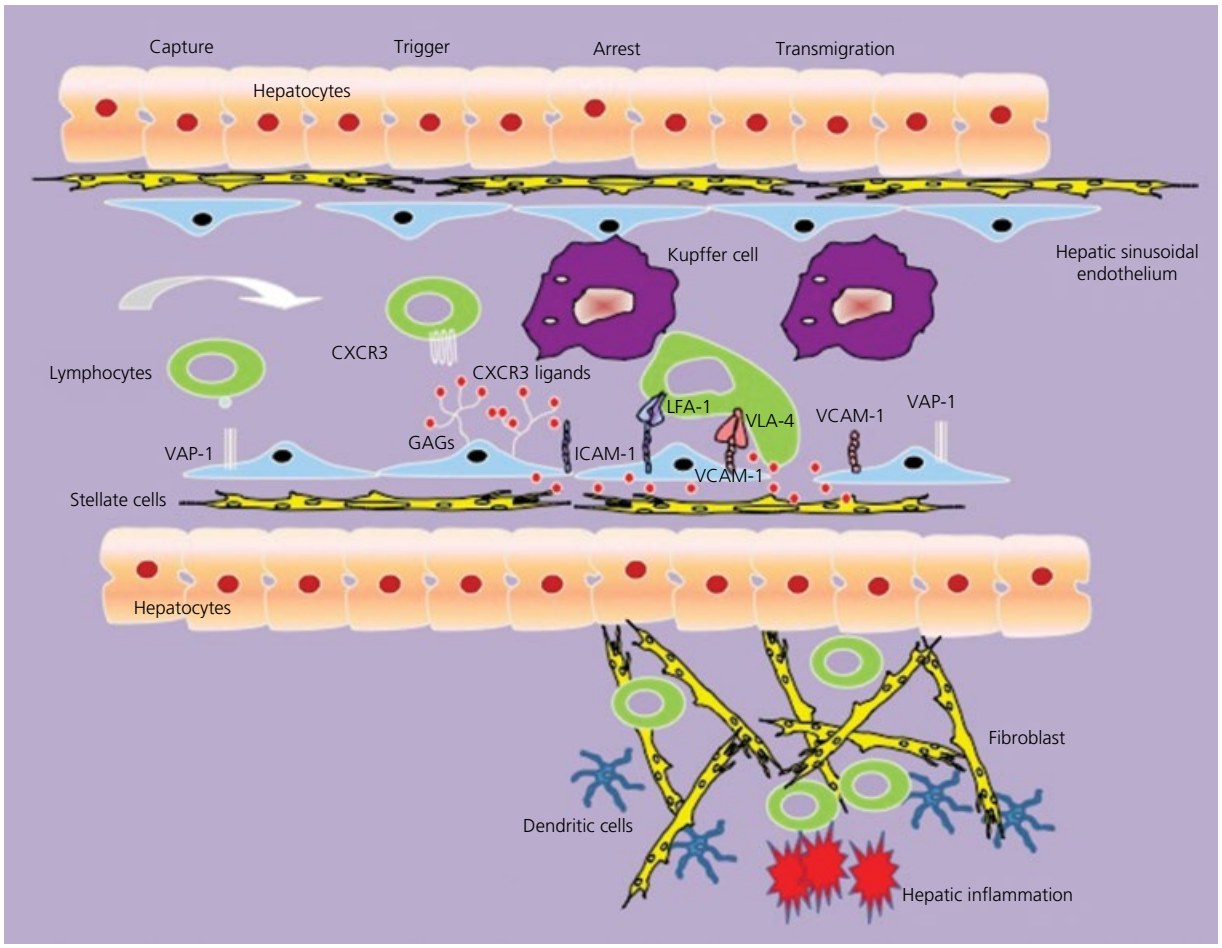

Figure 3.4 Multistep process of leukocyte recruitment at the hepatic sinusoids. First, rolling lymphocytes are captured, bringing the flowing cells into contact with the vessel wall to interact with the sinusoidal endothelium, which presents CXCR3 chemokines. Second, triggering or rolling involves chemokines – immobilized on the endothelial glycocalyx activating the chemokine receptor CXCR3 on the lymphocytes, which leads to conformational changes in integrins (LFA-1 and VLA-4). These integrins subsequently bind to the endothelial cell adhesion molecules ICAM-1 and VCAM-1 for firm adhesion/arrest. The final step involves transendothelial migration of the lymphocytes into the tissue and is mediated by chemokines, ICAM-1, and VAP-1. Once the cells are in the tissue, chemokine gradients and cell adhesion molecules guide post-endothelial migration to the site of inflammation on the fibroblast stromal framework. GAG, glycosaminoglycan; ICAM-1, intercellular cell adhesion molecule 1; LFA-1, lymphocyte functional antigen 1; VAL-4, very late antigen 4; VAP-1, vascular adhesion protein 1; VCAM-1, vascular cell adhesion molecule 1. (Adapted from Oo *et al.* 2010 [32].)

Table 3.1 Chemokines produced by cells of human liver and their cognate receptors. Immune cell recruitment can be across the portal endothelium, which is mediated by the chemokines CCL3–5, ligands for CCR5. Recruitment across the liver sinusoidal endothelium is mediated by the chemokines CXCL9–11 as well as CXCL16, which bind to the corresponding receptors CXCR3 and CXCR6. CCL20, CCL28, and CXCL16 expression on the biliary epithelium has been associated with the migration of CCR6 expressing Th17 [33] and CCR10 expressing Treg cells [34]. (Adapted from Oo *et al.* 2010 [32].)

Hepatic cells	Chemokines	Responsive chemokine receptors
Portal vessels	CCL3, CCL4, CCL5	CCR5
Liver sinusoids (hepatic sinusoidal endothelial cells)	CXCL9, CXCL10, CXCL11	CXCR3
	CXCL16	CXCR6
Biliary epithelium	CCL20	CCR6
	CCL28	CCR10
	CXCL16	CXCR6

and cathelicidin protect against pathogens by disturbing their membranes. We have now shown that biliary epithelial cells are a source of CCL20 to attract Th17 cells [33] and can also activate MAIT cells [26]. BECs also express low levels of the lymphocyte adhesion molecules VCAM-1, which contribute to the survival of lymphocytes around the biliary system [37]. Furthermore, BECs constitutively secrete low levels of IL-6, IL-8, and monocyte chemotactic protein 1 with a marked increase in secretion occurring during inflammatory states. These cytokines and chemokines attract neutrophils, monocytes, and T cells. Human BECs also express histocompatibility locus antigen (HLA) class I at a low level but in pathological biliary diseases they are expressed along with CD80 and CD86 co-stimulatory molecules, thus BECs can be behave as non-professional APCs in disease settings [38].

Intrahepatic microenvironment milieu

The human intrahepatic microenvironment is enriched with proinflammatory cytokines IL-6, IL-2, TNF-α, and IFN-γ. T cells drained from hepatic sinusoids could become activated in the liver tissue or in the draining portal lymph nodes when they encounter peptide–MHC from hepatic DCs. Naive T cells can then differentiate to Th1, Th2, and Th17 lineages depending on the cytokines and co-stimulatory molecules. Most importantly, from a translational point of view, lineage changes from regulatory to effector phenotypes with corresponding switches in functions depending on the microenvironment milieu could be detrimental. Many investigators including ourselves who intend to apply Treg-based immunoregulatory cell therapies for the treatment of liver diseases have tested the stability of Treg cells in the liver microenvironmental milieu extensively.

Hepatocellular carcinoma

The immune escape mechanism in hepatocellular carcinoma is also dependent on the tumor microenvironment. Both regulatory T cells and myeloid-derived suppressor cells (MDSCs) inhibit the functions of tumor-infiltrating lymphocytes. Immunosuppressive cytokines from these cells such as IL-10 and TGF-β inhibit the functions of tumor antigen-specific T cells. Tumor-associated macrophages and DCs are also more prone to acquiring a regulatory phenotype, which leads to tumor immune escape. Thus, many investigators are attempting a combination with immunogenic dendritic cell vaccination and manipulation of the tumor microenvironment to mount the immunity battle against hepatocellular carcinoma.

Gut and liver link

The unique positioning of the liver between the systemic and portal circulation augments the interaction between naive T cells and other hepatic cells and leads to disruption in the development of tolerance to commensal bacteria and other environmental agents. Interplay between the intestinal tract and the liver may explain the increased association with primary sclerosing cholangitis and inflammatory bowel diseases [39]. The gut–liver axis involves multiple inflammatory cell types, not only CD8 but also new subsets such as ILCs, MAIT cells, and MDSCs, which could link break down in mucosa immune defense and liver pathology. The variation in the gut microbiome is likely to be a factor in the pathogenesis of autoimmune and fatty liver diseases [40]. The intestinal microbiome plays a significant role in the development of autoimmune liver disease such as primary sclerosing cholangitis, which is generally associated with inflammatory bowel diseases. $\alpha_4\beta_7$-Integrin-expressing T cells are involved in the pathogenesis of both Crohn disease and ulcerative colitis and bind to their ligand MAdCAM-1. Blocking the $\alpha_4\beta_7$-integrin using a monoclonal antibody that targets the α4β7-integrin

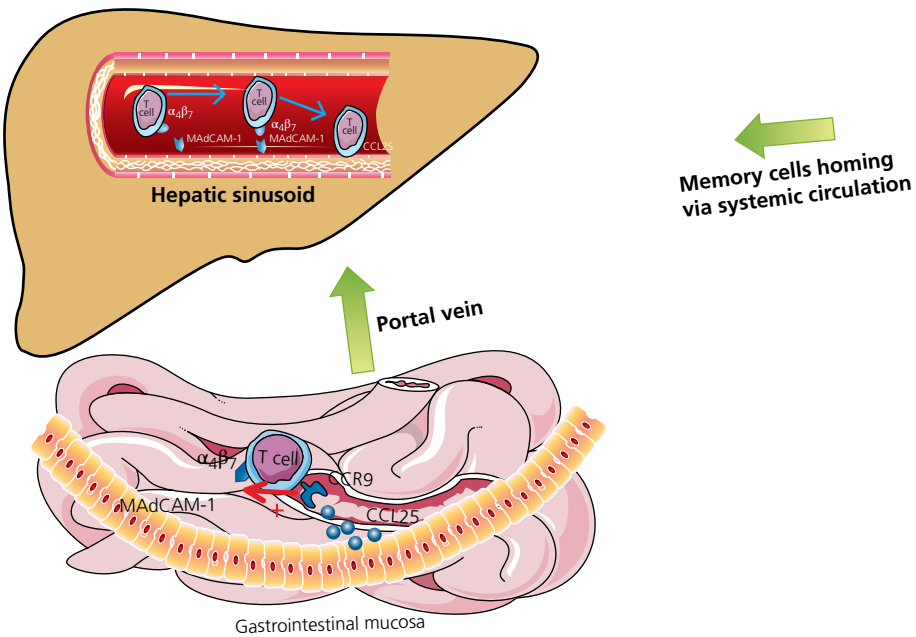

Figure 3.5 The gut–liver axis. Mucosal lymphocytes expressing CCR9 and α4β7-integrin home to the gut mucosa, which expresses chemokine CCL25 and cell adhesion molecule MAdCAM-1. These cells remain as long-lived memory T cells and they aberrantly home to the liver. This gut–liver link might underlie the pathogenesis mechanism of some liver diseases such as primary sclerosing cholangitis.

(vedolizumab) could significantly attenuate leukocyte extravasation and may decrease the severity of Crohn disease and/or ulcerative colitis (Figure 3.5).

Applications of immunological based therapies in liver diseases

The human liver immue system is complex and plays a pivotal role in many liver diseases. Thus we can manipulate immune cells in different pathways to treat many of our patients. Immune-based cytokines and cell therapies are necessary in the treatment of liver diseases and some of the therapies which are in early phase clinical trials are listed in the Table 3.2 and Figure 3.6.

Table 3.2 Immune cells or cytokine-based therapies applied in liver diseases and liver cancer.

Immune-based therapy in liver disease	
PD-1 block (to prevent apoptosis of effector cells)	Hepatocellular carcinoma
Ustekinumab (anti-p40 (IL-12 + IL23) block)	Primary biliary cirrhosis
Rituximab therapy (depletion of B cells)	Autoimmune hepatitis
Treg cell therapy	Autoimmune liver diseases
IL-2 low dose	Autoimmune liver diseases
Dendritic cell vaccination	Hepatocellular carcinoma
Mesenchymal stem cells and MDSC therapy	Autoimmune liver diseases
Gamma delta cells (Vγ9Vδ2)	Hepatocellular carcinoma

Conclusion

The liver is a complex immune organ able to promote both immune tolerance and to support active immune responses. Both tissue-resident and infiltrating immune cells, sinusoidal endothelium, biliary epithelial cells, and a network of myofibrobalst stromal cells act together to balance these processes. Through resident Kupffer cells, dendritic cells within the hepatic sinusoids, the liver acts as a vascular firewall and lymphocytes such as MAIT cells and innate lymphoid cells act as a biliary mucosal firewall in the portal tracts to guard against the constant influx of invading pathogens/commensal antigen load from the gut. An advancement in our understanding of liver immunology over the years has led to growing numbers of immune-based anti-inflammatory, antifibrotic, and anti-cancer clinical trials. Continuing research on hepatic immunology is crucial in order to advance these therapies further and identify novel translational therapies to treat patients with liver diseases.

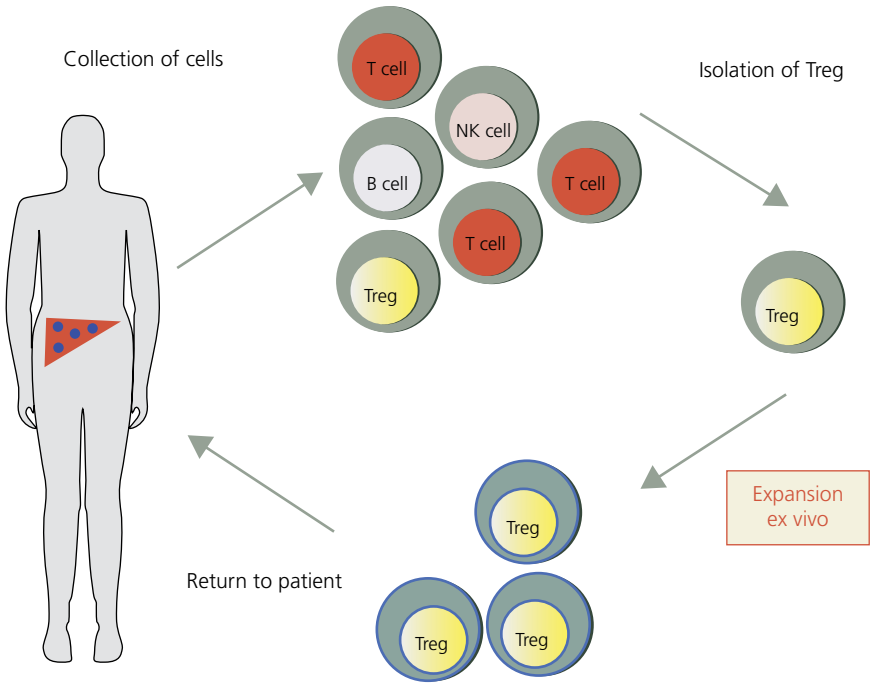

Figure 3.6 Diagram of possible future regulatory T-cell therapy. Tregs can be isolated in GMP (good manufacturing practice) laboratories using magnetic cell-sorting technology to obtain highly pure and functional cells. These Tregs can be expanded ex vivo with artificial antigen-presenting cells in the presence of IL-2, sirolimus, and retinoic acid to maintain stable Treg lineage. These cells can be reinfused back to the patient at the correct timing with optimal cell numbers to restore self-tolerance. This future cell therapy can potentially replace life-long immunosuppressive therapy. (Reproduced from Jeffery *et al.* 2016 *Front. Immunol.* http://dx.doi.org/10.3389/fimmu.2016.00334 © Jeffery, Braitch, Brown and Oo.)

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CHAPTER 4

Molecular Genetics and Liver Disease

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Key points

- Molecular genetics has revolutionized our approach to the diagnosis of liver diseases and advanced understanding of their pathophysiology.
- Molecular diagnostics allows clinicians to make an accurate diagnosis, and offer carrier testing for families and prenatal counseling for many patients with liver diseases.
- Novel DNA technologies may speed up the diagnostic process.
- Understanding the basics of molecular genetics is required to interpret laboratory tests.
- Many gene-based treatments for inherited liver diseases are in development and will become available in the next decade.

Recent progress in the understanding of molecular pathology underlying liver diseases would have been impossible without technological advances made in the field of human genetics. The tools and the informatics approaches used by geneticists has undergone dramatic transformation since the identification of the Wilson disease gene by Bull *et al.* in 1993 [1]. Whilst conventional Sanger sequencing was used to decipher the first human genome, discovery of the methods for next-generation sequencing (NGS) has allowed the analysis of thousands of exomes and genomes in the last 5 years [2]. Thus we are now starting to gain information about the true extent of genetic variability and heterogeneity of liver phenotypes in human populations using complementary approaches that employ clinical, epidemiological, and evolutionary studies.

Molecular genetic approaches are widely used in the research and clinical setting and include: (i) positional cloning of novel disease genes; (ii) diagnostic DNA screening for potentially pathogenic single nucleotide variants (SNVs); (iii) genome-wide association studies (GWASs) that allow simultaneous genotyping of the whole genome in a cohort of patients compared with controls in search for genetic risk factors; and (iv) NGS of selected genes, all exons, or the whole genome in individual patients.

Molecular characterization of inherited cholestatic disorders has made a particularly important contribution to the understanding of crucial pathways involved in the etiology of liver diseases, and studies in this field are increasingly adding to the knowledge of disease pathophysiology in hepatology. The availability of data from multinational projects such as the International HapMap Project [3] and the 1000 Genomes Project [4] provides controls for in silico analysis of the identified genetic variants and much easier prediction of their putative pathogenicity. Moreover, these projects have taken the genotype–phenotype correlation studies to a different level of complexity and promise to pave the way for a precision medicine approach to treatment in the future.

Basic terms in molecular genetics and clinical interpretation of mutations

Sanger sequencing. This is a method of DNA sequencing invented by Fred Sanger. It involves the use of chain-terminating dideoxynucleotides, which get incorporated by DNA polymerase during the polymerase chain reaction (PCR). Modern Sanger sequencing uses fluorescent nucleotides that can be detected by a laser.

Next generation sequencing (NGS). This term covers several types of novel DNA-sequencing technologies including Illumina (Solexa) sequencing, Roche 454 sequencing, Ion torrent: Proton/PGM sequencing, and SOLiD sequencing.

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These high-throughput sequencing methods generate sequence information much faster and in much larger volumes than Sanger sequencing.

Genome-wide association study (GWAS). This describes a research project that interrogates multiple genetic variants across the whole genome in multiple individuals (comparing study subjects and controls) to determine whether any of the variants may be associated with a particular trait (or disease). Modern GWASs typically investigate associations using single-nucleotide polymorphisms (SNPs) by applying DNA microarray technology.

Autozygosity (homozygosity) mapping. The term describes mapping of disease genes mutated in rare autosomal recessive disorders in inbred (consanguineous) populations. This method uses the assumption (correct in most but not all cases) that in inbred families the affected patients are likely to have inherited two recessive copies of the disease allele from the common ancestor. These types of alleles are called "identical-by-descent" (IBD) or "autozygous." The method uses genetic markers such as SNPs in order to identify homozygous regions. When the technique is applied to several affected patients with the same disease from different families, then shared regions of homozygosity found among the patients are likely to harbor the mutant disease gene.

Basic principles for the interpretation of molecular genetic tests

Most rare inherited liver disorders are inherited in an autosomal recessive manner, although there are exceptions like Alagille syndrome, which is inherited in an autosomal dominant manner. Even when the disease gene is known, not all mutations can be detected by conventional sequencing, and on average 5% of mutations may be missed. In most cases multiple mutations of many different types in the same gene or different genes can cause the same inherited disorder in different patients. For example, more than 400 mutations in either the *NPC1* or *NPC2* genes have been identified to cause Niemann–Pick type C disease.

In autosomal recessive disorders the identification of two alleles with previously demonstrated disease-causing mutations are considered consistent with a definitive diagnosis. The identification of disease-causing mutations allows carrier testing and antenatal diagnosis to be performed. Currently, the most frequently employed method of genetic analysis is Sanger sequencing, which determines DNA sequence of the coding exons, and intron–exon boundaries. This approach would miss the potential mutations located deep in the intronic sequence or the regulatory DNA sequence. Alternatively, targeted NGS methods may be applied. NGS has the potential to reduce costs associated with genetic testing for liver disease, particularly if the genes concerned are included on a multigene panel, for example as applied to "cholestasis" or "glycogen storage diseases" gene panels [5]. Sequence changes that result in the introduction of premature stop codons (i.e., nonsense, frameshift, and conserved splice-site mutations) produce truncated mRNA species that are usually targeted for breakdown (nonsense-mediated decay (NMD)) rather than translation into proteins. Sequence changes that result in small protein sequence changes (missense and in-frame mutations) may also be disease causing. However, missense and intronic variants are not necessarily disease causing, and many such polymorphisms exist. Additionally, many families have "private" sequence variants that have not been reported/published elsewhere. Therefore, in a number of suspected patients, confirmation of a diagnosis is not possible through genetic sequencing alone and additional testing maybe needed to confirm the diagnosis that interrogates the function of the mutant DNA or protein. The methods that target intronic sequence could identify variants that can disrupt RNA splicing and may also lead to NMD. In cases of missense or intronic changes of unknown significance, parental/familial DNA testing, the use of in silico protein and splicing prediction tools, and adherence to recently published guidelines on variant interpretation may assist in assigning their pathogenicity. Moreover the information source of the pathogenicity of mutations needs to be sought as some of the polymorphisms are reported as mutations in the databases.

Full genomic DNA sequencing is currently possible using NGS methods, but this may identify sequence variants that are difficult to interpret. Additionally, genomic rearrangements, such as exonic deletions or whole-gene deletions, are also reported with different frequency in various liver diseases. Although microarray-based comparative genome hybridization (array CGH) testing can pick up large deletions, testing for this type of mutation would generally require quantitative methodologies and is most frequently performed using multiplex ligation-dependent probe amplification (MLPA).

The interpretation of genetic results in some diseases may be complicated by the fact that pathogenic changes in two genes (digenic inheritance, e.g., Rotor syndrome) or even multiple genes (oligogenic inheritance), as in some ciliopathy cases, determine disease phenotype.

Advancing the understanding of liver biology and pathobiology

The identification of novel mutant genes causing rare mendelian liver disorders, although they directly affect only small numbers of patients, had major implications in the elucidation of liver pathophysiology. It led to further research in humans with liver diseases but also allowed studies in model organisms created by forward or reverse genetics approaches.

The highlights of the last three decades of gene cloning include the identification of genes with no previous known biological function (e.g., *CIRHIN*) [6], the elucidation of the molecular defects in candidate liver disease genes (e.g., *ABCB11*) [7], and providing unexpected insights into genetic involvement in human liver development and disease for genes with previously known functions.

Disease gene identification advanced our understanding of liver biology and pathobiology and aided correct disease classification. For example, multiple different hepatocyte transporter and associated protein defects were identified in liver disease patients that defined the involvement of these proteins in specific metal transport diseases such as Wilson disease, hemochromatosis, and hypermanganesemia [8].

Four subtypes of progressive familial intrahepatic cholestasis (PFIC) have been identified, with the first three being due to the defects in proteins involved in the transport of bile constituents; the most recently identified fourth type is due to a tight junction protein TJP2 defect [9]. Interestingly, milder abnormalities in all four proteins have been reported in attenuated forms of cholestasis such as benign recurrent intrahepatic cholestasis (BRIC) and hypercholanemia [10, 11]. It is now clear that PFIC and BRIC are extreme ends of the spectrum, whereas intermediate forms also exist. Furthermore, liver phenotypes similar to PFIC have been recognized as part of the multisystem syndromes arthrogryposis, renal dysfunction and cholestasis (ARC) syndrome and microvillous inclusion disease (MVID). Genetic defects recognized to cause ARC and MVID regulate a specific polarized canalicular membrane localization of the PFIC-associated transporters of bile constituents [12].

Other rare forms of cholestasis have been explained by mutations in the canalicular multispecific organic anion transporter gene (now termed *ABCC2*) (Dubin–Johnson syndrome) and a combined deficiency of the basolateral organic anion transporting polypeptide OATP1B1 and OATP1B3 (Rotor syndrome) [13].

Defects in the Jagged-Notch signaling pathway underlie the pathophysiology of a syndromic form of bile duct defect in Alagille syndrome. The majority of patients have mutation in *JAG1* and a small number of patients carry mutations in *NOTCH2*. Alagille syndrome has autosomal dominant inheritance and variable penetrance in the liver, as well as other features of the syndromic phenotype such as dysmorphic facies and cardiac abnormalities [14].

Most monogenic liver disease genes were identified through a combination of genome-wide linkage analysis and candidate gene sequencing. For example, to identify the mutant gene in patients with ARC syndrome, mutations were initially sought in the candidate genes such *ABCB11* and *ATP8B1* due to the similarity in liver phenotype. Autozygosity mapping study using genome-wide linkage markers in consanguineous ARC families identified the regions of the genome most likely to harbor the homozygous mutation due to "identity by decent" [15]. Sequencing of the candidate genes in the "autozygous" regions identified mutations in *VPS33B* in most of the patients. Identification of the second gene mutated in ARC was made using a candidate approach after the close interaction between *VPS33B* and the newly discovered protein VIPAR was found. Sequencing of *VIPAS39* encoding VIPAR identified mutations in the remaining patients with ARC syndrome [16].

More recently whole-exome sequencing has been used in combination with the linkage studies; this approach has significantly sped up disease gene identification.

A particular challenge for clinical geneticists and hepatologists is posed by the molecular diagnostics of congenital liver malformations including congenital hepatic fibrosis and cystic liver diseases. Recent studies identified multiple novel disease genes, many of which are linked to the function of primary cilia present on cholangiocytes, and the defects are termed "ciliopathies" [17]. These disorders can be inherited in autosomal recessive, autosomal dominant, or X-linked manner. Moreover, mutations in different ciliopathy-associated genes can be identified in the same patient and may contribute to the overall disease phenotype. The use of a genome-wide linkage approach to gene identification also led to discoveries of the numerous causes of fibrocystic liver disorders, which placed cilia and planar cell polarity defects at the core of pathological events for this group of congenital malformations. These discoveries provided a global link from liver disease to the understanding of human development and potential for therapies based on the knowledge of the signaling molecules involved.

Genetic diagnosis in the clinic

Until recently, mutation detection was predominantly used as a confirmatory diagnostic method in many liver disorders and not as an initial diagnostic procedure. With the much better availability of the newest sequencing methods, primary exon and exon–intron boundaries sequencing is now common as part of the routine diagnostic process. Conventional Sanger sequencing is still the most widely used technique, although NGS technologies are rapidly overtaking conventional sequencing as these approaches are more cost effective and accurate. Moreover, NGS methodologies can be used for gene panel sequencing, large-scale genetic analysis, and whole-exome and whole-genome analyses.

The identification of two previously demonstrated disease-causing mutations is considered diagnostic if the mutations are found on different alleles as confirmed by sequencing parental DNA. The mutations identified in patients with inherited liver diseases may be null (due to nonsense or splice-site point mutations, or large deletions and insertions) or hypomorphic (most commonly due to

missense mutations). Gain-of-function mutations have also been described in the *SLC40A1* gene encoding ferroportin that cause an autosomal dominant form of hemochromatosis [18].

More complex laboratory analysis is required in order to identify deep intronic and promoter mutations, or larger genomic sequence variants. Intronic mutations can result in splicing defects and promoter mutations affect expression of the genes. cDNA sequencing may be helpful in the detection of intronic variants and splicing defects. MLPA can be used to detect deletions of one or more exons. Furthermore, assessment of mRNA degradation (inhibition of nonsense-mediated mRNA decay processes) has also been used to investigate mutations resulting in aberrant splicing.

The expanded use of NGS as a powerful method for routine diagnosis of mendelian disorders will lead to the identification of an increased number of new variants, which may or may not be pathogenic and which are a common interpretation problem. This is true for many genes involved in liver disease, considering the size of genes and heterogeneity of mutations in most disorders.

Gene panel sequencing has a greater capacity and ability to sequence samples simultaneously compared with targeted gene sequencing, and thus can provide potential cost savings in certain cases. Whole-exome sequencing is also gradually entering the routine diagnostic arena. Although whole-genome sequencing is currently only available as part of many research projects, it is only a matter of time until it is also used in clinical practice. These methodologies may present significant challenges for interpretation due to the volume of data produced. The obvious benefits of these techniques include comprehensive molecular analysis in uncertain cases and, in the future, that they will be able to provide the unique genomic signature of each patient relevant to treatment. However, improvement in bioinformatics methods and a much deeper understanding of genotype–phenotype relationships will be needed.

Genetics and complex liver disorders

Large cohort studies have recently been employed with success to identify gene variants that influence human phenotypes at the population level. This work has been helped by the advent of methods such as linkage and association studies, which have allowed the detection of major genes. Although some studies use a candidate gene approach, which requires prior hypothesis, the development of molecular methods and informatics allow genome-wide studies for hypothesis-generating research. The quality of these studies is dependent on many factors including the choice of the statistical tests and stringency of the criteria. The original data are more convincing when they are replicated in independent experiments and in different ethnic groups. Further functional studies could also strengthen the evidence for a causal relationship between the identified genetic change and its pathogenicity.

Association studies aim to assess the contribution of genetic variants to the disease studied by comparing the distribution of these variants between affected (cases) and unaffected (controls) subjects. Until the advent of high-throughput genotyping, such studies investigated a limited number of variants of candidate genes.

Whole-exome sequencing can detect variations in 1–2% of the genome. Whole-genome sequencing can potentially detect mutations in the remaining parts of the genome, the function of which has not until now been studied. Large genetic epidemiology studies using whole-genome sequencing are likely to identify the contribution of various genes to liver disease phenotypes. Furthermore, it will be possible to adopt an interactome approach, where the interactions between multiple genes can contribute to phenotypic variation (e.g., the Human Gene Connectome) [19].

GWASs have been used since the mid-2000s following the development of high-throughput genotyping. The GWAS in gallstone disease detected a possible causal association between a SNP of cholesterol transporter ABCG5 and ABCG8 and gallstones. Pathogenic mutations in *ABCG5/ABCG8* were previously found to cause sitosterolemia, a condition that leads to the abnormal secretion of phytosterols such as sitosterol [20]. Clearly penetrance for gallstone development is much lower than in autosomal recessive sitosterolemia, which demonstrates the differences between mendelian and complex disorders, and indicates that additional factors are required for the gallstone disease pathogenesis.

Successes and future challenges

The identification of genetic causes of pediatric liver diseases has allowed the detection of accurate molecular diagnosis in approximately 50% of patients [14]. NGS is now more easily available and the costs are falling, making it clinically accessible. However, interpretation of the genetic data delivered by whole-exome sequencing and for whole-genome sequencing is not currently practical for most routine diagnostics. The number of variants identified in each individual is so large that the available bioinformatic predictive tools cannot correlate these with the complex disease phenotypes. Nevertheless, using gene mapping techniques it is now much easier to identify genetic causes of mendelian diseases.

Disease-specific treatment development has become possible for several enzyme deficiencies, most recently in lysosomal acid lipase deficiency [21].

Animal models of liver disease have demonstrated the feasibility of gene therapy using different vectors, so many genetic disorders may become targets for gene therapy development [22].

More work is required to determine the relationship between the susceptibility loci and liver diseases. Knowledge of the immune factors predisposing to liver disease prompted a study of a monoclonal antibody that targets the p40 subunit of interleukin 12 (IL-12) and IL-23; however, the initial trial was not successful and was terminated (https://clinicaltrials.gov/ct2/show/NCT01389973; last accessed June 2016). Thus knowledge of susceptibility factors is not yet easily translatable to clinical practice.

The development of technology that utilizes induced pluripotent stem cells (iPSc) generated from patients has produced healthy optimism for downstream studies of the disease pathophysiology [23]. There are robust protocols for iPSC differentiation into hepatocytes and more recently cholangiocytes. Work on different protocols for other liver cells is in progress. This technology will facilitate the study not only of mendelian disorders but also cell phenotypes specific for any individual patient's genomic signature.

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SECTION 2

Investigating the Liver

CHAPTER 5

Useful Investigations in the Assessment of Liver Disease

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Key points

- Investigating the liver relies on a multidisciplinary approach involving laboratory investigations, imaging studies, histopathology, microbiology, and molecular studies.
- The main purposes of the investigation of liver disease are to establish the diagnosis, stage the disease, detect complications, if any, and to detect associated conditions.
- Clinical presentations of liver disease are age-specific and include persistent neonatal jaundice, acute liver dysfunction, and chronic liver disease.
- At present, many imaging techniques provide valuable information in the investigation and diagnosis of pediatric liver disease. The rapid development of interventional radiology has also altered the management of many hepatic conditions.
- Liver biopsy remains a useful diagnostic procedure and may be the only definitive method of assessing the degree of inflammation and fibrosis.
- Many inborn errors of metabolism present with hepatomegaly and/or liver disease. It is therefore essential to screen for inborn errors of metabolism as part of the investigation of liver disease in both neonates and older children.
- Multisystem evaluation including assessment of the eyes, heart, and brain is important in many liver diseases in children.

The approach to the child with liver disease should be systematic and based on an accurate clinical history and a thorough physical examination. This chapter summarizes the key investigations. A summary of useful investigations for common phenotypes of clinical presentation of liver disease in infants and children is given. Further detail will be provided in individual chapters.

Investigating the liver relies on a multidisciplinary approach involving clinical chemistry, hematology, imaging studies, histopathology, and microbiology as well as molecular studies. Briefly, the principles of investigation should be to:

- Establish the diagnosis and exclude differential diagnoses.
- Stage disease and detect complications (see Chapter 21).
- Detect associated conditions, e.g., celiac disease or inflammatory bowel disease (see Chapter 22).

All investigations should start with an accurate history and physical examination. The clinical history should include:

- Details about the mother's pregnancy (drugs, alcohol, smoking, intercurrent illnesses, pruritus of pregnancy, hepatitis status, risk factors, e.g., drug abuse).
- Birth weight and gestational age.
- Vitamin K administration.
- Family history and consanguinity.
- History of the present illness should include:
  - date of jaundice
  - color of stools and urine
  - drug history, particularly parenteral nutrition
  - bleeding, petechiae, or bruising
  - feeding history and weight gain
  - diarrhea and vomiting
  - immunizations, if relevant.

Functions of the liver

It is essential to understand the many functions of the liver and to recognize the effects of hepatic dysfunction on other body systems (Table 5.1; see also Chapter 1).

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Table 5.1 Functions of the liver.

Function	Effect of dysfunction	Assessment
Metabolism/storage		
Carbohydrate/glycogen	Loss of glucose homeostasis	Hypoglycemia on fasting/stress
Lipid	Lipid accumulation in hepatocytes	High/low cholesterol
	↓ Oxidation of fatty acids	↑ Lactate
		↑ FFA : BOH ratio
		↑ Acylcarnitine
		Organic aciduria
Protein	↑ Catabolism	Low BCAA, urea
		↑ Ammonia
		↑ Tyrosine, ↑ phenylalanine, ↑ methionine
Synthesis		
Albumin	Loss of muscle mass	Low albumin
		Protein energy malnutrition
Factors II, VII, IX, X	Coagulopathy	Prolonged PT/PTT
Degradation		
Drugs	Prolonged drug effect, e.g., sedation	Clinical
Estrogens	Telangiectasia	Clinical
	Gynecomastia	
Toxic products	Encephalopathy	Abnormal EEG/clinical signs
Bile synthesis and excretion	Cholestasis	↑ Conjugated bilirubin
	Fat malabsorption	↑ GGT
		↑ ALP
		↑ Cholesterol
	Fat-soluble vitamin deficiency	Anthropometry
	Pruritus	
	Malnutrition	

ALP, alkaline phosphatase; BCAA, branched-chain amino acids; BOH, β-hydroxybutyrate; EEG, electroencephalogram; FFA, free fatty acids; GGT, γ-glutamyltranspeptidase; PT, prothrombin time; PTT, partial thromboplastin time.

Table 5.2 Basic biochemical and hematological tests of the liver.

Reference range of test	Abnormality
Synthetic function of the liver	
Glucose >4 mmol/L	Reduced in: acute or chronic liver failure/metabolic disease/ hypopituitarism
Albumin 35–50 g/L	Reduced in: chronic liver disease
Prothrombin time (PT) 33–37 s	Prolonged in: vitamin K deficiency reduced hepatic synthesis
Liver enzymes released from the liver	
Aminotransferases:	Elevated in: hepatocyte inflammation/damage
Aspartate (AST) <50 U/L	
Alanine (ALT) <40 U/L	
Liver enzymes detecting bile flow	
γ-Glutamyltranspeptidase (GGT) <30 U/L (age dependent)	Elevated in: biliary inflammation/ obstruction
Alkaline phosphatase (ALP) < 600 U/L (age dependent)	
Substance metabolized/transported by the liver	
Conjugated bilirubin <20 µmol/L	Elevated in: hepatocyte dysfunction or biliary obstruction
Substance cleared from the plasma by the liver	
Ammonia <50 µmol/L	Elevated in: abnormal protein catabolism/urea cycle defect/other inherited metabolic disease

Clinical chemistry and hematology

Biochemical and hematological tests to evaluate liver diseases can be divided into six categories (Table 5.2):

- Synthetic function of the liver.
- Liver enzymes released from injured liver.
- Liver enzymes and substances detecting the excretory function (bile flow) of the liver.
- Substances metabolized or transported by the liver
- Substances cleared from plasma by the liver.
- Other tests.

It should be recognized that some of these biochemical or hematological tests lack sensitivity and specificity. For example, serum alanine aminotransferase may be elevated in musculoskeletal conditions. Normal aminotransferase may be seen in some children with chronic liver disease. Finally, abnormal liver biochemical tests do not normally provide a specific diagnosis or indicate the severity of hepatic dysfunction. In addition, an abnormal liver biochemistry also rarely provides diagnostic information on individual diseases.

Synthetic function of the liver

The most useful tests of liver "function" are plasma albumin concentration and coagulation time.

Albumin is the principal form of protein in the serum and is only synthesized in the liver. In the absence of excessive urinary or gastrointestinal loss or prolonged starvation, a low serum albumin, which has a half-life of 20 days, indicates chronicity of liver disease. However, in a child with decompensated liver disease, an abrupt decrease in the serum albumin level maybe seen following an acute illness, such as a viral infection.

Deranged hemostasis is a common complication of liver disease. Mechanisms include: (i) diminished hepatic synthesis of coagulation factors II, V, VII, IX, X, and XI, prothrombin, and fibrinogen; (ii) dietary vitamin K deficiency or malabsorption; (iii) disseminated intravascular coagulopathy; and (iv) dysfibrinogenemia or increased fibrinogenolysis.

A prolonged prothrombin time (PT) is a common and affordable test, but is not specific for liver disease. Abnormal *coagulation*, especially PT after vitamin K deficiency is excluded, indicates significant hepatic dysfunction, either acute or chronic. A factor VII level that is less than 12% of normal may be predictive of irreversible fulminant liver failure (see Chapter 18).

Fasting hypoglycemia in the absence of other causes (e.g., hypopituitarism or hyperinsulinism) indicates poor hepatic function and is a guide to prognosis in acute liver failure. If these baseline investigations suggest hepatic dysfunction, then more specific investigations for metabolic disease are appropriate to consider (Table 5.3) [1]. Other causes of hypoglycemia include glycogen storage disease (see Chapters 9 and 19), disorders of fatty acid metabolism, or mitochondrial enzyme defects.

Table 5.3 Specific investigations of liver disease.

Second-line investigations	9 AM cortisol
Bacterial culture of blood and urine	Chromosomes/DNA
TORCH screen	Sweat test (>4 weeks)
Hepatitis A, B, C, E	**Older child (>2 years)**
α1-Antitrypsin level and phenotype	Copper, ceruloplasmin, urinary Copper
Abdominal ultrasound	C_3, C_4, ANA, SMA, LKM
Metabolic investigations	Immunoglobulins
Immunoreactive trypsin	EBV
Plasma lactate, BOH, FFA	**If indicated**
Ammonia	Radioisotope scan
Acylcarnitine	Liver biopsy for:
Serum iron and ferritin	histology
Plasma amino acids	electron microscopy
Cholesterol, triglyceride	enzyme analysis
α-Fetoprotein	immunohistochemistry
Endocrine tests	culture
Parathyroid hormone	**Others**
Wrist for bone age/rickets	Copper concentration
Urine	Skin biopsy
Reducing sugars	Ophthalmology
Organic acids	Cardiology
Amino acids	Bone marrow aspirate
Succinylacetone	Endoscopy
Bile salts	Endoscopic retrograde pancreatocholangiography
Neonate	Percutaneous transhepatic cholangiography
Galactose 1-phosphate uridyltransferase	Magnetic resonance pancreatocholangiography
Free T_4, TSH	

ANA, antinuclear antibody; BOH, β-hydroxybutyric acid; EBV, Epstein–Barr virus; FFA, free fatty acids; LKM, anti-liver, kidney, and microsomal antibody; SMA, antismooth muscle antibody; TORCH, toxoplasma, rubella, cytomegalovirus, herpes simplex; TSH, thyroid-stimulating hormone.

Liver enzymes released from the liver

Aminotransferases are intracellular enzymes, which are present in liver, heart, and skeletal muscles. Increases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) indicate hepatic necrosis irrespective of etiology (Table 5.2). ALT is more liver specific than AST but has a longer plasma half-life (approximately 24 h). A rise in AST is an early indication of liver damage and is a useful marker of rejection post-liver transplant. An isolated increase in AST maybe due to hemolysis (difficult venipuncture), acute rhabdomyolysis, myopathy, or cardiomyopathy. Elevated aminotransferases are often the first indication of the development of non-alcoholic fatty liver disease (NAFLD) in an obese child (see Chapter 15). Elevated AST and/or ALT are also found in muscular dystrophy and this diagnosis should be considered if there are no other signs of liver disease. These enzymes, however, may be normal in compensated cirrhosis (see Chapter 21).

Liver enzymes and substance detecting bile flow

Alkaline phosphatase is found in the liver, kidney, bone, placenta, and intestine. In children with liver disease, a raised alkaline phosphatase indicates biliary epithelial damage, malignant infiltration, cirrhosis, rejection, or osteopenia secondary to vitamin D deficiency. In a growing child, however, the potential contribution from bone makes alkaline phosphatase measurement less specific for liver pathology.

γ-Glutamyl transpeptidase (GGT) is present in biliary epithelia and hepatocytes, and also in the cell membrane of many other human organs, including the kidney, pancreas, spleen, brain, breast, and small intestine. As such, an elevated GGT is not specific for liver disease. In addition, the reference range is age related, with higher levels in neonates (up to 385 IU/L). It is elevated in many forms of liver damage. However, GGT does not increase in the serum of patients with bone disease or children with active bone growth, and thus is helpful in confirming the liver origin of a raised alkaline phosphatase. It may be normal in certain forms of intrahepatic cholestasis (progressive familial intrahepatic cholestasis 1, 2 and 4; PFIC 1, 2 and 4).

Urobilinogen refers to a group of colorless tetrapyrroles formed when unconjugated bilirubin (which is formed after the conjugated bilirubin secreted into the upper intestine is hydrolyzed to the unconjugated pigment) is reduced by intestinal bacteria. Excretion of urobilinogen in the urine and stool is reduced in biliary obstruction.

Substance metabolized/transported by the liver

Conjugated *bilirubin* is nearly always elevated in liver disease and is a particularly important investigation in the differential diagnosis of neonatal jaundice (see Chapter 8). The presence of bilirubin is always abnormal if it is detected in a fresh urine specimen.

Substances cleared from the plasma by the liver

Plasma ammonia and amino acids (particularly phenylalanine, tyrosine, and methionine) may be raised in either acute or chronic liver failure and are non-specific indications of hepatic dysfunction.

Specific investigations of the liver

In addition to baseline biochemical and hematological investigations, there are a host of second-line investigations available (Table 5.3). Selection of the most useful tests will depend on the age of the patient, clinical presentation, and chronicity of the illness.

Acute hepatic dysfunction may be secondary to sepsis, particularly urinary sepsis, inborn errors of metabolism, or endocrine disorders. It is usual to exclude sepsis by performing bacterial culture of the urine and/or blood cultures if appropriate, and to exclude known causes of viral hepatitis [2]. In an acutely unwell infant, or one with evidence of acute liver failure, galactosemia and tyrosinemia should be excluded (Table 5.4; see also Chapter 9). Other important causes of neonatal acute liver failure include perinatal infections, gestational alloimmune liver disease, mitochondrial hepatopathies, and other metabolic diseases [2].

In neonates, hypopituitarism may be difficult to exclude as thyroid function tests may be equivocal or in the low normal range. It is useful to perform a cortisol level test at 9 a.m. at the same time as measuring free thyroxine and thyroid-stimulating hormone (TSH).

α1-Antitrypsin deficiency is the commonest inherited metabolic liver disease and should always be excluded at any age. As α1-antitrypsin is an acute phase protein, it is necessary to measure both the concentration and phenotype in order to differentiate between homozygotes, heterozygotes, and an acute phase response.

Although cystic fibrosis is a rare cause of liver disease in the neonatal period, it should be considered in the differential diagnosis of neonatal liver disease, and excluded by performing an immunoreactive trypsin test, a sweat test, and mutation analysis if either is positive (see Chapter 16). Wilson disease rarely presents before the age of 3 years, but

Table 5.4 Causes and investigations of neonatal and childhood acute liver failure.

Diseases	Investigations
Neonates	
Infections	
Herpesviruses	Tissue culture, direct immunofluorescence of swabs or tissue Molecular technique (Note: serology is of no value in perinatal infection due to presence of maternal IgG to herpes simplex virus)
Adenovirus	Immunoassay or PCR to detect virus in stool, blood, or liver tissue
Echovirus	Tissue culture, direct immunofluorescence of swabs or tissue
Hepatitis B	Hepatitis B surface antigen (HBsAg), anti-HBc antibody
Metabolic	
Galactosemia	Galatose-1-phosphate uridyltransferase
Tyrosinemia	Serum tyrosine, methionine, α-fetoprotein, urine succinylacetone
Gestational alloimmune	Serum ferritin, extrahepatic siderosis (magnetic resonance imaging, or tissue biopsy showing hemosiderosis; oral or buccal mucosa)
liver disease	Liver biopsy: immunostaining of hepatocytes for the C5b-9 complex
(neonatal hemochromatosis)	
Mitochondrial	See Chapter 9
Other metabolic conditions	See Chapter 9
Ischemic	
Congenital heart disease	Chest X-ray, ECG and echocardiogram, cardiac enzymes (if myocarditis is suspected)
Older children	
Infections	
Viruses:	
Hepatitis A	Anti-HAV IgM
Hepatitis B	HBsAg, anti-HBc antibody
Hepatitis C	Anti-HCV, HCV PCR
Herpes viruses	Antibody
Epstein–Barr virus	Antibody
Bacterial	Leptospiral antibody if clinically indicated
Drugs	
Paracetamol	Paracetamol level (compare with normogram by Rumack and Matthews) [22]
Metabolic	
Wilson disease	Serum copper, ceruloplasmin, 24 h urine copper with penicillamin challenge when clinically indicated
Others	Serum organic acid, urine organic acid, urine reducing sugars
Autoimmune hepatitis	Raised plasma IgG in both forms Histology: interface hepatitis, bridging necrosis, dense mononuclear and plasma cell infiltration, hepatic regeneration with rosette formation

ECG, electrocardiograph; HAV, hepatitis A virus; HBc, hepatitis B core; HCV, hepatitis C virus; Ig, immunoglobulin; PCR, polymerase C reaction.

may mimic any form of liver disease and should always be excluded in older children (see Chapter 20). An autoimmune screen and immunoglobulin levels should detect 75% of children with autoimmune hepatitis (see Chapter 11).

The development of new technology, such as fast atom bombardment mass spectrometry and tandem mass spectrometry, has made it possible to identify specific metabolites in the urine and blood in a number of rare diseases, e.g., primary bile salt deficiencies (see Chapters 8 and 9). Other specific tests include measurement of carnitine and acylcarnitine in fatty acid oxidation disorders (see Chapters 9 and 19). These investigations are essential steps in the differential diagnosis of unresolved neonatal hepatitis.

Serum cholesterol is usually elevated in children with severe cholestasis – for example, in Alagille syndrome or biliary atresia – and provides supporting evidence of these diagnoses. In contrast, low or normal cholesterol is characteristic of bile acid transport disorders or of terminal liver disease.

An elevated plasma or urine tyrosine may indicate tyrosinemia type I, which should be confirmed by measurement of urinary succinylacetone. Definitive diagnosis requires assay of fumarylacetoacetase in skin fibroblasts or mutation analysis (see Chapter 9). Primitive hepatic cells synthesize α-fetoprotein. The levels are highest in the newborn (>1000 mg/L) and fall in the first few months of life. It may be a useful screening test in the diagnosis of tyrosinemia type I and hepatoblastoma, or for the detection of hepatocellular carcinoma in chronic carriers of hepatitis B and C.

Common clinical presentations of liver disease in infants and children

Persistent neonatal jaundice

Persistent jaundice (the presence of jaundice beyond 2 weeks of life) is a common clinical phenomenon in the neonatal period. Although physiological jaundice is the most common cause of jaundice in neonates, infants who developed severe or prolonged jaundice (14 days in term infants and 21 days in preterm infants) should always be investigated [3]. It is helpful to perform total, conjugated, and unconjugated bilirubin to classify jaundice into conjugated or unconjugated hyperbilirubinemia (Table 5.5).

1 *Unconjugated hyperbilirubinemia*. Common causes of unconjugated hyperbilirubinemia include hemolysis (ABO and Rhesus incompatibility), breast milk jaundice, sepsis, and Crigler–Najjar type I or II (Table 5.5).
2 *Conjugated hyperbilirubinemia*. A rise in conjugated bilirubin (conjugated bilirubin >17 μmol/L if total bilirubin <85 μmol/L; or conjugated bilirubin >20% of total if total bilirubin >85 μmol/L) always signifies underlying liver disease and warrants further assessment [4]. Causes of persistent conjugated hyperbilirubinemia in the newborn are manifold and it is imperative to have a focused and systemic approach to diagnose the underlying cause efficiently. A schematic approach to a child with neonatal liver disease is shown in Figure 5.1.

It is important to exclude surgical disorders such as biliary atresia in infants with neonatal cholestasis as early surgery is associated with a better outcome. Similarly, the outcome of bacterial infections and certain metabolic conditions (such as galactosemia) has improved with early identification and treatment, and hence they warrant rapid investigation. Although not usually posing a diagnostic dilemma, the successful management of pre-term infants as young as 25 weeks' gestation has increased the number of children treated with parenteral nutrition and a consequential rise in referrals of these infants with persistent jaundice. Other conditions to be considered that can present as neonatal cholestasis are listed in Table 5.6 (see Chapter 8).

Generally, the presence of the following clinical and laboratory features, together with results of imaging studies, should raise the suspicion of biliary atresia:

- General condition: usually well and thriving.
- Abdomen: liver – enlarged and firm in consistency; spleen – may be enlarged; ascites – rare, indicates severe portal hypertension.

Table 5.5 Common and uncommon causes of unconjugated hyperbilirubinemia in infancy.

Conditions	Frequency	Features/comments
Physiological jaundice	Very common	Usually benign, c. 8–20% of infants with physiological jaundice may have serum bilirubin >200 μmol/L
Hemolytic jaundice	Common	Causes include ABO and Rh incompatibilities, glucose-6-phosphate dehydrogenase (G6PD) deficiency, red cell membrane defects
Breast milk jaundice	Common	May overlap with physiological jaundice, and may last till 1–2 months; stools always pigmented; not to be confused with neonatal cholestasis
Sepsis	Common	Sick infants; blood, urine, cerebrospinal fluid cultures, chest X-ray
Hypothyroidism	Common	Thyroid function tests show high thyroid-stimulating hormone and low T_4
Gilbert syndrome	Common	Important cause of unconjugated hyperbilirubinemia, benign condition, polymorphism of the 5′ end of the promoter of the *UGT1A1* gene (homozygous insertion of the TA pair: genotype UGT1A1*28/*28)
Crigler–Najjar syndrome type 1	Rare	Autosomal recessive, mutations in the *UGT1A1* gene resulting in either truncated non-functional enzyme or non-recognition of the substrate bilirubin; rapid rise in unconjugated bilirubin early in life may lead to kernicterus
Crigler–Najjar syndrome type 2	Rare	Autosomal recessive, mutations have been reported in exon 1A1 of the *UGT1A1* gene; clinically less severe than type 1 disease and responsive to phenobarbital therapy

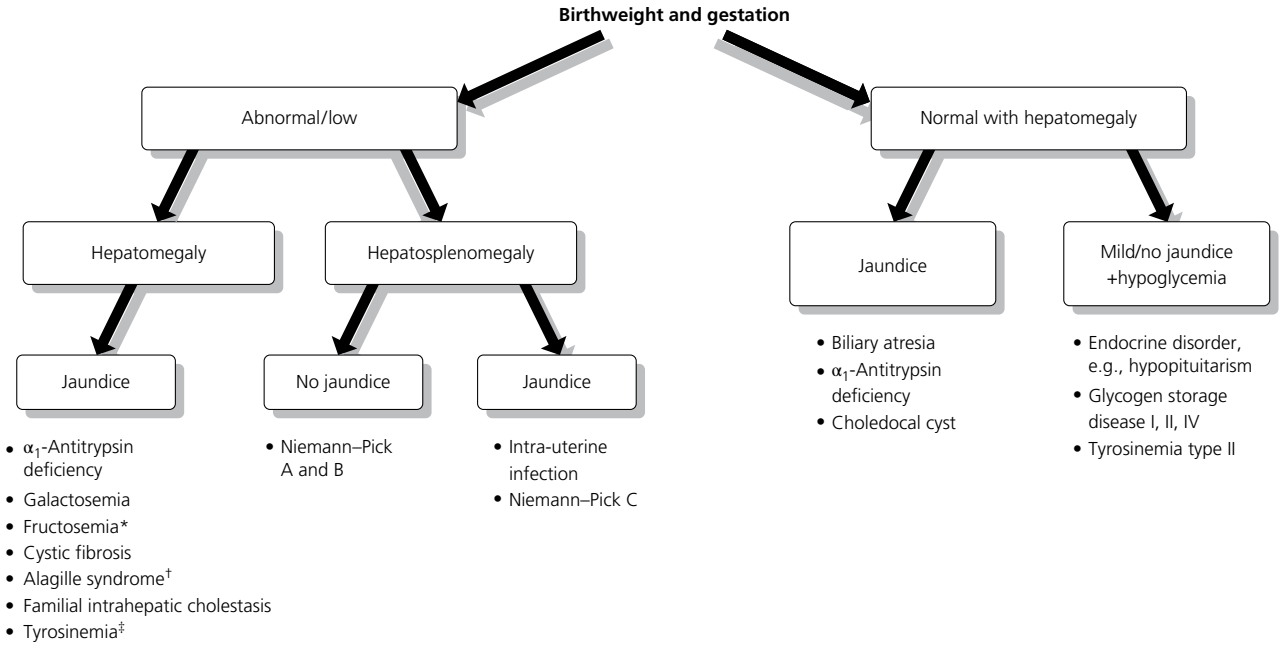

Figure 5.1 Approach to the diagnosis of neonatal liver disease. * Presents on weaning; † dysmorphic; ‡ jaundice may be mild.

Table 5.6 Differential diagnosis of infantile conjugated hyperbilirubinemia when biliary atresia has been excluded.

Categories	Differential diagnosis	Investigations	Results
Infective	Congenital infection	Serology for toxoplasma, rubella, CMV	Positive testing
	Bacteremia, urinary tract infection	buffy coat, herpes simplex, syphilis Urine microscopy, urine and blood culture	
Endocrine	Hypothyroidism	TFTs	Raised TSH, low T_4
	Hypopituitarism	TFTs, cortisol, glucose	Low TSH, cortisol, hypoglycemia
Metabolic (see Table 5.3)	Galactosemia	Urine-reducing substances	Positive reducing substances if on a galactose-containing diet
		Plasma Gal-1-PUT	Absent or reduced Gal-1-PUT detected
	Tyrosinemia	Urine succinyl acetone	High succinylacetone
		DNA	Mutations in *FAH*
	Storage disease, e.g., Niemann–Pick C	Liver biopsy, bone marrow biopsy, filipin staining	Storage cells on bone marrow and liver biopsy (can be difficult to see in young children), positive filipin staining of fibroblasts
		DNA	Mutation in *NPC1* and *NPC2*
	Bile salt synthesis disorders	Urinary bile salts (not accurate if on ursodeoxycholic acid)	Abnormal peaks on urine mass spectroscopy
		DNA	Mutation in *AKR1D1*
	Peroxisomal disorders	Plasma very long chain fatty acids	High levels of very long chain fatty acids
		DNA	Mutation in *PEX* genes
Genetic	α1-Antitrypsin deficiency	α1-Antitrypsin level and phenotype	Low α1-antitrypsin level and PiZZ or ZS phenotype
	Arthrogryposis, renal dysfunction, cholestasis (ARC) syndrome	GGT	Low GGT cholestasis
		DNA	Mutation in *VPS33B* or *VIPAR*
	Citrin deficiency	Plasma and urine amino acids	Increased plasma and urine citrulline and arginine
		DNA	Mutation in *SLC25A13*
Toxic	Intestinal failure-associated liver disease	Liver biopsy	Cholestasis with hepatocellular necrosis, abundant lipofuscin, fatty infiltration, mild giant cell transformation, portal tract infiltration, bile duct reaction, ± portal fibrosis

CMV, cytomegalovirus; GGT, γ-glutamyltranspeptidase; TFTs, thyroid function tests; TSH, thyroid stimulating hormone.

- Stool color – progresses to persistently pale (Figure 5.2).
- Clinical chemistry – raised conjugated bilirubin, ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and GGT; ALP and GGT are more elevated than AST and ALT.
- Hematology – usually normal.
- Abdominal ultrasound – absent or contracted gallbladder common; presence of triangular cord (a fibrous cone of tissue at the bifurcation of the portal vein); exclude choledochal cyst.
- Hepatobiliary scintigraphy – absence of biliary excretion of radioisotope.
- Histopathology – preservation of overall architecture, prominent bile ductular proliferation, canalicular and cellular bile stasis, and portal fibrosis.

Acute liver disease

Underlying causes and clinical presentation depends on the age, but the following clinical features are common: a prodrome of malaise, lethargy, and anorexia; and nausea, vomiting, or diarrhea. There may be weight loss, abdominal discomfort or tender hepatomegaly, splenomegaly, ascites (rarely, except for acute Budd–Chiari), rash, or joint pains. It is noteworthy that jaundice is not always present.

Important causes of neonatal liver failure include viral infections, metabolic liver disease, gestational alloimmune liver disease, and ischemic causes (see Table 5.4) [2]. It is important to obtain a detailed family history, presenting symptoms and signs, detailed antenatal history and delivery, and dietary and drug history.

The differential diagnosis of acute hepatitis in older children includes: viral hepatitis A, B, C, and E, seronegative hepatitis, autoimmune hepatitis, drug-induced liver injury, and metabolic liver disease especially Wilson disease [2].

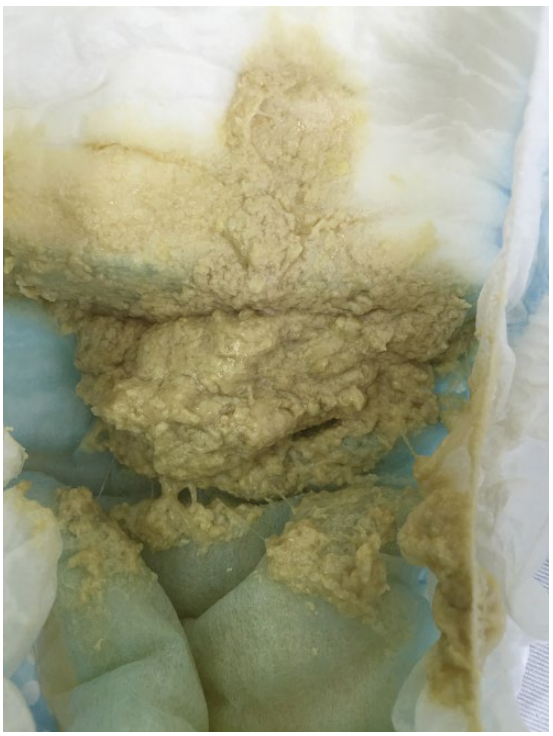

Figure 5.2 Typical appearance of pale stool.

Liver disease in older children

Liver disease in children older than 6 months may be acute or chronic. As in infancy, inherited disorders need to be excluded (see Table 5.4), but jaundice may not be a prominent feature. Acute or chronic liver disease may be due to infection, autoimmune disease, drug-induced hepatitis, and metabolic diseases (Table 5.7). Hepatomegaly is the most common mode of presentation of liver disease in older children. A schematic approach in an older child suspected of having liver disease presenting with an enlarged liver is shown in Figure 5.3.

Chronic liver disease

Chronic liver disease is frequently asymptomatic but can be detected through other analyses (Table 5.7), such as:

- Incidental detection of abnormal liver enzymes or hepatomegaly.
- Family screening for hepatitis B/C or metabolic disorders (Wilson disease).
- Analysis of transfusion recipient following diagnosis of donor infection.
- Coexistent disease, e.g., inflammatory bowel disease or celiac disease.
- Detection of a known toxic agent, e.g. methotrexate, in a patient.

When symptomatic, children may present with:

- Intermittent fatigue, anorexia, and weight loss.
- Abdominal discomfort.

Table 5.7 Age-specific investigations in chronic liver disease.

Age of patient	Investigation
Neonate	TORCHES screen Galactose 1-phosphate uridyl transferase Free T_4, TSH, morning cortisol Targeted DNA mutational analysis Sweat test (>4 weeks)
Older child (>2 years)	Copper, ceruloplasmin, urinary copper C3, C4, ANA, SMA, LKM, immunoglobulins EBV
If indicated	Liver biopsy for: histology, electron microscopy, enzyme analysis, immunohistochemistry, culture, copper concentration Skin biopsy, ophthalmology, cardiology, bone marrow aspirate Endoscopy, ERCP

ANA, antinuclear antibodies; C3, C4, complement components 3 and 4; EBV: Epstein–Barr virus; ERCP, endoscopic retrograde cholangiopancreatography; LKM, liver/kidney microsomal antibodies; SMA, smooth muscle antibodies; T_4, thyroxine; TORCHES, toxoplasma, rubella, cytomegalovirus, herpes simplex, syphilis; TSH, thyroid-stimulating hormone.

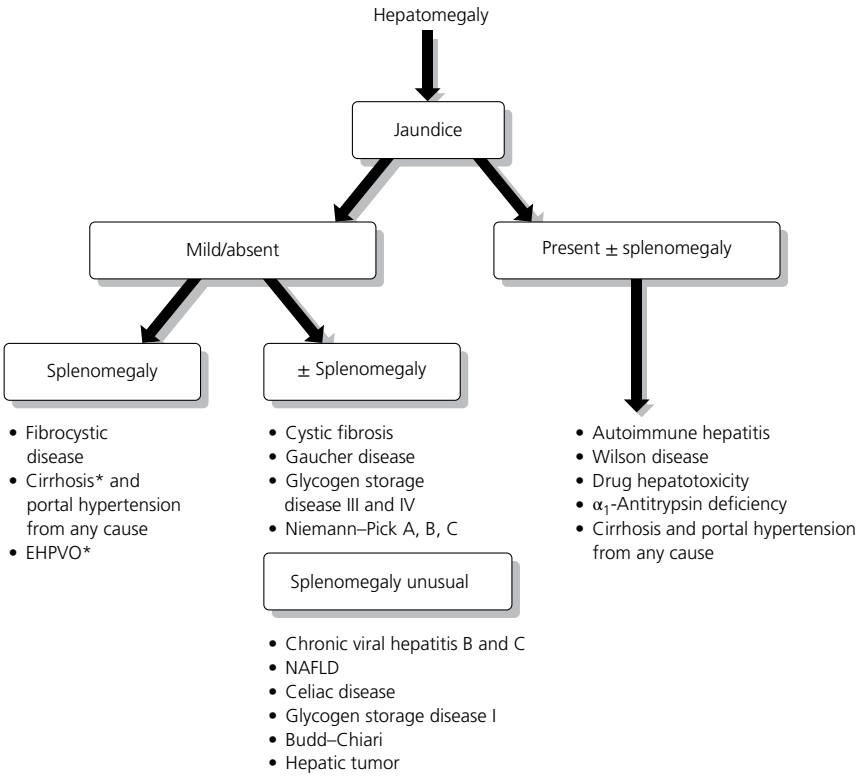

Figure 5.3 Approach to the older child with liver disease. * Liver may be small; EHPVO, extrahepatic vein obstruction; NAFLD, non-alcoholic fatty liver disease.

- Variable or fluctuating jaundice with pruritus and pale stools.
- Hematemesis or melena from variceal bleeding, especially with portal hypertension.

Investigating the liver

Imaging studies

Several imaging techniques provide valuable information in the investigation and diagnosis of pediatric liver disease, while the rapid development of interventional radiology has altered the management of many hepatic complications [3].

Radiography

Plain radiography of the abdomen will give an indication of liver and spleen size, but is rarely of diagnostic value and is not a routine investigation. Chest radiography may show skeletal abnormalities – for example, butterfly vertebrae in Alagille syndrome, rachitic rosary in rickets due to vitamin D malabsorption, a dilated heart secondary to fluid overload in end-stage liver disease, or evidence of congenital heart disease. Wrist and knee radiography will demonstrate bone age and/or the development of osteopenia or rickets.

Ultrasound

The development of Doppler ultrasound has been an important advance in the investigation of liver disease. It is the least invasive and the technique is portable, quick, and can be used to guide interventional procedures. Ultrasonic investigation of the abdomen provides information on the size and consistency of the liver, spleen, pancreas, and kidneys, on the size of the gallbladder, and on the presence of gallstones. It may identify tumors, hemangiomas, abscesses, or cysts within the liver, and it allows targeting of lesions for liver biopsy.

Ultrasound assessment of an infant with neonatal cholestasis

The gallbladder is best visualized after a 4–6 h fast. The following features are helpful in differentiating between biliary atresia (BA) and neonatal hepatitis [5]:

- *Liver parenchyma*: in BA, the liver size may be either normal or increased, and the echogenicity of the liver parenchyma may be either normal or increased.
- *Gallbladder*:
  - a small (<1.5 cm) or absent gallbladder after fasting suggests biliary atresia in the neonate, but may also be seen in severe intrahepatic cholestasis or (see Chapter 8)
  - a normal-sized gallbladder usually implies neonatal hepatitis; it may also be seen in BA when the atretic common bile duct is distal to the insertion of the cystic duct

  - a change in the size of the gallbladder after a milk feed suggests patency of the common bile ducts.
- *Intrahepatic duct:* dilated intrahepatic bile ducts are not a typical feature of BA in the neonate.
- *Other features*:
  - decreased visualization of the peripheral portal venous vasculature indicates fibrosis, a feature of BA
  - a triangular or tubular (triangular cord) structure may be seen in the porta hepatitis in BA
  - polysplenia may be seen in c. 10% of infants with BA
  - signs of advanced liver disease may be seen in older infants with unoperated BA: ascites, dilated veins at the splenic hilum, and collateral venous channels.

Other causes of neonatal cholestasis include:

- *Choledochal cyst*: most commonly appears as a simple cyst in the region of the common bile duct.
- *Inspissated bile syndrome*: biliary sludge may be seen within the gallbladder as low-level echoes within the lumen.

Other uses of ultrasound: an enlarged gallbladder may represent supportive evidence for primary sclerosing cholangitis.

Color-flow Doppler techniques

This technique allows rapid evaluation of vascular patency without the use of intravenous contrast material. Vessel velocity and flow direction can be determined by assigning color flow properties to the ultrasound image. The transducer detects moving blood and is arbitrarily assigned a red or blue color depending whether the blood is flowing toward or away from the transducer. This mode of imaging is particularly useful in pre- and post-transplant examinations to identify whether the portal vein, hepatic veins and artery, and splenic vessels are patent. Portal hypertension is suggested by the presence of ascites, splenomegaly, and splenic or gastric varices. It is also used in evaluating the vascular supply of hepatic masses and the flow direction and patency of surgically created portosystemic shunts.

Ultrasound may be less sensitive in identifying hepatic outflow obstruction after transplantation. This problem is related to "kinking" of the hepatic vein, which may not be apparent in a prone child who is fasted. A high index of suspicion and a low threshold for hepatic venography are required if clinical symptoms exist [6].

Duplex Doppler ultrasound

Duplex Doppler ultrasound converts an audible signal reflected from a selected region of interest on the ultrasound image into a continuous waveform. The flow rate can be accurately measured, the characteristics of the flow demonstrated, and the flow direction determined. It is useful in the follow-up of patients with transjugular intrahepatic portosystemic shunt (TIPSS), determining the increased resistance of flow in rejecting liver, or hepatic artery stenosis.

Contrast-enhanced ultrasound

Intravenous administration of an ultrasound-enhanced agent amplifies the signal produced by flowing blood. It has been used mainly in detecting subtle flow abnormalities and for distinguishing areas of abnormal flow relative to normal background parenchymal perfusion [7]. Example includes focal nodular hyperplasia. Presently, it is not widely used in children with hepatobiliary conditions [7].

Radioisotope scanning

Soluble radioisotopes such as technetium trimethyl 1-bromoiminodiacetic acid (TEBIDA), which are taken up well by hepatocytes despite elevated bilirubin levels, have been used to demonstrate either hepatic uptake or biliary excretion. Pre-treatment with phenobarbitone (5 mg/kg) or ursodeoxycholic acid (10–15 mg/kg) for 3–5 days prior to the investigation improves hepatic uptake of the isotope. Hepatic uptake is an index of hepatic function and may be patchy in inflammatory conditions, e.g., neonatal hepatitis.

Radioisotope scanning is most useful in the assessment of biliary excretion in the differential diagnosis of neonatal cholestasis. Under normal conditions, biliary excretion is completed within 4 h. Delayed excretion or no excretion after 24 h suggests severe intrahepatic cholestasis or biliary atresia. However, it must be emphasized that the absence of bowel activity is not specific for biliary atresia. Occasionally, a TEBIDA scan may also be used postoperatively after the Kasai procedure and is recognized as an early indicator of a successful operation.

Delayed biliary excretion or pooling in bile ducts is also a feature of cystic fibrosis liver disease (see Chapter 16).

In post-transplant patients, a TEBIDA scan may be of value in identifying the degree of biliary obstruction or a biliary leak.

Radioisotope scanning is of some value in the diagnosis of hepatic vein obstruction (Budd–Chiari syndrome), as poor uptake of the isotope is demonstrated in most of the liver except for the caudate lobe, which has a separate venous drainage. This method is rarely used now as ultrasound or venography is preferred.

Computed tomography

Computed tomography (CT) of the liver is useful for identifying and taking biopsies from hepatic tumors or space-occupying lesions of the liver. Intravenous contrast medium causes enhancement of vascular lesions and of the walls of abscesses and may be helpful in differentiating tumors from other solid masses. An important recent advance is the introduction of helical or spiral CT scanning, in which both the table and the roentgen ray tube move continuously to improve imaging. The use of CT angiography allows non-invasive evaluation of vascular structures. CT scans of the brain are helpful for the detection of cerebral edema in acute liver failure (see

Chapter 9) or for cerebral atrophy in certain metabolic conditions (see Chapters 5 and 19).

Endoscopic retrograde cholangiopancreatography

In this endoscopic technique, a fiberoptic duodenoscope is passed into the first part of the duodenum, the ampulla of Vater is identified, the pancreatic and biliary ducts are cannulated, and radiographic contrast medium is injected. The technique has an 80% success rate in skilled hands and is invaluable for the assessment of extrahepatic biliary disease in older children (e.g., choledochal cysts, primary sclerosing cholangitis) and the assessment of chronic pancreatitis. Although this technique should be of value in the differential diagnosis of neonatal cholestasis, technical difficulties in cannulating the bile ducts in small infants may lead to equivocal information being obtained. The development of a prototype fiberoptic duodenoscope (7.5 mm in diameter) has improved the diagnostic yield in this group of patients. In general, the diagnostic value of this technique has been superseded by that of magnetic resonance imaging (MRI), which is non-invasive, but ERCP retains an important role in therapy [8].

The removal of common bile duct stones, the insertion of biliary and pancreatic stents, and sphincterotomy are useful therapeutic procedures that can be performed at the same time as the diagnostic procedure, but have limited application in children.

Endoscopic ultrasound

Endoscopic ultrasound is an imaging modality that visualizes the lower biliary tree. The technique uses miniprobes (external diameter 2.6 mm) that are small enough to be passed through the operating channel of conventional pediatric duodenoscopes. The technique is well established in adult practice, with a wide range of applications. It is a highly sensitive and specific method of visualizing the lower biliary tree and demonstrating pathology in this area. In many instances, it has taken over the diagnostic role of ERCP in children with pancreaticobiliary pathology. This technique has been evaluated in pediatric practice recently, and has been found to be useful in biliary obstruction, such as biliary stones and biliary fibrosis (Caroli disease) as well as in pancreatic pathologies [9]. It may prove to be of value in the diagnosis of infants with neonatal cholestasis. Endoscopic ultrasound has also proved useful in the diagnosis of submucosal esophageal and gastric varices (Figure 5.4) [10].

Magnetic resonance imaging

Magnetic resonance imaging has now replaced hepatic angiography as the best way to stage or diagnose hepatic tumors and identify their vascular supply. It may provide valuable information about liver or brain consistency and the storage of heavy metals – for example, iron in hemochromatosis, copper in Wilson disease, and cerebral edema in acute liver failure [11].

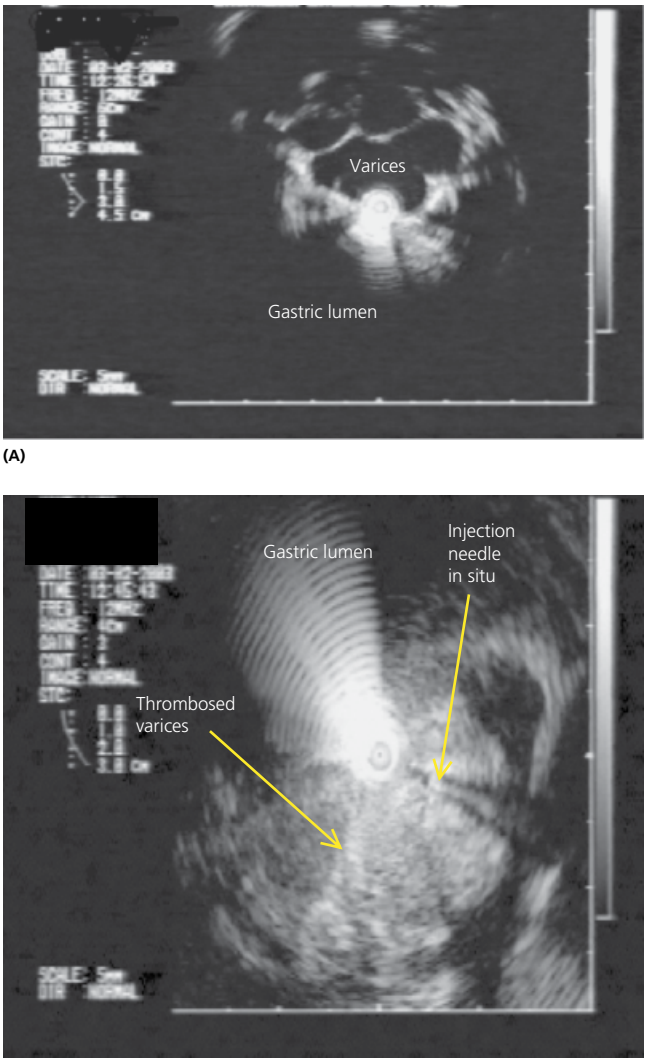

Figure 5.4 Endoscopic ultrasound is a useful way of detecting submucosal varices (A) before and (B) after injection with thrombin.

Magnetic resonance cholangiopancreatography

Magnetic resonance cholangiopancreatography (MRCP) is an extremely useful tool for evaluating a wide variety of disorders of the pancreaticobiliary system in pediatric patients of all ages [3]. MRCP has been shown to have good diagnostic accuracy, in which both intrahepatic and extrahepatic biliary duct abnormalities can be detected. MRCP has largely replaced ERCP and percutaneous transhepatic cholangiography (PTC) as diagnostic tools, although both ERCP and PTC will still be required for therapeutic procedures. The advantages of MRCP include the lack of ionizing radiation, the ability to image the hepatic and pancreatic parenchyma in addition to the pancreaticobiliary tree, and the capability for the generation of two-dimentional

multiplanar reformations and three-dimentional reconstructions [3]. MRCP is particularly useful in the assessment of bile duct injury (post-trauma, anastomotic leak, biloma), biliary and gallbladder stones, choledochal cyst, biliary stricture (in primary sclerosing cholangitis, anastomotic biliary strictures after liver transplantation), and congenital biliary anatomical variants.

Dynamic contrast-enhanced magnetic resonance imaging

This mode of MRI, using predominantly gadolinium excreted via the kidney, is used for the assessment of the vascular structure of hepatic tumors. It has been reported to be useful in distinguishing focal nodular hyperplasia and hepatic adenoma, in improving the detection of hepatic metastases, and better demonstrating the relationship of tumors and the biliary tree.

Non-contrast magnetic resonance angiography

This technique is mainly used in patients with renal insufficiency, while the principles and indications are similar to contrast-based MRI.

Magnetic resonance spectroscopy

Magnetic resonance spectroscopy is an emerging technique that may well be of value in the diagnosis of metabolic disorders, as intermediate metabolites such as lactate can be measured in the brain and other tissues [12].

Interventional imaging techniques

Percutaneous transhepatic cholangiography

This technique is useful for identifying biliary disease if the intrahepatic bile ducts are dilated secondary to obstruction and ERCP is impossible or unsuccessful. A thin needle (Chiba) is passed through the liver. The bile ducts or gallbladder are punctured and radiographic contrast medium is injected. External drainage of the biliary tree, dilation of biliary strictures, and insertion of biliary stents are all possible using this technique, and are useful both before and after transplantation. MRCP has largely replaced PTC as a diagnostic tool for biliary dilation, although both ERCP and PTC will still be required for therapeutic procedures.

Angiography

In many instances, the diagnostic role of hepatic arterial or venous angiography has been superseded by magnetic resonance angiography. However, catheter angiography is primarily undertaken in infants and children with the following disorders:

- Primary hepatic tumors for which complex surgical resection is being considered, e.g., autotransplantation.
- Cavernous transformation of the portal vein (also known as extrahepatic portal vein obstruction) prior to surgical shunting.
- Portal vein thrombosis in patients being considered for liver transplantation.
- If intervention is being considered for vascular stenoses or occlusion of the hepatic artery or vein, portal vein, or inferior vena cava following liver transplantation, or in the Budd–Chiari syndrome.
- Hepatic vascular anomalies such as life-threatening hemangiomas, arterioportal fistulae, arteriovenous malformations, and congenital portosystemic shunts.
- Refractory or life-threatening bleeding from gastrointestinal disease or following blunt abdominal trauma or liver biopsy.
- Invasive diagnostic imaging when angiography is required as part of the procedure, such as transvenous (transjugular) liver biopsy, portal vein sampling, and arterially stimulated hepatic vein sampling.
- Thrombolysis to restore flow to occluded blood vessels by the administration of recombinant tissue-plasminogen activator (tPA) (0.5 mg/kg/h for 6 h, or by direct clot infusion using a low-dose regimen).

Visualization of the celiac access and of the hepatic and splenic blood vessels is achieved by femoral artery catheterization and injection of radiographic contrast. This technique has two parts:

- The arterial phase, which provides information on the celiac axis, hepatic and splenic artery abnormalities, vascularization and anatomy of hepatic tumors, hepatic hemangiomas, or detection of hepatic artery thrombosis.
- The venous phase, which provides information about the patency of the portal, splenic, and superior mesenteric veins and the presence of portal hypertension by the identification of mesenteric, esophageal, or gastric varices.

In skilled hands, the investigation can be performed with little risk in infants. Femoral artery spasm or thrombosis is an occasional side effect, but rarely requires operative treatment.

Hepatic artery embolization is indicated for the treatment of hepatic hemangiomas (see Chapters 14 and 28) or in the control of liver hemorrhage from trauma or needle biopsy. Angioplasty for portal or hepatic vein obstruction is also feasible using angiographic techniques.

Selective angiography of the portal venous system

A wide variety of imaging methods is available for the assessment of portal hypertension, although most of the techniques, owing to their invasive nature, have not been properly studied in children. Selective angiography of the celiac axis, superior mesenteric artery, and splenic vein can be very useful in assessing extrahepatic vascular anatomy in children with suspected portal vein thrombosis. Splenic vein angiography, in which the splenic and portal veins are visualized by the injection of radiological contrast into the spleen, has largely been replaced by hepatic angiography. It may be useful for measuring splenic pulse pressures in the

evaluation of portal hypertension or if there is a post-transplant portal vein stenosis, but it carries a small risk of splenic rupture.

Estimation of portal venous pressure in portal hypertension

In patients suspecting of having portal hypertension, the wedged hepatic venous pressure (WHVP) and intrahepatic venous pressure have both been used as an estimate of the portal venous pressure. Intrahepatic venous pressure can be measured by means of using a Chiba needle inserted percutaneously under fluoscopic guidance into the liver parenchyma. The needle is filled with saline and connected to a recorder for measurement of interstitial pressure. This method has been advocated by some to be a simple and safe index of portal venous pressure. Some authors found there is a good correlation between pressure readings obtained via this method and the actual measurement of intravariceal venous pressure. WHVP can be determined by wedging a catheter in a hepatic vein, occluding it, and then measuring the pressure of proximal static blood. WHVP is not the actual hepatic portal vein pressure but reflects the hepatic sinusoidal pressure. WHVP slightly underestimates the portal pressure due to sinusoidal equilibration in patients without cirrhosis.

The hepatic venous pressure gradient (HVPG) is the pressure gradient between the WHVP and the free hepatic venous pressures. It is an estimate of the pressure gradient between the portal vein and the inferior vena cava. An HVPG of ≥10 mmHg defines clinically significant portal hypertension.

Transjugular intrahepatic portosystemic shunt

Life-threatening esophageal and gastric variceal bleeding occurs with portal hypertension secondary to liver disease. If the bleeding persists despite maximal medical and/or endoscopic therapy, a TIPSS procedure can be carried out. TIPSS is an intrahepatic portosystemic shunt, created with radiographic assistance, between the high-pressure portal vein and the lower pressure hepatic vein. Since 1982, technological advances have made this technique possible in pediatric practice. Hepatic encephalopathy is a common side effect after insertion of a TIPSS, particularly in adult patients, and especially in those who have previous hepatic encephalopathy or those with a more severe degree of disease.

Central venous access and related venous occlusive disease

Intensive treatment regimens such as parenteral nutrition require long-term central venous access, particularly in patients undergoing small bowel or combined liver and small bowel transplantation. Interventional radiological techniques are useful in establishing percutaneous placement of tunneled central venous lines or Hickman lines, maintaining central venous access, and managing the complications, such as thrombosis, in children of all ages.

Aspiration and drainage of fluid collections and abscesses

Imaging-guided (usually with ultrasound or occasionally CT) aspiration and drainage of fluid collections and abscesses in the peritoneal cavity, liver, spleen, pancreas, and retroperitoneum can be performed using percutaneous fine needle aspiration in all age groups. Complicated fluid collections such as abscesses and pancreatic pseudocysts may require drainage over a period of time.

Non-invasive assessment of hepatic fibrosis

Hepatic fibrosis occurs in response to chronic liver injury. Originally thought to be irreversible, hepatic fibrosis is now recognized as a dynamic process with the potential of significant resolution. Conventional biochemical and serological tests, when examined alone, are of little value for the assessment of fibrosis. Liver biopsy for histopathological assessment is considered as the gold standard for staging hepatic fibrosis. However, liver biopsy has many limitations, which include its invasive nature, potential complications, and the fact that it can only sample a small portion of the liver and is susceptible to sampling variation and inter- and intra-observer variability. Non-invasive tests of hepatic fibrosis attempt to predict the degree and stage of hepatic fibrosis that would be seen histologically. Currently there are two general categories of non-invasive tests for fibrosis: serological panels of tests and radiological tests.

Serological tests of assessing hepatic fibrosis

Serological tests, although more widely available, cannot supplant direct histological analysis. A variety of serological markers, which can be divided into direct and indirect, have been evaluated to predict the degree of fibrosis in the liver. In children, the most widely studied is the aspartate aminotransferase to platelet ratio index (APRI) [13]. The APRI is based on the AST level and platelet count: AST elevation (which is the AST level divided by the upper limit of normal (ULN) for the lab) and the platelet count per μL divided by 1000.

$$\text{APRI} = (\text{AST elevation/platelet count}) \times 100$$

Example: a child with an AST of 90 IU/L in a lab with an ULN of 45 IU/L, and a platelet count of 120,000/μL would have an APRI of:

$$\text{APRI} = (2/120) \times 100 = 1.67$$

The APRI has primarily been studied in patients with hepatitis C virus (HCV), human immunodeficiency virus (HIV), and HCV coinfection, and alcoholic liver disease in the adult population. In children, it has been evaluated in conditions such as biliary atresia, liver fibrosis, and cirrhosis in intestinal failure and non-alcoholic steatohepatitis. However, the results have been inconsistent and further evaluation is necessary [13].

Radiological assessment of hepatic fibrosis

Imaging methods for staging hepatic fibrosis include ultrasound-based transient elastography, magnetic resonance elastography, and acoustic radiation force impulse [14]. Radiological assessment of hepatic fibrosis has several advantages over conventional liver biopsy as the latter is prone to sampling error, is more costly, and places the patient at risk of procedure-related complications. Imaging methods provide a more global assessment of the hepatic parenchyma and are less invasive.

- *Ultrasound-based elastography*. One of the most commonly used ultrasound-based technique for assessing hepatic fibrosis is transient elastography or FibroScan [15]. It is a non-invasive method for the assessment of hepatic fibrosis. This device is based on one-dimensional transient elastography, a technique that uses both ultrasound (5 MHz) and low-frequency (50 Hz) elastic waves, whose propagation velocity is directly related to the elasticity. Liver elasticity measurements are reproducible and operator independent and correlate well with the degree of fibrosis on histology. The method has been evaluated in adults with hepatitis C [15], although not yet in children, but it has obvious advantages for the long-term follow-up of chronic disease.
- *Magnetic resonance elastrography*. In this method, a probe is placed against the patient's back. The probe emits low-frequency vibrations that pass through the liver and can be measured by the MRI spin echo sequence [16]. It is based on the principle that infiltrative processes such as hepatic fibrosis and tumors alter the mechanical properties of soft tissues and result in increased firmness. With vibrational stress, transmission of energy in the form of a shear wave will penetrate deeper into tissues with fibrosis, while softer tissues will dissipate that energy. A small pilot study was recently published on 35 children and adolescents with chronic liver disease (aged 4–20 years; 27 subjects had NAFLD) and has been found to be accurate and feasible in detecting significant hepatic fibrosis, including in severely obese children [16].

Liver biopsy and histopathology

Traditionally, the diagnosis of most liver diseases requires histological confirmation, and liver biopsies are thus a routine procedure in many specialist centers. However, in the era of genomic medicine, the availability of non-invasive methods such as mutational analysis, advanced imaging methods, and sophisticated biochemical assays have begun to compete with conventional investigational methods such as liver biopsy. Nevertheless liver biopsy remains useful in many conditions of the liver.

Routes of biopsy

An aspiration technique, using a Menghini needle (or disposable variant), has a complication risk of one in 1000 liver biopsies and may be performed under sedation with local anesthesia. In fibrotic or cirrhotic livers, a Tru-Cut needle, which removes a larger core, may be necessary [17]. Transjugular liver biopsies, in which the liver is biopsied through a special catheter passed from the internal jugular vein into the hepatic veins, is now possible for children weighing as little as 6 kg, and is the only safe way to perform a biopsy if coagulation times remain abnormal despite support (prothrombin time >5 s prolonged over control value) [18]. The complications of this potentially dangerous procedure are considerably reduced if it is performed by experts in specialized units under controlled conditions (Table 5.8) [19].

Table 5.8 Liver biopsy protocol.

- Obtain informed consent
- Investigations: FBC blood group and save serum for cross-match
- Antiplatelet therapy (except low-dose aspirin after liver transplant) should be discontinued 7–10 days before biopsy
- Patient with hypoglycemia: intravenous infusion of glucose
- Coagulation: prothrombin (PT; normal 12–15 s), partial thromboplastin (PTT; normal 33–37 s)
- Broad-spectrum antibiotics may be required if there are cardiac problems or concurrent dental extraction
- Fasting for 4 h

Coagulation support	
PT <4 s prolonged	No action
PT: >4–6 s prolonged (INR >1.5)	15 mL/kg FFP over 2.5 h
PT: >6 s prolonged	Correct with FFP; recheck PT/PTT at 1 h Reassess risk–benefit if no correction
FBC and platelets	
Platelet count: >80 × 10^9/L	No action
Platelet count: 40–80 × 10^9/L	10 mL/kg platelet infusion over 1 h
Platelet count: <40 × 10^9/L	10 mL/kg platelet infusion, recheck Reassess risk–benefit if correction >80 is impossible
Hemoglobin: <8.0 g/L	Transfuse to Hb >10 g/L pre-biopsy
*Procedure and sedation**	
Establish size of liver: ± mark site after ultrasound	
Apply local anesthetic cream to area of maximal dullness between 7th and 9th intercostal spaces (or ultrasound site)	
Oral midazolam	2.5 mg <1 year old 5.0 mg >1 year old
or	
Chloral hydrate 75 mg/kg	Lidocaine 1–2%
Local anesthetic	
Intravenous sedation	Pethidine 1–2 mg/kg Midazolam 0.1–0.75 mg/kg
Post-biopsy observations	
Blood pressure, pulse, respiration, and temperature:	
15 min for 2 h	
30 min for 2 h	
Hourly for 2 h	
4-hourly as required	
Chest radiograph/abdominal ultrasound may be required if bleeding is suspected. Laparotomy may be required	

NB most units now carry out liver biopsies in children under a general anesthetic.
FBC, full blood count; FFP, fresh frozen plasma; INR, international normalized ratio.

Preparation for biopsy

It is essential to have information about liver size and consistency and the presence of cysts or dilated bile ducts from ultrasound, and if necessary to have a "spot" marked on the abdomen to ensure an accurate biopsy. Correct information about coagulation parameters is vital. Prothrombin time should be within 3 s of control values; the platelet count should be $>80 \times 10^9$/L. The patient's blood group should be known, and it is prudent to cross-match a unit of blood prior to the procedure. Biopsy specimens should be obtained for routine histopathology, microbiology, electron microscopy, immunohistochemistry, and copper (if appropriate), and snap-frozen in liquid nitrogen for enzymatic or metabolic investigations. The interpretation of the histology may be difficult and requires considerable specialist expertise.

It is possible to carry out a liver biopsy as a day-case procedure in low-risk patients if the following criteria apply:

- Patient >1 year old.
- Bilirubin <200 µmol/L.
- No other organ dysfunction.
- No need for coagulation or platelet support.
- Access to emergency facilities for 24 h after the procedure.

Liver biopsy is contraindicated in the following circumstances:

- Abnormal coagulation parameters or thrombocytopenia (Table 5.8).
- Presence of grossly dilated bile ducts or large cysts.
- Angiomatous malformation of the liver.
- Extensive ascites.

In these circumstances, image-guided or transjugular liver biopsies should be performed if the diagnosis will change the management.

Complications of percutaneous liver biopsy

There are a number of complications of percutaneous liver biopsy (Table 5.9 and Box 5.1) but the main one is bleeding, and most deaths following a liver biopsy are due to intractable bleeding. However, subclinical bleeding (as evident on ultrasound imaging) is common, and intrahepatic and subcapsular hematomas with no hemodynamic compromise are seen in up to 23% of patients. Significant non-fatal bleeding (as seen with evidence of active bleeding, shock, and a hemoglobin drop of 2.0 g/L) occurs more frequently in children than adults. In adults, significant hemorrhage occurs in 0.3–0.5% of cases, whilst bleeding requiring transfusion is seen in up to 2.8% of children. Evidence of persistent bleeding following liver biopsy, despite medical support and blood transfusion, warrants urgent hepatic angiography and embolization or surgery. The following are of particular importance:

- Pneumothorax or hemopneumothorax, which are treated in a standard way.
- Infection (particularly if the biopsy is combined with another procedure, e.g., dental extraction).
- Perforation of the gallbladder or bile ducts leading to biliary peritonitis and the need for emergency surgery.

Table 5.9 Incidence of minor and major complications after liver biopsy. (Adapted from Dezsofi *et al*. [19].)

Complications (minor and major)	Incidence (adults and child)
Pain	84% in adults
Bleeding	0–18% adults; 2.8% children
Arteriovenous fistula	No data
Pneumothorax/hemothorax	0.2%
Organ perforation	0.07–1.25%
Biliary leak/hemobilia	0.6% children
Infection	12.5% in choledochojejunostomy
Death	0–0.4% in adults, 0.6% in children

Box 5.1 Risk factors for complications. (Adapted from Dezsofi *et al*. [19].)

- Risk factors for minor or major complications
- Low-molecular-weight heparin use
- Focal lesion
- Acute liver failure
- Infants aged <3 months
- Massive ascites
- Thrombocytopenia
- Previous malignancy or bone marrow transplantation
- Chronic renal failure
- Biliary tract dilation

Adequate monitoring of vital signs after biopsy is essential in order to detect complications such as hemorrhage or infection (Table 5.9) [19].

Metabolic investigations

Many inborn errors of metabolism present with hepatomegaly and/or liver disease. It is essential to screen for these diseases as part of the investigation of liver disease in neonates (see Table 5.3) and in older children (see Chapters 4, 8, 9, and 19).

Bone marrow aspiration

Bone marrow aspiration should be performed in infants with undiagnosed neonatal hepatitis with both hepatomegaly and splenomegaly, in order to exclude Niemann–Pick type C or familial hemophagocytosis (see Chapters 4 and 5), or at any age if a storage disorder is suspected.

Skin biopsy

This procedure should be performed if an inborn error of metabolism is being considered (e.g., Niemann–Pick type A, B, or C, or tyrosinemia type I) and the specimen should be stored frozen for future culture. It may also be necessary to obtain skin biopsies from parents or siblings.

Endoscopy

Upper gastrointestinal endoscopy (gastroscopy) using a flexible fiberoptic endoscope is now the best way to diagnose peptic ulcer disease or esophageal and gastric varices secondary to

portal hypertension. The technique is normally performed with the patient under sedation or general anesthetic. In children with hematemesis, gastroscopy not only provides rapid diagnosis, but also makes it possible to carry out therapy with variceal banding or endoscopic sclerotherapy for bleeding varices, or injection of bleeding ulcers with epinephrine or thrombin.

Capsule endoscopy

Wireless capsule endoscopy, which is a non-invasive tool for the investigation of the small intestine, has been used widely in children. In this technique, a wireless capsule is swallowed and images of the small intestine are obtained. It is of most value in the diagnosis of Crohn disease, obscure or occult gastrointestinal bleeding, polyposis syndromes, and protein-losing enteropathy due to lymphangiectasia. In liver disease, it may be useful for diagnosing small intestine varices.

Neurophysiology

Electroencephalography is mostly used in the assessment of hepatic encephalopathy. It will identify abnormal rhythms secondary to encephalopathy due to either acute or chronic liver failure or drug toxicity, such as post-transplant immunosuppression. It may also be of value for assessing brain death, as a flat electroencephalogram in the absence of sedation is an indication for the withdrawal of therapy. CT or MRI scans of the brain may identify cerebral edema, infarction, or hemorrhage.

Ophthalmology

A number of inherited conditions have associated visual lesions – for example, Kayser–Fleischer rings in Wilson disease, posterior embryotoxon or optic drusen in Alagille syndrome, and cherry-red spot in Niemann–Pick type A (Table 5.10). Ophthalmological examination may thus provide valuable diagnostic information and should be part of the assessment process. Children with Alagille syndrome have a higher than normal incidence of benign intracranial hypertension, and annual fundoscopy for papilledema is therefore essential [20].

Cardiology

A number of liver diseases have associated cardiovascular manifestations. These include Alagille syndrome, congenital rubella syndrome, and tyrosinemia (Table 5.11) [21]. It is therefore essential to include cardiovascular assessment.

Genetic tests (chromosome and DNA)

With the rapid development of molecular techniques for the diagnosis and detection of genetic diseases, samples for DNA analysis and/or chromosomes from both child and parent are essential.

The development of molecular biology has revolutionized the methodology for many complex diagnostic procedures, transforming many techniques into routine laboratory procedures, particularly in screening for rare neonatal diseases [22]. Progress in identifying specific genes and DNA sequencing has made it possible to diagnose many inborn errors of metabolism and inherited disease (e.g., Alagille syndrome, Wilson disease, tyrosinemia type I) and has led to the identification of mitochondrial disorders.

Advances in methodology for gene cloning and molecular cloning have been helpful in identifying viruses such as hepatitis C and G, while the polymerase chain reaction has been used to diagnose active infection and monitor patients with many different viral diseases, such as hepatitis C, cytomegalovirus, and Epstein–Barr virus. Diagnosis for autoimmune disorders has improved, with specific assays that use recombinant protein antigens (e.g., antinuclear antigens and liver/kidney microsomal antibodies). The rapid development of molecular techniques is certain to lead to further improvements in diagnostic methods and to a better understanding of pediatric liver disease.

Table 5.10 Ophthalmic lesions in liver disease.

Disease	Lesions	Comments
Galactosemia	Cataracts	
Congenital infections	Chorioretinal scar	Congenital toxoplasmosis, CMV, HSV, VZ infections
	Active chorioretinitis	Congenital toxoplasmosis, CMV, HSV, VZ infections
	Cataracts	Less specific than chorioretinitis and congenital rubella
Wilson disease	Kayser–Fleischer ring	Not specific for Wilson disease
Alagille syndrome	Posterior embryotoxon	Not specific for Alagille syndrome
	Optic disc drusen	Eye findings in Alagille syndrome usually not associated with functional significance
	Diffuse fundus hypopigmentation	
Niemann–Pick type A	Cherry-red spot	
Tay–Sachs disease	Cherry-red spot	

CMV, cytomegalovirus; HSV, herpes simplex virus; VZ, varicella zoster.

Table 5.11 Cardiovascular involvement in liver disease.

Conditions	Cardiac involvement
Congenital rubella syndrome	Present in 50% of infants infected in first 2 months of gestation Common: patent ductus arteriosus, pulmonary artery stenosis
Alagille syndrome	>90% have cardiac involvement Most common: peripheral pulmonary stenosis Others: tetralogy of Fallot, pulmonary valve stenosis, atrial septal defect, ventricular septal defect
Tyrosinemia	Myocardial hypertrophy, usually detected by echocardiography Low incidence of clinical heart disease
Wilson disease	Mainly electrocardiographic abnormalities in early adulthood; left ventricular hypertrophy, ST-wave depression, T-wave inversion
Chronic portal hypertension	Pulmonary artery hypertension, right ventricular hypertrophy

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SECTION 3

Supporting the Child and Family

CHAPTER 6

Multidisciplinary Approach to Liver Disease

6.1 The Role of the Multidisciplinary Team

Lindsay Hogg

6.2 The Role of the Dietitian

Sara Clarke and Kelly Guthrie

6.3 The Role of the Psychologist

Jacqueline Blyth

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CHAPTER 6.1

The Role of the Multidisciplinary Team

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Key points

- Multidisciplinary team working is the ideal way to assess and meet the needs of children with liver disease and their families within the hospital.
- Relevant information and literature should be produced for the parents and children as appropriate.
- Children should be prepared and counseled using skilled play therapists and psychologists.
- Attention should be paid to effective holistic rehabilitation and discharge planning with shared care for local centers.

Living with liver disease

Most parents expect their children to be well and to survive them and so chronic diseases that threaten children's autonomy and compromise their life expectancy challenge emotions and coping resources to the limit [1].

Most liver disease is life-limiting. Medical therapy may control symptoms and long-term complications and thus optimize the quality of life. However, for many children with liver disease, there is no absolute cure, and ongoing monitoring with regular hospital visits for blood tests or admission is required. Thus, from the time of diagnosis, both child and family have to adapt to a life that is different from their expectations. The child (that is, the infant to adolescent) with a chronic illness is automatically different from children of a similar age, while parents have an additional workload in caring for their child. "Normalizing" processes within this context should be encouraged, using the knowledge, skills, and active support of the different health professionals within the multidisciplinary team [2].

The child (depending on age) and family pass through numerous processes. Firstly, both parents and child need to come to terms with the shock of the diagnosis, as well as (for the parents) the grief of losing their child's good health. Parents have to take in much information relating to the diagnosis and prognosis, as well as learning new skills in caring for their child. Ideally, information should be shared with the parents and child at their own pace and in the context of their family and social setting.

Parents may find it difficult to accept the unpredictability of their child's disease and the effect on immediate life plans. If the child's condition deteriorates, with a reduction in physical mobility and increasing symptoms such as ascites and malnutrition, the intensity of the care required increases exponentially. As a result, the extra time and attention given to the child with liver disease may impact adversely on siblings and other members of the family [1].

As the child's disease progresses, parents need to detect subtle changes in their child's condition in order to seek appropriate help. As liver disease is relatively uncommon in the child population, many parents, children, and young people become "experts" in disease management and often know more about specific aspects of their disease than their local health-care team. This may be an added concern to the family, and it is vital that efficient communication systems are established with local services. Ideally, the child and family should experience a "seamless web" of care, treatment, and support centered on the needs of the child and his or her siblings and carers. Multidisciplinary working is an essential feature of this "seamless" care [3].

Effective team working

The multidisciplinary team will work most effectively if they follow a shared vision for the service. The National Service Framework for Children, Young People and Maternity Services in England advocates child-centered hospital services. Child-centered services are services that:

- Consider the "whole child," not simply the illness being treated.
- Treat children as children, and young people as young people.

- Are concerned with the overall experience for the child and family.
- Treat children, young people, and parents as partners in care.
- Integrate and coordinate services around the child and family's particular needs.
- Gradate smoothly into adult services at the right time.
- Work in partnership with children, young people, and parents to plan and shape services and to develop the workforce.

It works best if the skills and judgment of all team members are valued. Communication channels should be constantly appraised to ensure they are effective, so that shared decisions can be made. Staff support should be provided so that training issues can be addressed and debriefing can occur, as the nature of the work is stressful.

Consent to treatment

The United Nations Convention on the Rights of the Child states in Article 12 that governments should "assure to the child who is capable of forming his or her own views, the right to express those views freely, in all matters affecting the child, the views of the child being given weight in accordance with the age and maturity of the child."

For consent to be valid, it is expected that an individual is fully informed as to the alternatives, expected benefits, and the possible complications for a procedure or a research project and able to understand the implications of the decision. Consent may be given by a young person (in British law, this is usually 16 years of age) or by another on behalf of the child, such as parent or guardian. Emphasis should be placed on whether a child is competent to give consent. A child or young person who has the capacity to fully understand a decision affecting his or her life automatically has the capacity to make that decision, unless statute law states otherwise. This is the Gillick competence test after the Gillick case in 1985. This case set a precedent because it allowed under 16 year olds to consent to medical treatment provided they could show sufficient understanding and competence to make wise choices.

Although it may be difficult to define when a child or young person is competent, this can be overcome by establishing a framework to provide a clear process of information sharing and opportunities for shared decision-making, involving all members of the multidisciplinary team with the child and their parents [4]. The objectives of the framework are:

- For the team: to gain information about the child's knowledge, understanding, and experience of illness and health care in the context of the child's life.
- For the children and young people: to provide them with an opportunity to increase their knowledge and understanding of their disease and treatments and to express their feelings, fears, and expectations.
- To help them develop confidence in participating in decisions by providing opportunities for them to make choices in their care and treatment.
- For the parents: to help them to gain knowledge and understanding of their child's disease and treatment in order to make informed decisions with their child and as individuals.
- To enable parents to impart information to their child and siblings.
- To facilitate sharing information between the child and parent in order to help them make decisions together.

Members of the multidisciplinary team

Multidisciplinary team working is the ideal way to assess and meet the needs of children with liver disease and their families within the hospital. The composition of these teams will vary slightly from setting to setting within one country, and could vary greatly from one country to another. Individuals involved in a child's care should meet regularly to share information and to formulate a treatment plan that best meets a child's needs with the resources that are available to them.

The multidisciplinary team usually consists of the following members:

- Medical and surgical teams, led respectively by a consultant pediatric hepatologist and pediatric hepatobiliary and transplant surgeon.
- Ward nursing team, led by a senior nurse.
- Specialist nurse.
- Dietitian.
- Physiotherapist.
- Psychologist.
- Play specialist.
- Youth worker.
- Pharmacist.
- Family support worker.
- Schoolteacher.
- Chaplaincy.
- Secretarial and administrative support.

Similar teams will be found in the referring team, who will enter into a shared care agreement for the ongoing care of the child. All of these hospital-based teams will depend on the local primary health team for implementing parts of the treatment plan, such as prescribing medication, administering vaccinations, and support during normal childhood illness. This team will include:

- A general practitioner, or primary pediatrician.
- Health visitor.

- Practice nurse.
- Children's community nursing team.
- Community physiotherapist.
- Social services.
- Pharmacy.
- Other services, such as home tuition available through the education department.

Effective communication is the key to ensuring that children receive optimum care as they move between hospitals and the community.

Roles within the multidisciplinary team

Medical and surgical team

The medical and surgical teams should have sufficient consultant or specialist grades to provide 24-h cover for medical and surgical care for the child, support, advice, training, and teaching for the other medical staff involved in the care of the child. The consultants will provide the lead in developing the overall treatment plan for the child. Increasingly, this will involve asking advice from the hospital's wider team, such as:

- Microbiologist.
- Virologist.
- Radiologist.
- Gastroenterologist.
- Cardiologist.
- Respiratory physician.
- Neurologist.
- Histopathologist.
- Clinical scientists.

Communication between the team is essential. We have found the following structure helpful:

- Daily consultant-led ward rounds at which the needs of the children currently in hospital are discussed and new plans for investigation, treatment, and monitoring are made.
- Twice-weekly ward round with the multidisciplinary team, at which a business-like structure will ensure that the treatment plans for discharged children are fully known and a plan of follow-up care implemented. It also allows for planning for future admissions to the hospital and for information to be shared about children with problems in other hospitals or in the home and a plan made.
- Microbiologist and gastroenterologist attend one ward round a week.
- Weekly radiology and histopathology conferences.
- A weekly meeting to discuss specific issues for children being considered for transplantation and the management of children currently on the waiting list.
- Consultant outpatient reviews, for medical staff to provide feedback about how children seen as outpatients are progressing. Preparation can be made for the following week's clinics.
- Meetings for particularly challenging problems that need a face-to-face discussion between all relevant specialists.

Ward nursing team

This team needs to develop its own routines, which ensure that evidenced-based care is given to children. The senior nurses on the ward will take on development roles to ensure that nursing practice is continually changing to meet the needs of the children, such as:

- A teaching sister who coordinates the training needs of the nursing team in relation to new:
  - equipment
  - policy and procedure
  - ways of working.
- A student coordinator who coordinates the needs of nurses in various stages of training.
- A stoma nurse, who provides specific advice to children and develops the policies and provides training.
- An intravenous therapy nurse, who implements hospital policy and provides training.
- Other nurses may become link nurses with other groups in the hospital so that practice is kept up to date with:
  - tissue viability
  - moving and handling of the patient
  - nutritional care
  - safeguarding children
  - pain assessment and management
  - infection control.

It is important that nurses are given time to carry out these roles that improve care for children. Increasingly due to changes in medical training and careers, nurses are taking on duties that were traditionally seen as being the role of the doctor. The role of the advanced nurse has been developed to take on a job description that includes:

- Clinical assessment.
- Prescribing of medication.
- Venipuncture.
- Ordering of diagnostic investigations and monitoring tests.

Specialist nurse

Specialist nurses have a wider role than solely being on the ward. This allows them to build up a caseload of children whom they can support while in hospital, but also in the outpatient clinic and the home. This gives them the opportunity to act as a key worker for children and families. Their roles could include the following.

Provision of information

The need of each child and family is assessed and a plan of implementation is agreed in the multidisciplinary team. Information for the child is achieved by involving the schoolteacher and the

play specialist. The teacher involves the child with activities about the body in line with the national educational curriculum. This involves educational computer network-based activities for the older child. For the younger child, the play specialist uses a range of play-based activities, including anatomical puppets, books, and board games. The specialist nurse will indicate the specific disease and possible treatment options relevant to each child. The parents will have their own program of education on:

- The hospital and the ward.
- Facilities, including accommodation and provision of interpreters.
- Team members and roles.
- Reasons for investigations and what they involve.
- Basic understanding of the liver and its functions.
- Understanding the signs and symptoms of liver disease.
- When they can expect results.
- Treatments of liver disease.
- Looking after the child in the home.
- How to stay in contact.

Many units now have literature for explaining about procedures and the use of a questioning style will give a friendlier feel to the leaflet:

- Introduction – why are we writing this leaflet?
- Overview of the procedure – what does it involve, where will it take place, how will it help me?
- What are the benefits?
- What are the risks?
- Are there any alternatives?
- What happens afterwards?
- What care will my child need at home?
- Where can I get further information?
- Frequently asked questions.
- Space to write down questions.

The series should be reproduced for the younger and older child (Figure 6.1).

Useful leaflets include:

- Liver biopsy (percutaneous and transjugular procedures).
- Endoscopy and endoscopic ultrasound.
- Endoscopic variceal banding.
- Abdominal ultrasound.
- Endoscopic retrograde cholangiopancreatography (ERCP).
- Percutaneous transhepatic cholangiogram (PTC).
- Angiography.
- Transjugular intrahepatic portosystemic shunt (TIPS).
- Magnetic resonance imaging (MRI).
- Computed tomography (CT).

Each center should build up a set that reflects the investigations and treatments that are offered locally.

Disease and general support literature is well developed in the UK through the Children's Liver Disease Foundation (CLDF) many of which are available online (www. childliverdisease.org last checked June 2016). Guidelines should be used in producing disease specific literature:

- Introduction – why are we writing this leaflet?
- What is the condition? What is it called and what are its characteristics?
- How is it diagnosed?
- What causes it?
- How common is it and whom does it affect?
- What treatments are available?
- What is the outlook?
- Is there a support group?
- Where can you get further information?

Figure 6.1 The "Sam Series" is an example of children's information leaflets used to prepare them for procedures. The leaflets can be read to the child by a parent or nurse, or used as a basis for explanation.

Skills training and discharge planning
Discharge planning is an essential part of managing the child and family which is achieved with the ward-based team and members of the multidisciplinary team. A plan is agreed with the child and family to teach the skills that will be needed in the home for the family to look after the child safely. This will include the safe administration of medicines, enteral feeding products by nasogastric or gastrostomy routes, stoma care, management of fluid balance, and problem-solving skills. Discharge planning should begin early at the point of admission. With good planning and communication with the shared and primary health-care team, discharge can be facilitated at an early point.

Providing continuing care
The specialist nurse is best placed to provide this. In Birmingham, we started a nurse-led telephone consultation service in 2001, called Liver Direct, which proved very successful in managing the numerous calls that would come to the Liver Unit every day. Nurses are able to answer 90% of the calls. The availability of voicemail and email have extended the system. The service is used by shared care and primary health teams, who appreciate being able to discuss patients with individuals who are knowledgeable about their present condition and understand how services are run and managed.

Coordination of immunosuppression
Specialist nurses are able to provide the skills and knowledge to provide immunosuppression monitoring in an efficient and cost effective way are a key contact for parents and local teams for immunosuppression queries.

Coordination of vaccination
The management of vaccinations is a key part of keeping children with liver disease healthy. General practitioners and practice nurses are often hesitant about vaccinating children with liver disease. It is helpful if the specialist nurse produces a vaccination plan indicating which type of vaccine and the correct timing for children with liver disease, for those being prepared for transplantation, and for those after transplant. The letter should be copied to parents, who can ensure the plan is implemented.

Participation in outpatient and outreach clinics
The specialist nurse plays a key role in the management of new patients, helping the child and family with information and putting them at their ease. They are ideally placed to develop these clinics to meet the needs of special groups within a multidisciplinary framework which includes a consultant, specialist nurse, dietitian, psychologist, and physiotherapist. Specialized clinics might include an adolescent clinic, a metabolic clinic, a viral hepatitis clinic, or a transition clinic. Increasingly, children with stable liver disease and after transplantation are managed in outreach clinics in other cities. Children are seen by a consultant hepatologist, specialist nurse, and dietitian along with members of the local shared care team. The service is well received by families, as it reduces the travel burden. It is appreciated by the shared care teams, who have the benefit of discussion over specific and general management issues.

Play specialist

Play specialists work with children of all ages [5]. Their work involves:

- Using play to enable children to have a positive image of hospital.
- Preparing them to cope with admission, treatment, surgery, or other procedures.
- Enabling them to manage fears and anxieties.
- Offering coping strategies.
- Using specific play techniques to minimize stressful events by acting them out in advance or post-procedurally.
- Assessing the child's level of cognitive understanding and development.
- Education on condition and treatment.
- Basic pain management.

In our unit, the play specialists have a crucial role in:

- Preparing children for procedures and investigations.
- Helping children cope with invasive and non-invasive procedures.
- Preparing children for transplantation, enabling them to make informed decisions regarding their treatment and admission, and to support the consent process (Figure 6.2).
- Normalizing the day for children who are hospitalized for long periods.
- Using therapeutic play to help children express their fears and anxieties.
- Issues in relation to chronic illness.
- Supporting children post procedurally, emotionally and psychologically.

They work very closely with the specialist nurse who highlights any particular disease issue: with the hospital schoolteacher who may be able to use aspects of the National Curriculum to teach the child normal body anatomy and physiology; and with the psychologist who highlights particular aspects of psychological support that may be needed. They will also work with siblings, so that the whole family is informed about the treatment plan.

They will use a variety of different tools that will vary with each child. These will include:

- Various assessment tools that allow children to express what they already know and what they want us to know.
- Hospital play to assess past experiences and anxieties.
- Preparation books.
- Real medical equipment (Figure 6.3).

Figure 6.2 *Anita's New Liver* is a storybook for older children to prepare them for transplantation. It can be used in conjunction with the *New Liver Colouring Book*.

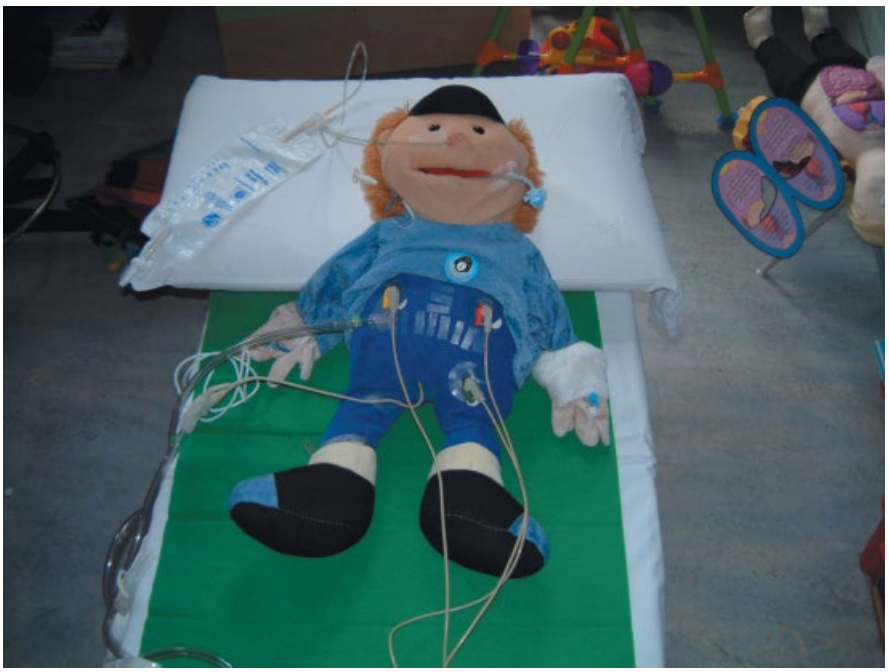

Figure 6.3 Children can be prepared for their postoperative experience using different play techniques. These puppets are very popular and allow the child to prepare for intensive care after surgery.

- Body work.
- Anatomical dolls, puppets, books, and body programs.
- Handling of medical equipment, visits to other wards.
- Developing the child's wish list about treatment.
- Preparing children for lifestyle changes (Figure 6.4).
- Offering coping strategies.

They will use the different skills of the adolescent play specialist and hospital youth worker when needed.

Figure 6.4 Life after transplantation may be different. It is helpful to encourage children to be independent and enjoy life using leaflets such as this one "Stay Well."

Family support team

Family support teams include a senior social worker and a family support worker. Issues of child safeguarding can thus be addressed promptly and with appropriate plans developed to keep children safe. The family support team's skills are in assessing the impact of the child's condition on social functioning, parenting capacity, assessing attachments, and facilitating help when needed. This may relate to help with claiming local and central government financial help, making applications to grant-awarding bodies, and liaising with local social services about ongoing practical support that may be available. Much of the time, our family support worker provide a listening ear and facilitate a weekly parent support group within the ward.

Physiotherapist

The physiotherapist has a vital role in assessing the effects of liver disease and various treatments on movement. This is especially important in pediatrics, when developmental delay can occur quite readily. Once baseline assessments have been made, the physiotherapist will institute a treatment plan with the child and family, communicating this to the multidisciplinary team so that the plan can be supported. The role encompasses working on the ward, in the pediatric intensive care unit, in outpatients, and facilitating continuing support either in other hospitals or in the community. The role increasingly involves health promotion and preventative health care, as obese children are seen in our clinics and ward with fatty liver disease. The long wait on the transplant list for many children means that respiratory and physical rehabilitation in children is longer and more challenging.

Pharmacist

Drug therapy in liver disease and after transplantation has become increasingly complex. The challenge in pediatrics is to have a preparation that can be taken by children and that ultimately is available in the local community. The ward-based pharmacy team is now a valuable part of the team. Ward-based pharmacy technicians are an innovation to improve patient safety while ensuring that child-friendly medication is available and that families have their take-home medication available before discharge. This enables training to be given in the complex medication regimens that are often needed in treating liver disease and after transplantation. The specialist nurse team facilitates home delivery of certain medicines if general practitioners are not willing to prescribe – unlicensed medication, or when a medicine does not have a liquid formulation that a local pharmacist can access.

Chaplain

Hospital chaplaincy has developed in recent years to reflect a multi-faith society. The chaplaincy provides spiritual support for patients, staff, and relatives through chaplains and faith community representatives. They should be easily contacted, mentioned in ward literature and valued as team members. Some chaplains attend weekly psychosocial meetings with other members of the multidisciplinary team. They offer religious and pastoral care, opportunity for children and families to continue their spiritual observances while in hospital, and support in times of crisis – in particular, when end-of-life decisions are to be made. They facilitate memorial services within and outside the hospital, which is appreciated by the wider hospital community and those in our service. They are often part of the ethics advisory group in the hospital.

Shared care protocols for patient management

Developing these documents is an important part of the multidisciplinary team role. Local teams need the support that these documents provide, enabling them to provide the continuity of care that children need. The scope of each of these documents should provide the following:

- An overview of the specific liver disease and its treatment.
- An overview of the management of specific complications.
- Specific information about the medication involved in treatment including dosage and side effects.
- Contact information.

These should be available for primary and shared care teams and will supplement discharge summaries and outpatient and other correspondence.

CHAPTER 6.2

The Role of the Dietitian

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Key points

- Malnutrition is the most common co-morbidity associated with pediatric liver disease.
- Energy intake should be increased to 150–200% of the estimated average requirement.
- Cholestatic infants require a high-calorie, medium-chain triglyceride formula.
- Early initiation of nasogastric feeding is indicated for children not meeting nutritional requirements.
- Water and fat-soluble vitamins should be supplemented.
- Post-transplant catch up growth can be achieved.

The liver has a central role in energy metabolism, nutritional homeostasis, and absorption of nutrients. Although pediatric liver disease is rare, it has significant effects on growth and development due to the effect of malnutrition and anorexia. The pathophysiology of malnutrition in liver disease is complex and multifactorial (see Chapters 8 and 21). The most severe cases tend to be in infants with chronic cholestatic liver disease, who are particularly vulnerable to the effects of malnutrition because of their high energy and growth requirements. Insufficient dietary intake is a factor that can be rectified with proven nutritional support strategies. Nutrition is not only important in helping these children to grow, but also maximizes the success of liver transplantation, should the disease progress. Nutritional management depends upon the type of disease: acute, chronic, or caused by an inborn error of metabolism. The role of the dietitian is to provide expert advice and practical support to prevent or treat malnutrition in children with liver disease. This section will consider nutritional requirements and provide a guide to nutritional intervention.

Prevalence of malnutrition

It is essential for nutritional management to be initiated promptly as malnutrition is extremely common in children with chronic liver disease. Malnutrition is increased by diminished bile flow leading to a significant reduction in the absorption of energy-rich lipids and poor utilization of long-chain triglycerides. Malnutrition becomes more prominent as liver disease progresses; particularly cholestasis advances and is the most common comorbidity associated with liver disease [6] which makes it an important risk factor for survival. Portal hypertension, organomegaly, and ascites contribute to the difficulties in feeding children with liver disease leading to the progression of anorexia and malnutrition.

Severe malnutrition (weight and/or height less than 2 standard deviations below the mean) with loss of fat stores and muscle wasting used to affect 60% of infants with liver disease. Good nutrition is critical in preventing further damage to the liver by increasing energy available for synthesis, storage, and detoxification functions.

At particular risk for developing malnutrition include children under 2 years of age with severe cholestasis, those with progressive liver disease such as biliary atresia or end-stage liver failure awaiting liver transplantation, and children with recurrent hepatic complications such as ascites and variceal bleeds.

Aggressive nutritional management plays an important role in the care of these children as a better nutritional state is associated with better survival both before and after liver transplantation.

Factors which contribute to the development of malnutrition in patients with liver disease are highlighted in Box 6.1.

Reduced energy intake

Insufficient dietary intake, or anorexia, is a well-known symptom in children with chronic and acute liver disease. Due to ill health or anorexia, children may take less than the recommended requirements needed or less than is appropriate for their increased energy requirements due to excess energy expenditure thereby leading to loss of body stores and lean body mass as determined by anthropometry. The necessity to change children onto unpalatable unfamiliar feeds, discussed further in this chapter, can also contribute to reduced oral intake.

In children with chronic liver disease, the complications of portal hypertension, such as ascites and hepatosplenomegaly, can lead to malabsorption of nutrients. The treatment of ascites, which can necessitate a fluid restriction, can exacerbate anorexia and reduce energy intake further thus further compromising nutritional status. In practice, a discussion should be held between the dietitian and clinician as to the level of fluid restriction and negotiate with the clinician and patient how much volume is allowed for oral drinks and how much remains for enteral feeding.

Increased energy expenditure

In pediatric liver disease the risk of faltering growth is high and cholestatic children have a resting energy expenditure of up to 129% of that suggested by equations to predict calorie requirements. In infants younger than 12 months this can be as high as 150% [7].

Mechanisms implicated include portosystemic shunting and ascites; abnormal intermediate metabolism: and energy demands of specific complications, such as sepsis and variceal hemorrhage.

Children with end-stage liver disease have a hypermetabolic state with increased metabolic activity in the body cell mass with excess lipid oxidation during fasting and at rest. This confirms the necessity for appropriate nutrition and limiting fasting in these children. Dietetic interventions recommended to meet high nutritional needs will be discussed further in this chapter.

It is recommended that all children being assessed for liver transplantation receive a full nutritional assessment as part of their preparation for listing.

Box 6.1 Malnutrition in pediatric liver disease.

- Reduced dietary intake/anorexia
- Early satiety due to organomegaly and ascites
- Malabsorption/impaired digestion of fat and vitamins Increased energy expenditure
- Increased nutritional requirements
- Poor palatability of feeds and diet prescribed Impaired metabolic pathways
- Disease-related pancreatic insufficiency
- Taste changes related to medications and biochemical disturbances
- Behavioral feeding problems

Consequences of malnutrition

Many different nutrient deficiencies occur in children with chronic liver disease (Table 6.1). Malnutrition itself can lead to increased liver dysfunction as a result of the energy involved in synthesis, storage, and detoxification. Malnutrition can lead to lengthy hospital stays and some children may be an inpatient until post-transplantation in an attempt to improve their nutritional status.

Carbohydrate metabolism

Under normal circumstances, the liver receives portal vein blood rich in absorbed glucose, which can be stored in the liver as glycogen or circulated to extrahepatic tissues, especially muscle, where lactate, pyruvate, and alanine are generated by glycolysis. In children with liver disease, this substrate supply and use can be abnormal. The loss of glycogen stores in chronic liver disease leads to fasting hypoglycemia and an inability to meet energy demands. In addition, any significant hepatocyte loss, especially in acute liver failure has an immediate effect on glucose metabolism, leading to hypoglycemia.

Table 6.1 Clinical manifestations of malnutrition in pediatric liver disease.

Nutritional deficit	Clinical manifestation
Protein-energy malnutrition	Stunting, muscle wasting, motor/development delay
Fat malabsorption	Steatorrhea, loss of fat stores
Essential fatty acid deficiency	Peeling rash
Vitamin A deficiency	Conjunctival and corneal drying, night blindness, dry skin
Vitamin E deficiency	Peripheral neuropathy, ophthalmoplegia, ataxia, hemolysis
Vitamin D deficiency	Osteopenia, rickets, fractures, reduced bone density, muscle hypotonia
Vitamin K deficiency	Bruising, epistaxis, coagulopathy
Zinc deficiency	Acrodermatitis, anorexia, poor growth
Hypercholesterolemia	Xanthomata
Immunosuppression	Systemic infections secondary to reduced cell-mediated immunity

The treatment of hypoglycemia is dietary: providing a supply of glucose to maintain normal blood glucose levels and suppress counter-regulatory responses. Infants benefit from having smaller, more frequent feeds, often 2-hourly, orally or enterally. If ineffective at maintaining blood sugar levels continuous enteral feeding is commenced. In older children, encouragement of regular meals and snacks consisting of complex carbohydrates can contribute to the stabilization of glucose levels. If this regimen fails to maintain blood sugar levels then continuous enteral feeding of a glucose polymer solution may be required.

Protein metabolism

Amino acids are absorbed by the intestine directly into the portal vein and transferred to the liver, where they are synthesized into protein or used for energy. The liver is responsible for approximately 10% of plasma protein synthesis.

Non-essential amino acids are oxidized in both liver and muscle. The seven essential aromatic amino acids (AAAs; arginine, histidine, lysine, methionine, phenylalanine, tryptophan, and valine) are metabolized in the liver, whereas the three essential branched-chain amino acids (BCAAs; leucine, isoleucine, and valine) are metabolized predominantly in muscle and pass unaltered through the liver to the periphery, where their uptake is regulated by insulin. Abnormal protein use by the liver leads a rise in AAAs and a reduction in BCAAs. An abnormal ratio of BCAAs to AAAs correlates with histologic damage and encephalopathy in children. Protein requirements and examination of the addition of BCAAs to infant formulas will be discussed further in this chapter.

Fat metabolism

Most dietary fat is in the form of long-chain triglycerides (LCT) which are a high energy source. Fat digestion starts with emulsification in the stomach: it is followed by hydrolysis of triglycerides by pancreatic lipase in the intestinal lumen and then micellar solubilization of di- and monoglycerides by bile acids, which are then transported into enterocytes. Once they are in the enterocytes, fatty acids are re-esterified and chylomicrons are formed and removed via the lymphatics through the portal system to the liver and other tissue.

In contrast, medium-chain triglycerides (MCTs) do not depend on micellar solubilization for absorption and can be transferred directly from the enterocytes to the portal circulation without esterification allowing MCTs to be used directly by the liver for energy.

In all forms of chronic liver disease, there is a reduction in the synthesis and secretion of bile salts, although this is more severe in cholestatic diseases such as biliary atresia and in children with progressive familial cholestasis who have a bile salt transport defect. Up to 50% of LCTs, fat-soluble vitamins (ADEK) and essential polyunsaturated fatty acids (PUFAs) may not be absorbed because of reduced biliary secretion and reduction in intraluminal bile concentration. In contrast, 96% of water-soluble lipids, such as MCTs which do not depend on bile solubility, are absorbed even in cholestatic infants and form the basis for energy replacement [8].

Fat malabsorption can also be affected by portal hypertension, which leads to congested gastric and intestinal mucosa, and by small bowel overgrowth in the Roux-en-Y blind loop created by a Kasai portoenterostomy. In addition, therapies such as cholestyramine to reduce pruritus can exacerbate steatorrhea because it binds bile salts, decreasing micellar solubilization.

Dietary fats significantly contribute to energy intake, providing 9 kcal per 1 g consumed hence malabsorption can lead to negative energy balance.

In most children, pancreatic function is normal, however those with Alagille syndrome can be found to have reduced levels of pancreatic lipase [8].

Long-chain polyunsaturated fatty acids

Long-chain polyunsaturated fatty acids (LCPs) such as arachidonic and docosahexaenoic acid (DHA) are essential nutrients in neonates and infants. LCPs, in particular DHA, play a major role in the development of visual acuity and mental development in the first year of life, particularly in preterm infants [9]. The main source of LCPs is maternal, in the last trimester of pregnancy and through breastfeeding, because breast milk is a rich source of LCPs and contains both arachidonic acid and DHA in a combination of phospholipid and triglyceride forms.

Children with cholestatic liver disease have normal LCP and DHA levels at birth but can become deficient within 8–12 weeks from malabsorption of LCTs, from prescription of formula feeds rich in MCTs, or as a result of inadequate liver desaturase enzyme activity.

Fat-soluble deficiency

Chronic liver disease affects vitamin absorption, metabolism, and storage. Reduction in bile salt secretion leads to malabsorption of the fat-soluble vitamins A, D, E, and K. Fat-soluble deficiency can develop within 6–12 weeks of birth, depending on body stores and availability of vitamin supplementation. Despite supplementation of all fat-soluble vitamins deficiency has been found to be as high as 30% [10].

Trace elements and metals

Biochemical deficiencies of water-soluble vitamins such as thiamine and pyroxene can potentially lead to cardiomyopathy and peripheral neuropathy. Trace metal deficiencies include iron deficiency secondary to gastrointestinal bleeding or diminished intake and zinc and selenium deficiencies caused by reduced enteral intake, malabsorption or increased losses.

Table 6.2 Anthropometric assessment.

Measurement	Unit	Technique	Frequency
Weight	kg	Calibrated infant or chair scales. Infants under 2 years should be undressed	Daily – 4 weekly
Length/height	cm	Length board for infants under 2 (requires two people to do this); stadiometer for >2 years old	4 weeks
Head circumference	cm	Measure around forehead, above ears <2 years old	4 weeks
Girth	cm	Measure at umbilical line	Daily (if has ascites)
Mid upper arm circumference	cm	Midway point from shoulder to elbow. Measure circumference	4–6 weeks
Triceps skinfold	mm	At point of mid upper arm, back of arm	4–6 weeks

Nutritional assessment

The role of the dietitian is to offer dietetic support for each child with acute, chronic, or metabolic liver disease. This is achieved through nutritional assessment which includes the collection of anthropometrical and biochemical data in addition to clinical, social, and cultural information.

Nutritional status is monitored closely throughout the disease using nutritional parameters such as growth charts and anthropometrical standards; however both require careful interpretation in this group of patients. Serial anthropometric measurements can be useful in identifying early malnutrition as standard weight and height measurements are not always reliable in this population due to misinterpretation caused by fluid overload, ascites and organomegaly. Regular abdominal girth measurements can be useful in showing the likelihood of weight gain being attributable to ascites.

Monthly anthropometrical measurements such as mid-arm circumference and triceps skinfold (TSF) should be used to assess malnutrition by calculating mid-arm muscle area. This reflects muscle mass and is sensitive to nutritional status [11]. Children, particularly those listed for liver transplantation, should have anthropometrical measurements checked monthly. Height is likely to be stunted whilst the child is malnourished, although in infancy this is a late marker of growth failure.

Close nutritional monitoring highlights the need for when aggressive nutritional management such as nasogastric feeding or parenteral nutrition is required (Table 6.2).

Nutritional therapy (Figure 6.5)

Cholestatic liver disease is divided into two groups: those presenting in infancy and those presenting in the older child. Growth during infancy is of paramount importance consequently placing the younger children at greater risk of receiving sub optimal nutrition and poor outcomes if not addressed promptly. The aim of nutritional therapy is prevent or treat malnutrition by providing adequate calories for energy and sufficient nitrogen for protein synthesis, restore plasma amino acid imbalance, prevent vitamin and trace element deficiency, and achieve normal growth and activity.

Energy requirements

As resting energy expenditure is higher than that of a healthy disease free child, it is important to increase the energy intake of the estimated average requirement (EAR) to 150–200% [11]. Cholestatic infants in the early stages of disease will compensate for malabsorption and increased energy requirements by increasing their intake, often consuming 120–200% more formula than the recommended intake for age.

With ongoing cholestasis, energy intake falls due to progressive liver disease and strategies to increase the nutrient density of feeds must be employed. This can be achieved by using ready to feed, nutrient dense MCT formula (Infatrini Peptisorb®, Nutricia Advanced Medical Nutrition UK), which provides enhanced protein and energy intakes (100 kcal/100 mL, 10% energy from protein). Alternatively, MCT containing formula can be concentrated from 13% standard dilution to 15–19% dilution. This increases the energy density from 67 kcal to 80–100 kcal/100 mL and the protein from 1.9 g/100 mL to 2.24–2.82 g/100 mL. Concentrating infant formula feeds increases the intake of all nutrients and maintains the delicate balance between energy, protein, and micronutrients. The practice of concentrating formula should only be undertaken by an experienced pediatric dietitian who can ensure appropriate monitoring of intake and growth (Table 6.3).

There are multiple high-calorie, nutrient dense nutritional supplements available for those over the age of 1 year (Fortini®, Nutricia Advanced Medical Nutrition; PaediaSure Plus®, Abbott Nutrition) which can be effective in enhancing nutritional intake by providing 300 kcal/200 mL bottle. MCT-containing supplements are also available (PaediaSure Peptide®, Abbott Nutrition and Peptamen Junior®, Nestlé Healthcare Nutrition). Unfortunately, long-term compliance in taking these drinks is poor. If there is no response to an increase in energy intake alone, enteral feeding by nasogastric tube as either daytime bolus feeds or continuous overnight feeds is beneficial.

Fat requirements

Fat is a major source of energy for infants; however as malabsorption occurs in cholestatic liver disease with fat-soluble vitamin and essential fatty acid (EFA) deficiencies, the fat

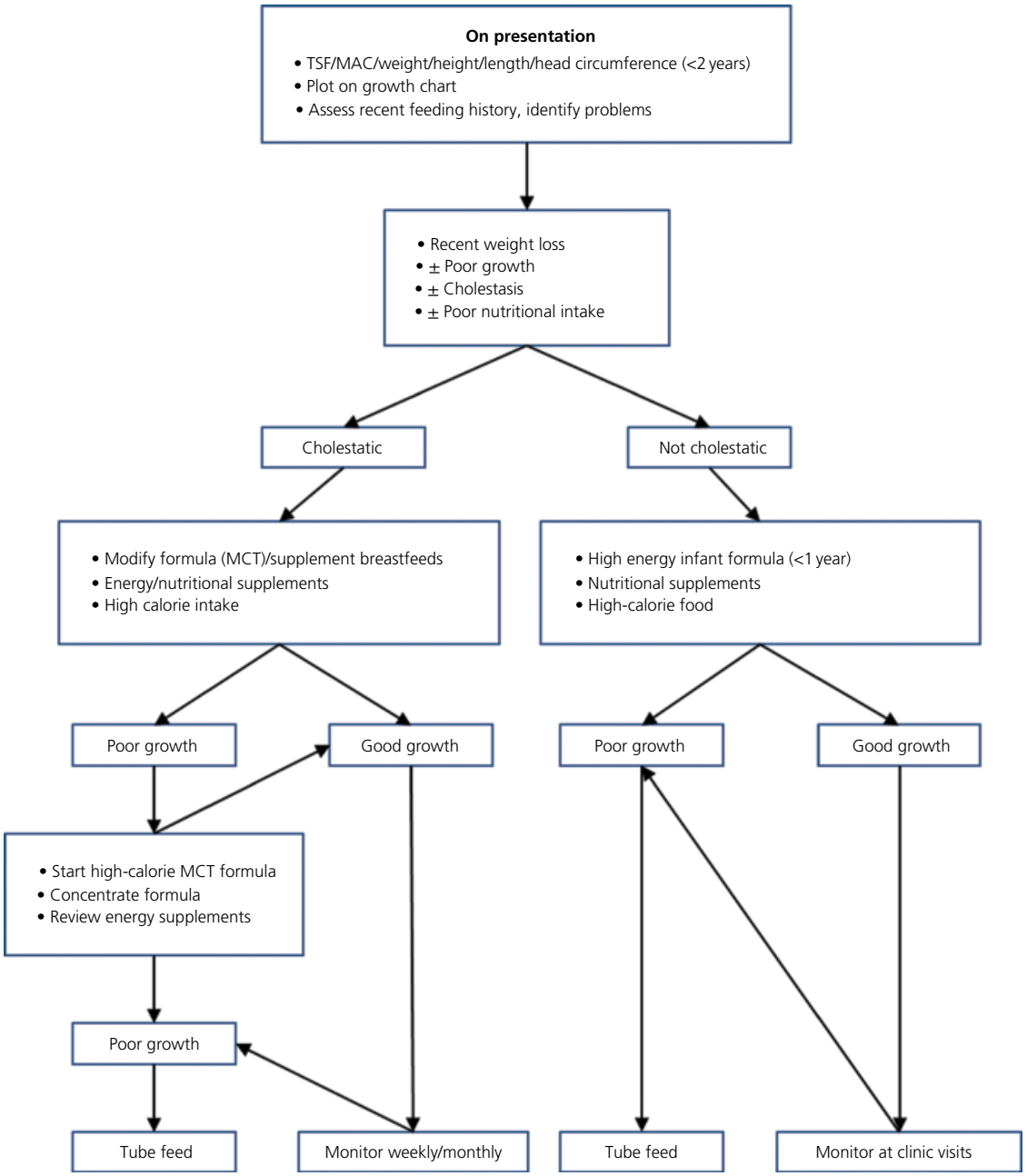

Figure 6.5 Dietetic assessment, treatment, and monitoring for children with liver disease. MAC, membrane attack complex; TSF, triceps skinfold; MCT, medium-chain triglyceride.

Table 6.3 Nutritional supplements (composition per 100 mL).

Formula	Manufacturer	Energy (kcal)	Protein (g)	Fat (% medium-chain triglycerides)	Sodium (mmol)	Essential fatty acids omega 6 : omega 3
Pregestimil®	Mead Johnston Nutritionals	68	1.9	55	1.4	Yes 16.5 : 1
Pepti-junior®	Cow & Gate	67	1.8	50	0.9	Yes 64 : 1
Infatrini Peptisorb	Nutricia Advanced Medical Nutrition	100	2.6	50	1.1	Yes 418 : 1
Modular feed	Scientific Hospital Supplies International	Flexible	Flexible	Flexible	Flexible	None

content of the diet requires careful consideration. Low-fat diets are no longer considered appropriate but an increase in MCT content can reduce steatorrhea [12].

If bile flow is absent or interrupted from the liver into the gut, fat emulsification and digestion are reduced. Progressive liver disease and ongoing cholestasis necessitate a change of formula to increase nutrient density and to change the fat source. General indications for a change to MCT formula are total bilirubin >100 mol/L or conjugated bilirubin >70 mol/L.

Infant formulae are changed from a standard LCT-containing formula to a specialized MCT formula [12]. Current MCT formulas are hydrolyzed protein infant formulas such as Peptijunior (Cow & Gate), Pregestimil (Mead Johnson), or Infatrini Peptisorb (Nutricia Advanced Medical Nutrition) which contain 50% MCT to maximize fat absorption and improve steatorrhea.

Overall total fat should contribute 30–60% of dietary intake of which the optimal percentage to prevent malabsorption and growth failure is 30–70% of fat as MCT. EFA deficiency occurs with intakes of MCT greater than 80% [13] and infant formulas which contain greater than 80% MCT are not recommended.

Cholestatic breastfed infants will demand frequent feeds as breast milk is high in LCT, often leaving the mother exhausted. While continuing breastfeeding is recommended, offering an MCT-containing formula as alternative complimentary feeds reduces the demand on the mother.

MCT does not contain the EFAs linoleic acid (C18:2) and α-linolenic acid (C18:3) which are required for growth and brain and eye development. EFA deficiency is common in cholestatic children as a consequence of fat malabsorption and as a result of low intake from the use of MCT feeds. Advice from the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) states that infant formula should contain 4.5–10.8% energy as linoleic acid, with the ratio of α-linoleic to α-linolenic being 1.5 : 15. It is suggested that >10% total energy should be provided as PUFA in children with cholestasis [14]. The LCTs or PUFAs can be supplemented by the addition of walnut oil to provide 1–2% of total energy. Older children can receive dietary sources from eggs (rich in arachidonic acid) or fish oil (rich in DHA).

Supplementation of fat-soluble vitamins is required due to malabsorption in cholestatic children. Levels are monitored via blood tests and doses of fat-soluble vitamins adjusted as necessary to maintain levels within normal ranges.

Protein requirements

Protein requirements are higher in advanced liver disease as a result of increased rates of amino acid oxidation, gluconeogenesis, proteolysis, and the loss of protein in the gastrointestinal tract; however nutritional supplementation outcomes remain inconclusive. Infants with advanced liver disease require 2–3 g/kg/day, although this will be even higher in children with biliary atresia receiving treatment for cholangitis (up to 4 g/kg/day) to prevent a negative protein balance. Children tolerate increased protein intakes compared with adults because of metabolic processes associated with growth.

The effect of chronic liver disease is altered amino acid metabolism with lower levels of BCAA and increasing levels of aromatic amino acids. Despite this, there is insufficient evidence to support additional BCAA supplementation. An important consideration when examining the use of BCAAs should be the expensive cost and poor palatability of these feeds which may further decrease energy intake.

Protein restrictions to control hyperammonemia and encephalopathy are not justified; medical management should be the first-line treatment. In cases of severe encephalopathy protein should be restricted to no lower than the recommended nutritional intake (RNI) to ensure nutrition is not further compromised.

Carbohydrate requirements

Carbohydrate is the major source of dietary energy and should contribute 60% of the daily calorie intake of the child. Reducing substances in the urine may highlight a need to change to a lactose free feed, such as Pepti-junior if there is carbohydrate malabsorption.

Carbohydrate polymers are frequently used in nutrition support, usually without any adverse side effects. As polymers are increased, diarrhea may develop, due to an increase in osmotic load. Increasing polymers slowly in small increments may reduce intolerance. The addition of carbohydrate sources is generally more palatable and more easily accepted by patients than supplementing with a fat or protein sources.

Vitamin and mineral supplementation

Vitamin malabsorption is a major nutritional problem specific to cholestasis. Both water- and fat-soluble vitamins should be supplemented in children with chronic liver disease. Supplementation should be based on plasma levels of selenium, zinc, calcium, and magnesium. Iron supplementation might be required in children with chronic blood loss and calcium and phosphate if rickets is diagnosed. Fat-soluble vitamins are essential for all children with prolonged or cholestatic liver disease. Most children will be maintained adequately on oral fat-soluble vitamins, but monthly intramuscular administration is occasionally required for children with severe cholestasis.

Monitoring is essential and doses should be adjusted accordingly.

Mode of delivery

Whenever possible the oral route should be used for nutrition, unless it is not possible to provide adequate energy. Enteral nutritional supplementation is cost effective, maintains gastrointestinal tract immunity, reduces bacterial overgrowth, and maintains the integrity of the gut barrier to microorganisms.

Infants should be introduced to weaning solids from 6 months of age. This helps to support the development of oral motor skills regardless of how little volume is consumed or contributed to overall nutritional requirements. Texture progression is also encouraged.

Children with advanced liver disease will not be able to consume orally the volume of formula or nutritional supplements necessary to prevent of reverse malnutrition. Early nasogastric feeding is indicated for children who have a poor response to an increase in nutritional intake or become anorexic. Nasogastric feeding can be very effective in feeding children with liver disease and acceptance is enhanced by explanation and support by the multidisciplinary team pre and post initiation.

The benefits include reduced parental anxiety, better feed tolerance, and parents often report an improved sense of well-being in infants. Nocturnal feeding over 10–12 h is the preferred choice as it allows nutritional supplementation whilst supporting normal feeding in the daytime and reducing the risk of oral aversion. Continuous overnight feeding is often better tolerated than bolus feeding in patients with organomegaly. The nasogastric feeding regimen should provide 50% of EAR.

For infants with severe liver disease, the duration of feeds may be increased to 24-h feeding if hypoglycemia or severe malabsorption is present or if feed intolerance is a problem. If vomiting is an issue, a continuous feed via a nasojejunal tube, which requires radiological insertion, may be warranted. Under normal circumstances, it should be possible to provide nocturnal or continuous enteral feeding at home with appropriate support from a nutritional care team including a dietitian, a liaison nurse, pediatric community nurses, and clinicians.

Gastrostomy tube feeding is rarely performed in children with chronic liver disease as placement has difficulties due to organomegaly, risk of peritoneal infection with ascites, and stomal variceal bleeding associated with portal hypertension. Use of a soft silk nasogastric tube is safe.

Parenteral nutrition should be considered when enteral feeding is ineffective or not possible and should be used for short-term purposes only. Parenteral nutrition is beneficial if maximizing calories of enteral feeds results in malabsorption, severe intolerance and poor weight gain or secondary to recurrent variceal bleeding or abdominal sepsis which are associated with marked catabolism and weight loss. Attempts should be made to still offer trophic feeding of up to 10 mL/kg alongside parenteral nutrition to promote gut integrity and assist in protecting the liver. Enteral feeds may be re-graded up to full volumes as tolerated. Standard amino acid and lipid solutions are well tolerated in stable patients, and lipids can be particularly beneficial in achieving adequate calorie intake. Mixed lipid emulsions (e.g. soya bean oil, MCT, olive oil, fish oil emulsion; SMOF) have demonstrated reduced effects of oxidative stress, immune responses, and inflammation.

Patients receiving parenteral nutrition require careful biochemical monitoring and attention to fluid and electrolyte balance to avoid fluid overload.

Post-transplantation

It is well documented that good nutrition can maximize the success of a liver transplant; however many children do undergo transplantation in a malnourished condition. Post-transplant growth can be achieved by good nutrition, good graft function, and lack of concurrent illnesses like rejection or sepsis.

Feeding post-transplant should start as soon as possible, ideally within 3–5 days. Parenteral nutrition should be started if delays to feeding arise due to postoperative complications. It is important to ensure sufficient energy intake (at least 120% EAR) postoperatively. Children who are fed orally preoperatively can progress onto light diet (e.g., soup, yoghurt, jelly) once tolerating clear fluids and normal diet can be reintroduced over the first 5–7 days. Children previously fed MCT infant formula or feeds can be changed to standard or high-calorie age-appropriate feeds containing LCTs either enterally or orally as bilirubin levels normalize and bile flow is re-established.

Damage to lymph vessels during transplantation caused by dissection of lymph glands can lead to chylous ascites. This appears in as a milky solution in abdominal drain output. Dietetic treatment involves commencement of a very low-fat diet (<10% LCT as total fat) for a period of 6 weeks. Infant formula with a low LCT content such as Monogen® (Nutricia Advanced Medical Nutrition) can be used for those under 1 year of age; a modular feed is required for older enterally fed patients to ensure an adequate MCT : LCT fat ratio is achieved. Modular feeds are produced from separate components consisting of fat, protein, carbohydrate, and vitamin and minerals. The benefits of modular feeds are their flexibility; ingredients and quantities are able to be adapted to meet specific needs of the child. A modular feed is often required for older children with a chyle leak as there are no standard feeds available low in LCT and high in MCT. Although beneficial, they can be lengthy to prepare, require complex feed calculations and potential for errors in making them is high. Juice-based oral nutritional supplements are suitable for those children that accept oral nutrition support.

Children malnourished at the time of transplant may continue to rely on nutrition support from nasogastric feeds for some months after transplant until oral intake is sufficient to meet nutritional requirements and good weight gain has been demonstrated. Weaning of nasogastric feeds may be delayed by factors including organ rejection, sepsis, severe malnutrition pre-ptransplant, and behavioral feeding problems. Weaning of enteral feeds usually starts with one to two nights free from overnight feeds per week with further nights ceasing as weight and intake improves.

Loose stools post-transplant are common. Possible causes include infection, magnesium supplementation or initiation

of mycophenolate mofetil (MMF). Enteral feeds may benefit from being switched to non-fiber containing and children having a diet rich in sugary foods should be encouraged to lower overall refined sugar intake.

Initial weight gain can be rapid because of the effects of corticosteroids on appetite and salt and water retention, but most children return to a normal weight once these are reduced or discontinued. Linear growth can be delayed by 6–24 months depending on the rate of hepatic complications and the withdrawal or reduction of corticosteroids. Appetite and oral intake needs careful monitoring as steroids are weaned; some children will experience reduced appetite and may require high-calorie nutritional supplements.

Acute liver disease

The dietetic goal in the treatment of acute liver failure is to prevent hypoglycemia and maintain nutritional status. In the presence of hypoglycemia continuous feeding is initiated, with glucose polymers in addition if enteral feeds alone do not provide adequate carbohydrate volumes. Some children may additionally require intravenous administration of dextrose. A modular feed may be required in instances when children are fluid restricted but continue to have high glucose needs. As with chronic liver disease, protein and energy requirements should also aim to be met to prevent catabolism.

Cystic fibrosis related liver disease

A small proportion of children with cystic fibrosis will develop liver disease (see Chapter 16). Malnutrition is common in cystic fibrosis related liver disease (CFRLD). Dietetic interventions are similar; nutrition support aiming for 150% of EAR, oral nutritional supplements, and overnight enteral feeding. Sodium requirements are already high in this population so that sodium restrictions as being unnecessary. An increase in malabsorption is generally related to portal hypertension rather than inadequate supply of pancreatic enzyme replacement. As with all children with progressive liver disease the ultimate goal is to ensure nutritional is optimal for liver transplantation.

Conclusion

In summary, early nutritional assessment is essential for all children with liver disease due to the common symptom of anorexia. Many studies have highlighted malnutrition as a risk factor for survival. Appropriate nutrition support to meet the high nutritional requirements of this population are necessary to prevent faltering growth, particularly in those under 2 years of age who are more vulnerable to an energy deficit. Cholestatic infants with a conjugated bilirubin >70 mol/L should be changed to a feed containing MCT to prevent ongoing fat malabsorption which can lead to wasting. Energy intakes should be a minimum of 150% of the EAR. Anthropometrical serial measurements are more useful in monitoring nutritional status than regular weighing and highlight when more aggressive nutritional support, such as enteral feeding or parenteral nutrition is necessary. Many children do require enteral feeding as liver disease progresses and the need for transplant becomes apparent. A better nutritional state is associated with better survival both before and after liver transplantation.

CHAPTER 6.3

The Role of the Psychologist

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Key points

- Liver disease may effect child development and impair cognitive functioning and require psychological assessment and support.
- Mental health problems in chronically ill children include separation anxiety, depression, and post-traumatic stress.
- Children need expert preparation for medical and surgical procedures with both the psychologist and play specialist.
- Psychologists have an essential role to play in supporting the child and family in all these difficult situations.
- It is essential that the dying child and their family should receive psychological, spiritual, and religious support, and that palliative care is planned by the multidisciplinary team.

Child development and psychosocial functioning

Depending on their diagnosis, children suffer from a range of symptom which may have a direct impact on their physical, emotional, social, and cognitive development. Children diagnosed in infancy, which is a particularly vulnerable stage of neurological development, are at greater risk of being adversely affected by the illness and its treatment, and by the impact of multiple hospitalizations, all of which will affect the growing brain [15] and impact on children's physical and psychosocial development and behavior.

As a consequence of their illness many children miss a great deal of time away from school. This may be due to repeated or lengthy periods in hospital, or because parents are concerned about letting their child go to school in fear of them being hurt or bullied. For example, children have been teased about having nasogastric tubes, being of small stature, or because they may be struggling to keep up with the work. This can often lead to low self-esteem as well as both internal and external behavior problems, particularly if children find it difficult to express their emotions.

Behavior problems

Often a referral to the psychologist is made when the child is an inpatient and either displaying externalizing behaviors (acting aggressively towards staff; refusing treatment such as blood tests), or internalizing behaviors (withdrawal, depression). In these situations, the role of the psychologist is to conduct a detailed assessment to determine what the underlying cause may be for the child's presenting problem. For example, the child may be fearful of needles and require psychological therapy to help reduce their anxiety or psycho-education to help them understand their liver disease and the need for treatment.

Cognitive functioning

One of the most important areas of function in children and adolescents is academic performance, as this reflects their developmental status and prepares them for independent functioning in adulthood. Impaired cognitive development, below average performance, and inconsistent school attendance have all been documented in chronic childhood illness [16]. There are a number of factors associated with chronic disease likely to have an adverse effect on cognitive function. Some of these include the impact of the illness and its treatment on the growing brain, particularly when disease onset is during infancy, and the impact of multiple hospitalizations on the child's psychosocial development and behavior.

Furthermore, many children with liver disease are malnourished from birth which is a vulnerable period of neurological development. Although transplantation reverses liver failure, children are still exposed to neurotoxic medications and require prolonged periods of hospitalization. The end result may be expressed as poor school performance

and the requirement for an educational health and care plan (EHCP), formerly known as a special educational needs assessment, which the psychologist will be able to perform.

By assessing children's cognitive abilities the psychologist may be able to identify whether or not they have a significant learning difficulty and if they are therefore able to communicate effectively or understand what is happening to them. Following this the psychologist can provide a detailed report regarding the child's overall cognitive abilities and make informed recommendations as to how best support them at school. For children who demonstrate difficulties, the most helpful times for administering repeat cognitive assessments include when specific concerns are noticed, and at times of transition, such as the transition to junior and secondary school.

Hepatic encephalopathy

Some children with chronic liver disease demonstrate behavior problems or neurological symptoms associated with abnormal brain function due to hepatic encephalopathy (HE). Minimal hepatic encephalopathy is accompanied by a broad spectrum of cognitive symptoms (see also Chapter 21). Children with minimal or mild encephalopathy may have more subtle abnormalities and demonstrate changes in logical thinking, personality, mood, and behavior. In the event that mild hepatic encephalopathy is suspected the psychologist can conduct a range of specific cognitive and psychometric tests. It is important to ensure that any cognitive problems the child may be having are specifically related to HE since complaints can have multiple origins and may reflect any number of psycho-affective or other health problems [17].

Developmental, intellectual, and neuropsychological assessment

Developmental assessment involves the administration of standardized tests (e.g., the Bayley Scales of Infant Development, the Griffiths Mental Developmental Scales) to babies and preschool aged children in order to measure their developmental ability. Intellectual assessment involves the administration of norm-referenced tests (e.g., the Wechsler Preschool and Primary Scale of Intelligence III, the Wechsler Intelligence Scale for Children IV) to preschool and school-aged children in order to measure their general intellectual ability. A neuropsychological assessment involves the administration of standardized tests of intellectual ability, learning, memory and attention, and the interpretation of these measures in the light of known brain functioning.

Attachment

Parent–child relationship are affected by psychosocial and sociodemographic risk factors that undermine its quality and play a negative role in the child's short- and long-term psychological health. Attachment develops through parental attunement to their child's needs, which in turn helps to establish their understanding of relationships and the foundations of their verbal and non-verbal communication.

By giving meaning to their child's feelings and body signals, attuned parents help to build their child's self-awareness. Similarly, by modulating their child's stress, they contribute to the programming of the child's stress systems, with lifelong implications for the development of self-regulation, behavioral control, and physical and psychological health [18].

Attachment insecurity has lifelong implications for children's personal, social, and professional relationships, stress regulation, and physical and psychological health. Without it, our ability as professionals to protect children by preventing illness and disease, and promoting their optimal development, will be seriously challenged. Attachment insecurity has also been proposed as an individual vulnerability factor that can have a seriously negative impact on children's experience and expression of pain, disability, psychological distress (anxiety and depression), and treatment compliance, all of which can lead to a poorer outcome. Attachment avoidance has also been associated with opioid abuse.

Having an understanding of attachment theory allows psychologists to provide other professionals with a unique, simple, and pragmatically useful model for appreciating the particular ways that children feel and react when stressed, and how other professionals (nurses, doctors, anesthetists, phlebotomists) can help to manage that distress.

Pain

Insecure attachment style is associated with chronic widespread pain, hypochondriac beliefs, hypervigilance to pain, increased pain-related fears, reduced pain thresholds [19], and poor pain coping, increased psychological distress, frequent attendances to GP practices, and admissions to hospital. These findings suggest that children with insecure attachments are more likely to develop pain and once pain develops they are more likely to perceive it as more intense, disabling, and distressing.

Research indicates that conflicts generated between children and their parents during the first 2 years of life become reactivated again during the teen years when adolescents struggle to gain control and may attempt to demonstrate this by becoming non-compliant with treatment. Similarly Goldberg *et al.* [20] found an increase in behavior problems in older medically compromised children who presented

with behavior problems associated with attachment problems as early as 2–3 years of age.

Mental health problems include separation anxiety, childhood depression (especially if there is a family history or if a parent experienced it at a very early age), conduct disorder (CD; aggressive antisocial behavior believed to be a defense against anxiety and attempt to recapture mother–infant relationship; the result of maternal deprivation; and failure to internalize controls), oppositional defiant disorder (ODD; most likely a combination of an inherited predisposition and environmental and parental influences).

From a care planning perspective psychologists play an important role in identifying children at risk of developing attachment difficulties. By making observations of parent–child interactions the psychologist is able to build up a profile of the child's attachment and highlight the importance of early intervention and prevention. Some families may only require a little encouragement to help them develop healthy relationships whereas for others, the psychologist may recommend any one of a range of interventions aimed at helping children deprived of early sensitive care.

Along with medical treatment, research demonstrates that psychological therapy is one of the most beneficial treatment methods in terms of assisting families and children with a chronic illness [21]. Interventions include support groups, one-to-one psychological treatment, patient education, and cognitive–behavioral skills training. These interventions have been found to have positive effects on the health status and psychological functioning of the child and other family members. Interventions which also focus on behavior management of the child as well as relational issues between various family members and the chronically ill child have been shown to leave family members feeling less anxious, burdened, and depressed [21].

Post-traumatic stress symptoms and post-traumatic stress disorder

For children and adolescents who struggle to cope with stress or pain, especially children who have experienced numerous invasive procedures, the psychologist has an important role to play in assessing and treating children who may be suffering from post-traumatic stress symptoms (PTSS) or even post-traumatic stress disorder (PTSD). Some procedures that children and adolescents with liver disease go through are experienced as so traumatic that they overwhelm their ability to cope. These events may impact upon their thoughts of themselves as well as of the world, leaving the memory of the event deeply encoded and in a different way from their normal memories. That is, rather than occasionally thinking about the event, the child is inclined to continuously re-experience the event; this includes the associated pain and fear associated with the trauma, which causes the child to fear the memory of the event as well as the event itself. PTSD has elements of intrusive thoughts, emotional numbing, social withdrawal and isolation, alterations in cognitive processing, and changes in hyperarousal.

A number of children with liver disease who are required to undergo anesthesia (e.g., for endoscopy or liver biopsy), can experience this as traumatic or life-threatening event as they are afraid either that they may not survive the procedure, or that they simply will not wake up. Many of these children, even if they do not cross the traditional threshold for PTSD diagnosis, may still suffer from significant functional impairment.

Immediately after a traumatic event, children are likely to be confused, frightened, and distressed. The initial goal of intervention is to ensure that the child is provided with a safe environment where they can experience routine and consistency in their daily activities. By helping parents and staff adopt an approach of respect, compassion, and containment the psychologist can ensure that the child experiences a sense of security and predictability. With respect to therapeutic intervention cognitive–behavioral therapy (CBT), especially trauma-focussed CBT (TF-CBT) [22] is the first-line treatment for PTSD. Relaxation training and medication may be helpful in enabling the child to do this.

The initial aims of therapy are to educate the child and family about the physiological and cognitive effects of the trauma, and to teach the child some strategies for dealing with both of these aspects of their distress on their physical and cognitive well-being.

Family functioning and adjustment to chronic illness

Although there are a variety of community services available to support families, they remain the primary caregivers for children with a chronic illness. The most common challenges of parenting children with a chronic illness include: high levels of stress and tension in the family, disrupted family relations, difficulties with time management, interruptions to or cancelled family activities, feeling disconnected from social networks, and problems communicating with the child's school [21].

Other factors include disruptions to daily routines, interference with parents' careers, problems with the child's developmental transitions, friendships, school performance, poor parenting strategies, and sibling relationships. If the family views any of the stressors as unmanageable, or if they are unable to attain supportive resources, family relationships may weaken and the negative effects of stress can accumulate.

Chronic illness impacts upon all members of a family with some parents experiencing significant periods of anxiety or depression. Ellenwood and Jenkins [23] note that mothers of

chronically ill children may become neurotic, introverted, and lack self-confidence, whereas fathers of chronically ill children may form long-term personality changes.

Researchers have noted that psychological interventions that focus on the chronically ill child and their family members appears to be the most effective in reducing depression among both the child who has the chronic illness and their family members [24]. It is also imperative that siblings are included in the treatment program [25].

When a child suffers from chronic illness, sibling relationships may become highly fractious because the chronically ill child may be perceived as needing more protection and attention by the parents in order to survive. Many healthy siblings suffer from high amounts of anxiety, which stems from the worry, guilt, shame, and competitive feelings they hold towards their chronically ill siblings. On the plus side, some siblings develop a number of positive qualities and are able to identify and describe one or more personal strengths that developed as a result of their coping with the challenges of having a sibling with a chronic illness.

Altered physical appearance

Most children with liver disease experience abnormal changes in their due to disease processes, e.g., jaundice, or as a consequence of medical or surgical treatment, e.g., cushingoid features, hirsutism, or abdominal scarring. Treatment adherence may be compromised by a patient's wish to avoid the negative side effects on physical appearance, especially steroids. Worries about altered appearance are likely to peak at adolescence when many young people are concerned about how they look and the association with peer group acceptance.

The psychological effects of growing up with a distinctive appearance may have significant psychological effects. For instance, children and adolescents describe themselves and their peers according to their physical appearance, while there is evidence to suggest that individuals with an atypical appearance experience social avoidance which may lead to decreased social competence, teasing and low-esteem.

Children with liver disease may also experience difficulty in establishing peer relationships because of their frequent hospital stays. Social competence in childhood predicts to the formation of adult social relationships and may be seen as a component of an individual's quality of life. Lack of social competence is a risk factor for social isolation, low self-esteem and depression.

There are a number of ways in which psychologists can help patients with abnormal physical appearance. Firstly, it is useful to enable patients to develop age-appropriate ways of explaining their altered appearance. Assistance with social skill development and coping strategies for teasing may also be indicated and role play with video feedback can be a useful adjunct. For preschool children this work can be carried out with the child's parents with the expectation that the child will imitate their parents' behavior. Secondly, patients can be involved in preparing photographs, video materials, and written text to send to their school prior to their return. Healthy peers will habituate to the patient's abnormal appearance, have an opportunity to ask questions and their desire to stare is reduced. Thirdly, cognitive therapy may be useful for adolescents whose abnormal appearance is associated with depression, social anxiety, or poor treatment adherence.

Liver transplantation

Transplant and surgical teams need to take into account both a child's cognitive and emotional development when making decisions about how much, and in what way children should be informed about the disease, transplantation procedure, and medical regimen. Assessment may reveal anxieties about medical procedures which impact on the transplant process, for example, procedural anxiety and needle phobia.

The psychological techniques described above, such as education, play therapy, distraction, relaxation, behavioral therapy, and CBT, will all help children cope with medical procedures.

In specific cases it may be useful to assess the cognitive functioning of the parents to determine whether they will require additional support in being able to comprehend and recall details of the child's medical regimen.

Children may experience depressive and anxiety disorders during the transplant process due to psychological stressors, medications, physiological disturbances, and post-traumatic stress (see earlier). Adults tend to underestimate the child's post-transplant emotional symptoms, possibly because they focus on the improved prognosis, whereas the children focus on the concrete experience of illness. In addition some children experience changes in mood related to death anxiety, guilt due to perceived and real burdens on the family, survival guilt when other transplant patients have died, changes in family dynamics, and body image concerns.

Consequently, it is important to monitor the child's mental state from pre-transplant to post-transplant, enabling early psychological intervention if these symptoms reach clinically diagnostic levels.

Treatment adherence

Children with liver disease pre- or post-transplant need to adhere to drug regimens with negative side effects, dietary restrictions, or repeated invasive procedures. Adherence to a specific treatment is affected by a number of interrelated variables including the child's developmental status, disease knowledge, health beliefs, and family support. Non-adherence

to medical therapy is a serious problem for adolescents and is one of the most common causes of chronic graft rejection in this population (see Chapters 31 and 35 for more details).

End-of-life and palliative care

Hospital deaths are inevitable and dying children have distinct needs. An important aspect of caring for a dying child is to recognize that patients have changing medical, psychological, religious, and spiritual needs at every stage of end-of-life care and that family carers' needs are also significant. Whilst hospices can provide support, they offer limited space and in some families there is a cultural expectation that death will happen in hospital. Such evidence that exists indicates the need for the dying child and their family to be supported by a multidisciplinary team.

The World Health Organization and Department of Health in the UK highlight that the dying child and their family should receive psychological, spiritual, and religious support. However, death is a difficult subject to manage and difficulties tend to be magnified. Issues of communication are fraught with difficulties and parents may be reluctant to allow clinicians to talk openly about end of life to their child. There is a clear need for multidisciplinary training in order to equip professionals with the skills to manage these situations. Since psychological, religious, and spiritual needs are often culturally defined this imposes wider imperatives to understand the diverse needs of this group of patients.

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SECTION 4

Liver Disease in Pregnancy

CHAPTER 7

The Effects of Liver Disease in Pregnancy on Mother and Child

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Key points

- Hepatic conditions may be specifically related to pregnancy.
- Neonatal liver pathology may occur secondary to maternal disease.
- Contraception and pregnancy in those with chronic liver disease and following liver transplantation requires special consideration to ensure the safety of the mother and child.

The rate of teenage pregnancy (15–17 year olds) in the UK is one of the highest in Europe with a rate of 19.7 per 1000 live births in 2014. The trend in the rate however has been steadily declining with a reduction by one-third in the past 10 years. In comparison the Nordic countries have much lower rates with Denmark and Sweden having rates of 7 per 1000. Bulgaria is the highest at 50 per 1000. It is therefore important for pediatricians to consider the potential for conception in young women with chronic liver disease or those who have had a liver transplant, advise on contraception, and manage any ensuing pregnancy. Pediatricians should also be aware of hepatic complications specific to contraception and pregnancy, some of which require immediate action. Increased understanding of the pathophysiology of neonatal liver disease has led to a greater awareness of the influence of the mother on the neonatal liver, either via the fetal–placental unit or through inheritance [1, 2].

Physiological changes to the liver and biliary system during normal pregnancy

Thirty-five percent of the cardiac output normally goes to the liver; however, due to the redirection of blood to the uterus during pregnancy, this reduces to 27%, whilst the total blood volume increases, resulting in a relative decrease in the serum concentration of liver enzymes. Table 7.1 lists the liver-related changes seen in normal pregnancy.

Hepatic conditions specific to pregnancy

Liver disease is a rare complication of pregnancy, but when it occurs, morbidity to both the mother and fetus can be severe and may prove fatal [3–5] (Table 7.2).

Hyperemesis gravidarum

In 0.2–3% of pregnancies, the symptoms of nausea and vomiting in the first trimester are severe, leading to dehydration and malnutrition. It is more common in multiple gestations and with fetal anomalies. Raised transaminases (seldom exceeding 200 IU/dL) occur in up to 50% of the hospitalized cohort. Both conjugated and unconjugated bilirubin may be mildly raised, as may alkaline phosphatase (ALP). Liver biopsy is not normally indicated and may be normal or have fatty changes. Hypotheses to explain the transaminitis include a hepatic response to starvation, which due to the associated increased fatty acid load may unmask a heterozygous defect of mitochondrial fatty acid oxidation in the mother (long-chain 3-hydroxyacyl-acetyl-coenzyme A (CoA) dehydrogenase and carnitine palmitoyltransferase I (CPT I)).

With hydration and nutritional therapy, the transaminases tend to settle without any further maternal pathology. The neonate may be small for gestational age, but no long-term detriment to the fetus has been reported.

Diseases of the Liver and Biliary System in Children, Fourth Edition. Edited by Deirdre A. Kelly.

Table 7.1 Physiological changes in liver investigations during normal pregnancy.

Clinical features	Liver and spleen are not palpable Palmar erythema and telangiectasia
Serum bilirubin	Normal or low
Serum albumin	Low
Prothrombin time	Normal
ALT, AST, and GGT	Normal
Alkaline phosphatase	Increases steadily throughout pregnancy, with a sharp rise in the last month. This reflects increased production by the placental syncytiotrophoblast and skeletal maturation of the fetus. It returns to normal within 2 weeks of delivery
Total bile acids	Normal or only mildly elevated
Urea	Low
Triglyceride and cholesterol	Increase throughout pregnancy
Gallbladder motility	Decreases Biliary lithogenicity increases
α-Fetoprotein	No changes
Liver biopsy	Normal histopathology Electron microscopy: proliferation of the smooth endoplasmic reticulum and giant mitochondria with increased paracrystalline inclusions, reflecting the increased protein and energy requirements

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase.

HELLP syndrome

This acronym was first coined in 1982 by Weinstein to describe patients with *h*emolysis, *e*levated *l*iver *e*nzymes and *l*ow *p*latelets. It is associated with substantial maternal and perinatal morbidity and mortality. The diagnosis is made on clinical suspicion and supportive laboratory findings.

Pathogenesis

The exact cause of the condition is unknown. Hypotheses are:

- Placental ischemia due to inadequate invasion of the cytotrophoblast, leading to the production of systemic toxic vasoactive factors causing endotheliosis in end organs including the liver.
- A thrombotic tendency, with detection of anticardiolipin antibodies being the most common finding.
- An immunological reaction that causes cytokine-mediated endothelial damage.
- Many different genetic polymorphisms have been implicated in HELLP syndrome suggesting a complex genetic interaction. Polymorphisms that have been identified include changes in chromosome 12q, *TLR4*, factor V Leiden, *MTHFR* (C677T) and polymorphisms in the glucocorticoid receptor.

Table 7.2 The incidence of liver disease specific to pregnancy and the trimesters in which each is most likely to occur.

	Intrahepatic cholestasis of pregnancy (ICP)	Pre-eclampsia/hemolysis, elevated liver enzymes and low platelet syndrome (PE/HELLP)	Acute fatty liver of pregnancy (AFLP)
Incidence	1.5%	PE: 5–7% HELLP: 0.2–0.6%	0.005–0.01%
Onset	(2–) 3 trimesters Multiple pregnancy	3 (postpartum)	3 (postpartum) First pregnancy
Family history	Often	No	Sometimes
Hypersensitivity	Contraceptives	No	No
Pre-eclampsia	No	Always (edema)	50% (edema)
Symptoms	Pruritus	Abdominal pain Nausea Vomiting Headache	As in HELLP Icterus Ascites Encephalopathy
Investigations	Bile acids Bilirubin (↑) Transaminases ↑ Coagulation (↓)	Hemolysis ↑ + Platelets ↓ Bilirubin (↑) Transaminases ↑–↑↑ (Coagulation ↓↓↓)	Platelets (↓)–↓↓↓ Bilirubin (↑)–↑↑↑ Transaminases ↑–↑↑↑ Coagulation (↓)–↓↓↓
Imaging (MRI/US)	Normal (gallstones)	Hematoma rupture/infarction	Fatty liver
Histology	Normal Mild cholestasis	Necrosis Bleeding	Diffuse fatty infiltration Microvesicular fat
Mortality	Fetus 0.4–1.4% Mother 0%	Fetus 10% Mother 1–10%	Fetus 10–20% Mother 1–10%
Recurrence	45–70%	4–19%	Long-chain 3-hydroxyacyl-CoA dehydrogenase defect

MRI, magnetic resonance imaging; US, ultrasound.

Demographics

A pregnancy may be complicated by HELLP syndrome in a mother of any age, though it is more common in those older than 35 years, or ethnicity. It affects primiparous or multiparous women, with an incidence of 0.11% of liveborn deliveries. The majority (70%) are diagnosed in the third trimester, while the remainder is identified during the first 72 h postpartum.

Maternal clinical features

- Malaise.
- Right upper quadrant pain or tenderness on palpation.
- Blood pressure is raised above the pre-pregnancy baseline (19% of mothers have pre-existing chronic hypertension), but there may only be mildly raised nausea and vomiting.
- Features of hypertension, such as headache, edema, proteinuria, blurred vision, and hyperreflexia may be the presenting conditions in 10%.
- Disseminated intravascular coagulation (DIC) and renal failure occur in severe disease (7%).
- Placental abruption (16%).
- Pulmonary edema (6%) is more common following abruption.
- Hepatic rupture presenting as profound shock occurs in 1% with HELLP syndrome.
- Postpartum bleeding may be severe in the setting of thrombocytopenia.

Sixty percent of women will require intensive care with 10% of cases resulting in severe morbidity for the mother or child.

Laboratory findings

Hemolysis

The only constant diagnostic feature is decreased serum haptoglobins. Markers of microangiopathic hemolytic anemia (burr cells and schistocytes) and reticulocytosis with polychromasia, anisocytosis, and poikilocytosis are also frequently present.

Liver transaminases

These are typically raised two to three standard deviations above the mean.

Thrombocytopenia

A platelet count of <100,000/μL is taken to define thrombocytopenia. The platelet count continues to drop to a nadir at 24–48 h after delivery. Although the platelet count may respond to steroid therapy, other features (elevated liver enzymes, hemolysis, hypertension, and oliguria) are often not benefited.

Histology of the liver

Liver biopsy is not necessary if there is diagnostic certainty. Liver involvement is similar to the findings in pre-eclampsia (PE). The main features are periportal neutrophilic infiltrates, hepatocyte necrosis, fibrin microthrombi in portal vessels, and fibrin deposition within the sinusoids (Figure 7.1). Fatty change in hepatocytes has also been described, as in acute fatty liver of pregnancy (AFLP), suggesting that the three entities may fall within the spectrum of the same disease.

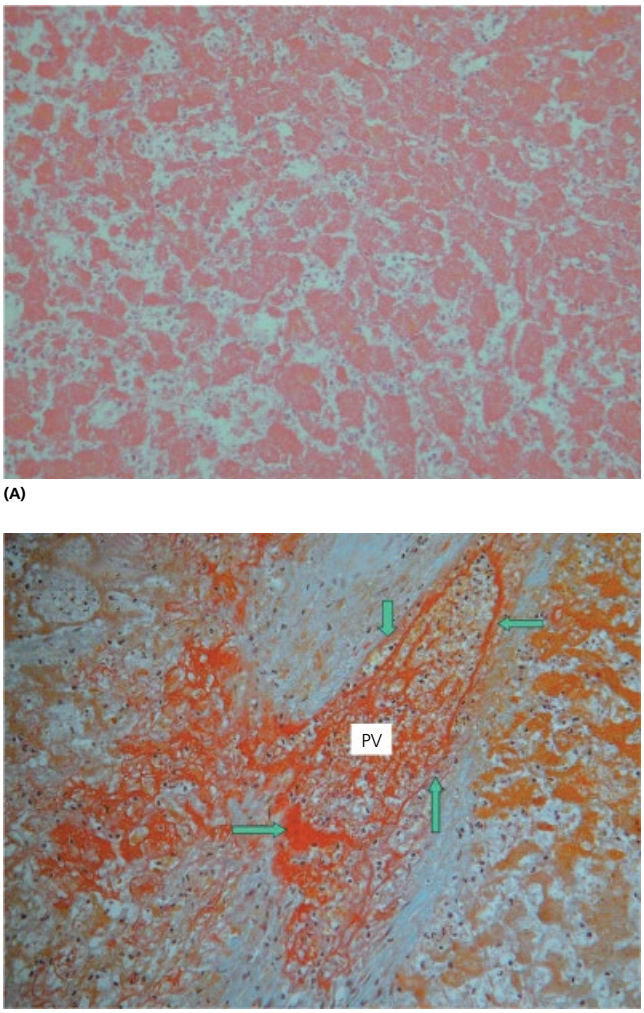

Figure 7.1 Liver histology from the resected infarcted liver in hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome. (A) An area of coagulative ischemic-type necrosis and focal infiltration by neutrophils. (H&E, original magnification × 40.) (B) Martius scarlet-blue stain, showing fibrin in the periportal sinusoids (stained red) and occluding a portal vein branch (PV).

Imaging

Ultrasonography and computed tomography (CT) are normal in HELLP syndrome unless it is complicated by hepatic rupture or infarction (Figure 7.2). Serial CT scans are the best method of monitoring the size and extent of the rupture. This will also aid planning of surgery if required.

Management of the mother

- The risk to the mother and the fetus increases with prolongation of the pregnancy, so that aggressive management and delivery are generally advisable. Vaginal delivery is possible, but a cesarean section is often necessary. The majority of

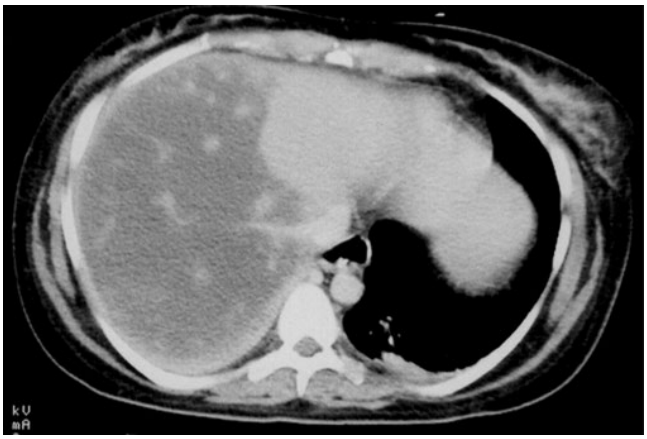

Figure 7.2 The liver in hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome. Infarction of the liver is a rare complication of HELLP syndrome. This computed tomographic image of the liver shows hemi-infarction of the right lobe. The main portal vein is patent, but the right portal vein branch has been occluded. Treatment is usually conservative.

deliveries will be preterm via cesarean section (42–98%), with an increased risk (7–14%) of wound hematoma and infection. Complete normalization of laboratory indices has been reported in some women with continuation of pregnancy, but there is a higher risk of stillbirth. In a study of 25 patients, it was found that steroids temporarily stabilized HELLP syndrome by increasing the platelet count, reducing liver enzymes, and increasing urine output.

- Blood transfusions may be required. The indication for platelet support is controversial, as there is rapid platelet consumption, with no incremental rise. When cesarean sections were studied, bleeding from the wound site was not seen to have diminished.
- Hypertension should be controlled with standard medication.
- Renal support with continuous venovenous hemofiltration.
- In those who develop a liver hematoma, no intervention is required if the mother is hemodynamically stable. However, rupture is a major surgical emergency requiring evacuation and drainage of the blood, packing for tamponade, or suturing by experienced hepatobiliary surgeons.
- Hepatic infarction may lead to hepatic insufficiency, requiring management of acute liver failure.

Seventeen liver transplants for HELLP syndrome have been reported, the main indication being liver necrosis and liver failure following rupture. Two patients required total hepatectomy prior to transplantation, to control bleeding. Fourteen of the patients were alive at follow-up, suggesting that liver transplantation is appropriate in this setting.

Fetal presentation

- One-third will have intrauterine growth restriction.
- Placental abruption may lead to fetal death without expedient delivery.
- In those born alive, perinatal mortality has been reported as between 5–20%; it is related to the complications of preterm delivery, and is comparable with those born preterm in the absence of maternal HELLP syndrome. The mean gestational age at delivery is 32 weeks, ranging from 24 to 39 weeks' gestation.
- The neonate may have thrombocytopenia and leukopenia, but these findings are not specific to HELLP syndrome and may also be found in PE.
- There is no increase in liver enzymes in neonates born to mothers with HELLP syndrome.

Management of the neonate

As with PE, up to half of the babies born to mothers with HELLP syndrome will be small for gestational age. The long-term outcome for the infant depends on the extent of prematurity. The neonate should be managed initially on a neonatal intensive care unit.

Risk in future pregnancies

There is a risk of recurrence in subsequent pregnancies of 3–19% for HELLP syndrome and a 23% risk for PE.

Acute fatty liver of pregnancy

AFLP is a consequence of defective mitochondrial fatty acid β-oxidation in the infant, with a high potential for both maternal and fetal morbidity and mortality [6].

Mitochondrial β-oxidation

Mitochondrial long-chain fatty acid β-oxidation provides the major source of energy for cardiac and skeletal muscle and intermediary metabolism of ketone production by the liver in the fasting state. A trifunctional protein catalyzes the final three steps in the formation of acetyl-CoA from long-chain fats. Acetyl-CoA then enters the tricarboxylic acid pathway, which results in energy generation by the liver. The three enzymatic steps for the trifunctional protein are:

1. 2,3-enoyl-CoA hydratase.
2. Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD).
3. Long-chain 3-keto-acyl-CoA thiolase.

Two biochemical disease phenotypes have been described:

1. Complete deficiency of all three enzymes of the trifunctional protein.
2. Isolated deficiency of LCHAD.

Genetic analysis

A mutation resulting in a complete trifunctional protein deficiency of the fetus causes disease in the child only and has no effect on the mother.

- Nineteen percent of infants born to women with AFLP have mutations leading to LCHAD deficiency in the fetus, which causes disease in the child and may result in AFLP in the mother.

- A fetus carrying the G1528C mutation (amino acid residue E474Q; homozygous or compound heterozygote) results in disease in the child and AFLP in the mother in 79% of cases (whether the mutation was inherited from the mother or father).
- A fetus with other hitherto detected mutations of LCHAD does not cause AFLP in the mother.
- A heterozygous fetus (one wild type allele) does not cause AFLP in the mother. Infants born to mothers with AFLP should always be screened for LCHAD deficiency. Not all cases of AFLP can be attributed to fetal LCHAD deficiency, and other causes of abnormal mitochondrial oxidation therefore need to be considered, including drugs (aspirin and tetracyclines have been reported as causing AFLP). A case report of CPT I deficiency identified two siblings with mild symptoms whose mother developed AFLP whilst carrying them.
- AFLP has also been described as the presenting feature of maternal previously undiagnosed, medium-chain acyl-CoA dehydrogenase deficiency, with no effect on the fetus.

Maternal presentation

AFLP usually affects women in the third trimester of pregnancy, although it has been reported exceptionally as early as 26 weeks' gestation. The clinical picture can vary between almost asymptomatic forms and fulminant liver failure.

AFLP affects women of any ethnic group or age and is more common in multiple pregnancy and in women with PE. In contrast to PE it can equally affect first or subsequent pregnancies. The reported incidence has ranged from 1 in 13,000 pregnancies to 1 in 6659, and in a recent prospective study from Swansea, Wales, it reached 1 in 1000. In the latter study, six or more of the following features were accepted as being diagnostic of AFLP:

- Vomiting.
- Vague abdominal pain.
- Polydipsia/polyuria.
- Encephalopathy.
- Elevated bilirubin (bilirubin rises in severe AFLP and in contrast to HELLP syndrome is not related to hemolysis).
- Hypoglycemia (profound).
- Elevated urate.
- Leukocytosis (common).
- Ascites or bright liver on ultrasound scan.
- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are moderately raised (rarely above 10 times normal).
- Elevated ammonia.
- Renal impairment.
- Coagulopathy (with disseminated intravascular coagulopathy (DIC) in 10%).
- Microvesicular steatosis on liver biopsy.

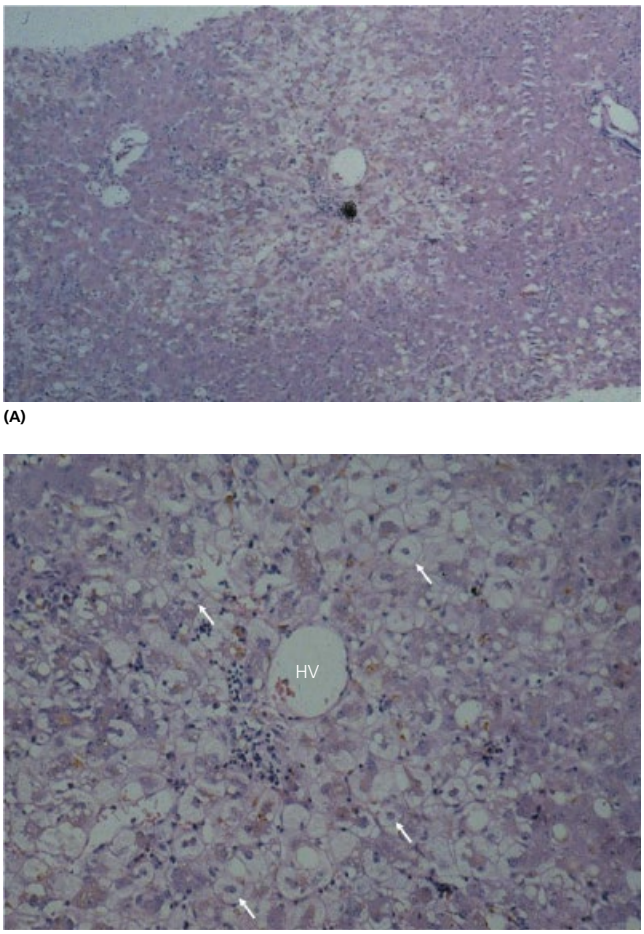

Figure 7.3 Acute fatty liver of pregnancy. (A) The typical distribution of fat accumulation in zones 2 and 3 surrounding the hepatic venule (HV), with relative sparing of zone 1 hepatocytes surrounding portal tracts. (B) The microvesicular nature of the fatty droplets, which accumulate without displacing the nucleus from its central position (arrows).

Histology of the liver

Liver biopsy is not normally required in the acute situation, but if performed should be via the transjugular route. Biopsy within the week following parturition will allow confirmation of the diagnosis.

The liver is typically small, with microvesicular steatosis in a pan-acinar distribution or centrilobular with minimal inflammation (Figure 7.3). Other changes are intrahepatic cholestasis and extramedullary hemopoiesis, and when there is loss of hepatocytes, Kupffer cells aggregate. Following delivery, the lipid rapidly mobilizes to become undetectable after a month, with normal hepatic architecture and no chronic changes.

Imaging

Ultrasonography

There is increased echogenicity, reflecting the increased fat in the liver.

CT scan
There is decreased attenuation of the liver in comparison with the spleen (usually the spleen is of lower attenuation than the liver).

Maternal management
Prompt diagnosis with expeditious delivery is recommended, as resolution of symptoms can only occur following delivery. However in up to 25% of cases there is continued progression of clinical features following delivery and therefore careful monitoring during the first month after delivery is mandatory. Delivery may be vaginal, but in the face of acute liver failure requires emergency cesarean section. Meticulous management of liver failure and encephalopathy should lead to resolution of the symptoms with no long-term sequelae. Maternal death is related to the severity of liver disease, with raised intracranial pressure and gastrointestinal bleeding being reported as contributing to death in 12%. In those who develop fulminant hepatic failure, successful liver transplantation has been performed. Most mothers recover completely. However, in women with LCHAD mutations, the risk of recurrent AFLP is 20–70%. These women need careful surveillance during pregnancy with advice to follow a low-fat, high-carbohydrate diet and to avoid fasting.

Clinical features in the child
Fetal presentation
Late maternal diagnosis increases fetal mortality. There is an increased incidence of prematurity, asphyxia, intrauterine growth retardation, and intrauterine death, which may be related to uteroplacental insufficiency.

Infancy
There is a genotype–phenotype correlation. Symptoms develop when there are periods of fasting or intercurrent illness.

Those with isolated LCHAD deficiency present with:
- Hepatic failure, which may lead to death.
- Hepatomegaly.
- Non-ketotic hypoglycemia.
- Encephalopathy.
- Sudden death.
- Hypocalcemia and cholestasis.

Those with complete trifunctional protein deficiency present with:
- Dilated cardiomyopathy.
- Peripheral neuropathy and myopathy.
- Rhabdomyolysis.
- Peripheral neuropathy and retinitis pigmentosa in long-term survivors

Laboratory investigations during the acute episode show:
- Hypoglycemia with low ketones.
- Elevated transaminases.
- Elevated prothrombin time.
- Increased ammonia.
- Increased urate and urea.
- Elevated plasma acyl carnitines.
- Urinary organic acids show an increase in 3-hydroxy dicarboxylic acids.
- Secondary carnitine deficiency.

Liver histology
There is usually microvesicular fat deposition in hepatocytes, although some macrovesicular deposition may be present.

Genetics
Mutation analysis of the trifunctional protein should be carried out.

Treatment of the child
All children born to mothers with AFLP should be treated as having LCHAD deficiency until the molecular investigations have been completed:
- Frequent feeding, which includes overnight nasogastric feeding to avoid prolonged fasting.
- Low-fat and high-carbohydrate diet to reduce the long-chain fatty acid load.
- Medium-chain fats should replace most long-chain fats in the diet.
- Early intervention with intravenous fluids during intercurrent illness.
- Treatment of acute liver failure may be required.

Despite optimal treatment, sudden death can still occur and is most likely in the setting of an intercurrent illness, which may only be mild. Parents should be counseled as to the need for intravenous glucose even during mild infections.

Screening
All children born to mothers with AFLP should be screened for LCHAD deficiency by molecular analysis.

In mothers whose child is a compound heterozygote or homozygote for LCHAD deficiency, there is a 1 in 4 risk in future pregnancies. Chorionic villus sampling in subsequent at-risk pregnancies has been used to assess the risk of AFLP in pregnancy and LCHAD deficiency in the fetus (see Chapters 9 and 19).

Intrahepatic cholestasis of pregnancy
Intrahepatic cholestasis of pregnancy (ICP; also known as obstetric cholestasis) is the most common liver disease in pregnancy [7] and is characterized by:
- Otherwise unexplained pruritus in the late second and the third trimester of pregnancy.
- Elevated bile acids (≥10–14 μmol/L; repeated testing might be necessary) and/or transaminases.
- Spontaneous relief of symptoms and complete normalization of biochemical aberrations within a few weeks after delivery.

Diagnosis

- Pruritus is the main presenting symptom causing discomfort, insomnia, and fatigue.
- Transaminases (ALT/AST) are elevated in about 80% of cases. ALP levels are of limited diagnostic value due to large amounts of the placental isoform in the third trimester. Levels of γ-glutamyl transpeptidase (GGT) are commonly normal. Elevation of bilirubin is found in 10–20% of women with ICP and may indicate a severe form. Overt jaundice is more suggestive of viral hepatitis.
- The resolution of symptoms and normalization of hepatic biochemistry after delivery help distinguish ICP from other liver conditions, such as hepatitis C or primary biliary cirrhosis that may first be unmasked in pregnancy. The mother should be followed up to ensure normalization of liver function test 6–12 weeks after delivery, irrespective of persisting pruritus, and referred to a hepatologist for further evaluation if required [8].

Demographics

In Europe, ICP occurs in 0.1–1.5% of pregnancies. The incidence is highest in Chile, the Baltic states, Scandinavia, and Bolivia, with up to 15% of pregnancies being associated with ICP. Over the past decade, a trend toward lower incidence has been observed in Chile, possibly due to increased micronutrients such as selenium in an improved diet.

ICP is more common in advanced maternal age and likely to recur in subsequent pregnancies and is five times more common in multiple pregnancies. It may be sporadic or run in families. There is seasonal variability, with more cases occurring in winter.

Pathogenesis

The sex hormones play a major role in the pathogenesis of ICP. Specific alterations in progesterone and bile acid metabolism have been defined. It has been suggested that there is a combination of increased synthesis and impaired biliary excretion of sulfated progesterone metabolites. The impaired biliary excretion occurs in individuals who have otherwise subclinical mutations within genes coding for hepatobiliary transport proteins, whose capacity for efficient biliary excretion is exceeded in the hormonal milieu of pregnancy.

Genetics

The increased incidence in certain populations, family clusterings, and recurrence in subsequent pregnancies supports a genetic etiology. This led to the investigation of genes involved in other cholestatic disorders, progressive familial intrahepatic cholestasis (PFIC) and benign recurrent intrahepatic cholestasis (BRIC).

- PFIC type 1 is caused by mutations in the *ATP8B1* gene. The gene encodes an aminophospholipid P-type adenosine triphosphatase (ATPase) that transports phosphatidylserine and phosphatidylethanolamine from one side of the membrane lipid bilayer to another. In PFIC1 and BRIC1, GGT is normal or low. Two *ATP8B1* mutations (D70N and R867C) have been found in four women with ICP and normal GGT.
- Bile salt export pump (BSEP) deficiency is caused by mutations in the *ABCB11* gene leading to PFIC2 and BRIC2, also with low or normal GGT. In a large cohort a key role for ICP susceptibility has been identified for common variation around the *ABCB11* (V444A polymorphism) and *ABCB4* loci.
- The *ABCB4* or multidrug resistance 3 (*MDR3*) gene encodes the hepatobiliary phospholipid transporter, mutations in which cause PFIC3, which differs from PFIC1 and PFIC2 by having a raised GGT.
- Bile acids are ligands to the nuclear receptor farnesoid X receptor (FXR) that in addition to bile acid homeostasis also regulates glucose and lipid metabolism. *ABCB11* and *ABCB4* are among FXR target genes, and in some cases functional variants in the *FXR* gene were found to be associated with a predisposition to ICP.
- Overall, heterozygous mutations and polymorphisms in *ABCB4*, *ABCB11*, *ATP8B1*, and *FXR* were found in up to 14% of women with ICP [9].

Hormonal

A hormonal role in the etiology of ICP is suggested by the observation that symptoms are most prevalent in the third trimester of pregnancy when sex hormones are at their highest, in multiple pregnancies, the induction of cholestasis by oral contraceptive pills containing estrogen in a high dose or by progesterone treatment. When the hormone levels fall after delivery, symptoms tend to improve rapidly.

There is a significant alteration in the ratio of different progesterone isomers in ICP in comparison with other causes of liver disease in pregnancy, the cause and effect of which are not known. Sulfated progesterone metabolites were recently found to inhibit the hepatic uptake of bile acids and also to decrease their excretion via inhibition of FXR-dependent pathways [10].

The role of bile acids as the cause of pruritus is unclear. Serum bile acid levels only weakly reflect the degree of itch, but are of important prognostic value. Decreased urinary excretion of sulfated progesterone metabolites, indirectly indicating enhanced biliary excretion during treatment with ursodeoxycholic acid (UDCA), was associated with improvement of pruritus whereas bile acids were not. Recent studies indicate that pruritus in cholestatic liver disease including ICP may be caused by phospholipid metabolites such as lysophosphatic acid.

Clinical presentation

ICP usually presents in the second or third trimester, but may occasionally occur as early as the first.

- Cholestatic pruritus presents as an irresistible desire to scratch with no skin changes to be seen except for excoriations, with the palms and soles of the feet especially

affected, and with highest intensity in the night, which causes insomnia and fatigue.
- Excoriation may be seen on clinical examination after longstanding pruritus.
- Steatorrhea and fat-soluble vitamin malabsorption are observed after longstanding cholestasis.
- There are no signs of chronic liver disease, and the liver is of normal size and not tender.
- Jaundice develops in 10%.

Histology

Liver biopsy is rarely undertaken unless there is diagnostic uncertainty.

Intracellular bile pigment and canalicular bile plugs are seen in the absence of any other histological abnormalities. Electron microscopy shows dilated canaliculi with loss of microvilli.

Maternal management

Management consists of [11]:
- Relief of pruritus.
- The guidelines of the European Association for the Study of Liver (EASL) recommend UDCA (10–20 mg/kg/day) as the first-line treatment for ICP as it also improves liver function tests in many cases. A number of randomized trials however have not shown improvements in maternal or fetal outcome with UDCA.
- Dexamethasone is not recommended for the treatment of pruritus but may be the preferred steroid administered for lung maturation in very early delivery (<33 weeks of gestation). Cholestyramine, which is first-line treatment in other forms of cholestatic pruritus, is not sufficiently effective in ICP and thus not recommend. It may also worsen malabsorption of fat-soluble vitamins.
- Rifampicin is highly effective as second-line treatment in women not sufficiently responding to UDCA, but has not been subjected to a controlled trial. After a start dose of 600 mg/day for 3–4 days, rifampicin may be tapered down provided that pruritus improves. If there is no improvement after 1 week of treatment, rifampicin should be stopped.
- Fat-soluble vitamin supplementation, should be given to those with longstanding cholestasis, alongside nutritional support. Postpartum bleeding has been reported in this group, which may be secondary to vitamin K deficiency.
- Delivery of the fetus. Induction of labor at gestational weeks 37–38 has been advocated to prevent obstetric complications by prolonged pregnancy and to possibly reduce the risk of stillbirth, since stillbirth in ICP in previous studies tended to cluster at this age of gestation. This kind of active management of ICP is already common practice in many countries, despite lack of evidence. Induction of labor at gestational weeks 37–38 does not result in higher rates of emergency cesarean section.

Effect on the child

Risks to the child include [12]:
- Increased risk of preterm delivery (19–60%).
- Fetal distress (22–41%), as indicated by meconium liquor and asphyxia.
- Fetal loss (0.4–1.6%).
- In the largest prospective observational study from Sweden that identified 693 cases of ICP among 45 485 pregnancies, a 1–2% increase in the risk of spontaneous preterm labor, asphyxial events (defined as operative delivery due to asphyxia, Apgar score <7 at 5 min or arterial cord pH <7.05), or meconium staining of the amniotic fluid and/or placenta and membranes was observed for every additional μmol/L of maternal serum bile acids. However, this study did not find an increase in adverse outcomes in mild ICP and moderately elevated bile acid levels (10–40 μmol/L) as compared to women with pruritus but normal bile acid levels (<10 μmol/L). Thus the authors concluded significant increased risk for the fetus to occur at maternal serum bile acids >40 μmol/L.
- A recent large prospective population-based case–control study from the UK confirmed the increased risk of intrauterine fetal death at least in severe ICP, defined as serum bile acids >40 μmol/L (aOR 2.58, 95% CI, 1.03 – 6.49). Doubling of serum bile acid levels correlated with a 200% increase in the risk of intrauterine fetal death.
- The pathogenesis of fetal complications is not fully understood. In stillborns, the postmortem findings are non-specific. The fetus is usually well grown, and surveillance of the fetal–placental unit is normal, suggesting that placental insufficiency is not the cause. Evidence of a possible pathogenetic role for bile acids comes from *in vitro* and laboratory animal studies. Elevated bile acids might contribute to preterm delivery by increasing oxytocin activity and may have a role in unexplained intrauterine fetal death by inducing vasoconstriction of chorionic veins, oxidative stress in the placenta and increased apoptosis in the fetal liver, or triggering of arrhythmia in fetal cardiomyocytes. ICP-typical changes in placenta morphology have been described.
- Methods used to monitor the fetus, such as umbilical artery Doppler ultrasound, are poor predictors of fetal outcome. Neonatal resuscitation facilities and intensive care unit should be available at delivery. In those neonates who are born healthy, there are no long-term complications.

Long-term maternal complications

ICP resolves within a few weeks of delivery, with no longlasting effects. However, studies from Finland and Sweden showed that women with ICP have a 3–5 times increased risk of hepatobiliary diseases, such as hepatitis C, cirrhosis, and gallstones. This increased risk was found both before and after ICP diagnosis and was not related to age at first ICP, number of earlier pregnancies, or smoking

status. In an extension of the Swedish study that included in total more than 125,000 pregnancies, a 2.5 times higher risk for cancer in the biliary tree and even a 3.5 times increased risk of liver cancer was found for women once diagnosed with ICP. Even after adjusting for a diagnosis of hepatitis C, which was very strongly associated with liver cancer, women with ICP were still at 2.5 times increased risk of later liver malignancy [13]. Women with ICP were also found to have about 25% increased risk to be later diagnosed with immune-mediated diseases, in particular diabetes mellitus and Crohn disease but not ulcerative colitis. There was also a small increased risk of later cardiovascular disease, in particular if the woman with ICP also suffered from PE. Of note, in addition to PE, ICP is also associated with gestational diabetes. Both are common complications of pregnancy that increase the risk of ICP about three-fold [14].

Neonatal liver pathology secondary to maternal disease

Gestational alloimmune liver disease (see also Chapter 10)

Gestational alloimmune liver disease (GALD) presents with neonatal liver failure or fetal death due to intrahepatic and extrahepatic (but sparing the reticuloendothelial system) siderosis [15]. Historically the disease was known as neonatal hemochromatosis due to the high iron and ferritin levels. The term GALD now recognizes the underlying alloimmune-mediated pathology.

Pathogenesis

There is complement-induced liver injury mediated by maternal alloantibodies. This is seen by the identification of C5b-9 complement complex on immunohistochemistry of the neonatal liver.

Maternal factors that have been associated with GALD are systemic lupus and anti-Ro anti-La antibodies. The disease is rare in firstborn infants. Following the birth of an affected child there is a high recurrence rate in subsequent pregnancies of up to 80%. However there is variability and normal neonates have been reported in between affected siblings. The mother is usually well throughout the pregnancy.

Diagnosis

This is suspected in a neonate with liver failure that may have progressed to cirrhosis (confirming that the pathology was initiated *in utero*). The clinical features are variable with some infants only having mild disease. The diagnosis is made by demonstrating siderosis in the liver and extrahepatic sites such as in biopsies of minor salivary glands. Abdominal magnetic resonance imaging demonstrates loss of signal on T2-weighted images of the liver, indicating iron accumulation, whilst the spleen is normal (the reticuloendothelial system is spared). The signal intensity of the pancreas has been reported as variable. Histology of the liver demonstrates intracellular iron that spares the Kupffer cells and stains positively for the terminal membrane attack complement complex C5b-9.

Treatment

This is as follows.

- Neonate: untreated, the disease is fatal in up to 60% of neonates. A combination of intravenous immunoglobulin (IVIG) and plasma exchange reduces the antibody load leading to stabilization or improvement in the liver failure. If commenced early, an antioxidant cocktail (selenium, desferrioxamine, *N*-acetylcysteine, and prostaglandin E_1) improves the outcome in those with mild features, but the majority require liver transplantation. The outcome of transplantation for GALD is comparable with transplantation for other causes of neonatal liver failure and there is no recurrence of disease in the child.
- Mother: it is advisable that from 18 weeks' gestation in subsequent pregnancies, the mother should receive IVIG on a weekly basis (1 g/kg). This has been reported to modify the disease process, with either mild or no signs of neonatal hemochromatosis in subsequent newborns.

Viral hepatitis in pregnancy

Acute viral hepatitis secondary to hepatitis A, B, or C, herpes simplex virus (HSV) or cytomegalovirus (CMV) is the most common cause of jaundice during pregnancy. There is no increase in the incidence of acute hepatitis A, B, or C in pregnancy as compared to the normal population. An acute hepatitis may precipitate preterm labor but otherwise there is no detriment to the fetus and hence no recommendations for abortion or cesarean section are necessary. In contrast, hepatitis E, which is endemic in large areas of Asia, Africa, and Central America, is very serious for pregnant women with an increased risk of a fulminant course and a maternal mortality rate as high as 25%.

Hepatitis A

Acute hepatitis A has no increased risk to the mother. Treatment is supportive. Late hepatitis A can contribute to premature birth. Neonates born to mothers with acute hepatitis A in the month prior to delivery may acquire self-limiting infection. In acute hepatitis A in the third trimester, the child must receive hyperimmune globulin within 2 days after birth.

Fetal ascites and calcification have also been seen. Meconium peritonitis of the fetus has been reported following maternal infection, leading to ileal perforation and requiring resection in the newborn period.

Hepatitis B (see Chapter 13)
Mothers with hepatitis B are usually asymptomatic during pregnancy, although there are case reports of maternal liver transplant during pregnancy for acute liver failure due to hepatitis B.

Hepatitis B vertical transmission usually occurs at the time of delivery on exposure to blood and rarely in utero as the placenta provides an immune barrier. The transmission rate is dependent on the mother's infectivity at the time of delivery. Mothers who develop acute hepatitis B in the last trimester have a 70% chance of infecting their infant whilst mothers who are HBeAg negative with low viral load, the transmission rate is lower. All babies born to mothers with hepatitis B however should be vaccinated and receive hepatitis B immunoglobulins (HBIG) which provide protection against perinatal transmission in over 95% of cases. Without successful vaccination, the majority of infected infants become "healthy carriers," but a minority of those born to mothers who despite being HBeAg negative, have high hepatitis B virus DNA titers due to the precore mutant form of the virus, have a high risk of fulminant hepatic failure from 6 weeks to 6 months of age.

Antiviral treatment with lamivudine or tenofovir should be considered in the third trimester for mothers with high virus concentrations (HBV DNA log 7–8 IU) and are considered as low risk, even in the first trimester. Only limited data are available on the use of adefovir, dipivoxil, or entecavir during pregnancy, hence their use is not recommended.

Breastfeeding should be encouraged for infants receiving HBIG and vaccination. However, there is insufficient evidence that breastfeeding is safe in mothers receiving antiviral therapy and thus mothers on lamivudine, telbivudine, or tenofovir should be discouraged from breastfeeding.

Hepatitis C (see Chapter 13)
Mothers with hepatitis C are usually asymptomatic during pregnancy. Treatment of chronic hepatitis C with pegylated interferon plus ribavirin is contraindicated during pregnancy. Ribavirin is known to be teratogenic and can be traced in blood 4 weeks after exposure, so an exposure-free period of at least 6 months is recommended before conception. Highly effective new interferon- and ribavirin-free hepatitis C virus treatment regiments have not yet been tested in pregnancy. If the mother is coinfected with human immunodeficiency virus (HIV) retroviral drugs should be continued so as to avoid HIV infection of the baby during birth.

There is a 3–5% vertical transmission rate, with transmission occurring either *in utero* or during delivery. Infectivity is not related to genotype but is related to viral load; it is increased if there are $>10^7$ RNA copies/mL and if the mother has an acute infection and is hepatitis C virus IgM positive, has concomitant infection with HIV, prolonged rupture of membranes, and the use of invasive monitoring. Cesarean section does not reduce transmission rates. Immunoprophylaxis for the newborn is not available. Although hepatitis C virus is found in breast milk, breastfeeding is considered to be safe for babies born to hepatitis C positive mothers and should be encouraged.

Hepatitis C does not cause acute liver failure in infancy, but carriers progress to a chronic hepatitis C virus infection, with the risk of fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) in adulthood.

Hepatitis E (see Chapter 13)
Hepatitis E is a self-limiting viral infection spread by the fecal–oral route. Hepatitis E is found worldwide and different genotypes of the hepatitis E virus determine differences in epidemiology. Genotype 1 is usually seen in developing countries and causes community-level outbreaks while genotype 3 is usually seen in the developed countries and does not cause outbreaks. In pregnant women, hepatitis E has a high mortality rate, which increases throughout pregnancy with up to a 27% mortality rate in the third trimester. The fetal outcome is poor, with one series reporting a 70% mortality rate due to stillbirth and maternal death. China has produced and licenced the first vaccine to prevent hepatitis E virus infection, although it is not yet available globally.

Herpes simplex virus
Both HSV type 1 and type 2 can cause severe disease in the neonate, either through direct shedding at the time of delivery or in primary transplacental infection, leading to miscarriage, stillbirth, and congenital malformations.

Primary herpes infection may be more severe in pregnant women, with dissemination leading to hepatitis, encephalitis, thrombocytopenia, coagulopathy, and a mortality rate of up to 50%. Early delivery of the child, followed by liver transplant, have been reported for fulminant liver failure in pregnancy.

The incidence in the UK is rising with current rates being 17.5 per 100 000 live births.

Genital herpes has the highest risk to the neonate at the time of delivery, due to direct viral shedding.

In the neonate, it may present as:
- Localized infection of the skin, eyes, and mucous membranes.
- Infection of the central nervous system, which has a 5% mortality rate, with 50% of survivors having neurological impairment.
- Early-onset disseminated infection, presenting with acute liver failure, with a high mortality rate unresponsive to antiviral therapy in the setting of multiorgan failure.
- Skin lesions are often not seen until late in the disease.

Neonatal herpes prevention
Active genital herpes should be treated with aciclovir or valaciclovir in the weeks prior to delivery. Women with active genital infection at the time of delivery should be offered a cesarean section, as the likelihood of neonatal transmission is high.

A high index of suspicion is needed in a septic neonate to commence aciclovir treatment early before the onset of multi-organ failure. Single cases have been reported of liver transplantation being a successful treatment in herpes simplex infection isolated to the liver; however in most cases the infection is disseminated and transplantation is contraindicated.

Contraception

Hepatic complications of combined oral contraceptives

The combined oral contraceptive pill (COCP) has an efficacy of 99.9%. It reduces menstrual irregularities and premenstrual tension, as well as reducing the risk of fibroids, benign breast disease, and ovarian and endometrial cancer.

No pre-existing liver disease

Cholestasis

This occurs in 10 per 100,000 in the West, and as with ICP (see earlier), it has a higher incidence in Chile and Scandinavia (25 per 100,000). It is more common in those who have had ICP, or in whom there is a family history of jaundice in pregnancy or whilst taking the COCP. It tends to occur within 3 months of commencing an estrogen-containing COCP.

Hepatic adenomas

There is an increased risk of adenoma formation, which is associated with the length of use of the COCP and the strength of the estrogen component. With the introduction of COCPs containing lower estrogen levels, the incidence of adenoma has fallen. Adenomas may regress when the COCP is stopped but return again following reintroduction of the COCP or during pregnancy. Hepatic adenoma constitutes a contraindication to pregnancy, and in patients suitable for resection should be removed before conception occurs. Adenomas caused by estrogens are vascular and may rupture, with hemorrhage into the adenoma or peritoneal cavity.

Focal nodular hyperplasia

Of those with focal nodular hyperplasia (FNH) 50–75% use the COCP, suggesting that estrogen may be a contributing factor. FNH does not increase any risks during pregnancy.

Hepatocellular carcinoma

There is an increased risk of HCC with long-term use of COCPs containing high levels of estrogen. Cofactors such as hepatitis B and alcohol may increase the risk. Tumors may regress following cessation of the COCP.

Budd–Chiari syndrome

The procoagulant effect of the COCP increases the risk of Budd–Chiari syndrome 2.5-fold. Nine percent of cases of Budd–Chiari are attributed to COCP use. In a case series of 13 women taking the COCP who developed hepatic vein thrombosis, 10 had an underlying prothrombotic condition, suggesting that the development of Budd–Chiari syndrome should prompt investigation for other thrombophilic disorders. Budd–Chiari syndrome is associated with factor V Leiden (FVL) mutations whereas G20210A prothrombin mutation has not been identified.

Women with established liver disease

Fertility may be reduced in advanced chronic liver disease, but contraception is an important issue for the majority.

Gallstones

There is an alteration in the composition of bile in those taking the COCP, with an increase in cholic acid and a decrease in chenodeoxycholic acid, which may increase the risk of cholelithiasis. There is no consensus from studies as to whether this is the case, but due to the potential risk it is advised that those with a history of biliary colic should avoid the COCP. There is a very small increased risk of developing symptomatic gallstone disease in those taking the COCP. However, clinically meaningful recommendation cannot be given based on this finding.

Hepatocellular carcinoma

The cirrhotic liver is at risk of developing HCC. Some studies have reported an increased incidence of HCC in chronic liver disease in those using the COCP, but this has not been verified in subsequent studies. Due to this uncertainty, it is recommended that COCPs should be avoided in chronic liver disease.

After transplantation

Delay conception for 1 year following liver transplantation. COCPs have been used safely if there have been no thrombotic complications in the peritransplantation period. An increase in ciclosporin levels has been reported in concomitant use of the COCP, and levels should therefore be monitored closely.

Other forms of contraception in liver disease

Progesterone-only oral and depot contraception is the preferred contraceptive in all forms of liver disease, as it avoids the estrogen component of the combined contraceptive. The pill has to be taken within the same 3 h every day to ensure efficacy. There are no hepatic complications. The progesterone-only pill is safe following liver transplantation and is recommended when there have been thrombotic complications after transplantation.

Intrauterine device

The intrauterine device (IUD) is 99% effective for 1–5 years. It can be used in all forms of liver disease except for Wilson disease, in which the copper coil component of the

IUD may potentially increase serum copper levels. In the post-transplant patient, there have been reported cases of IUD failure due to immunosuppressants reducing the immunological mechanism of the IUD. There is also a potentially increased risk of urinary tract infections.

Barrier contraception

In the context of hepatitis B and C infection, this form of contraception should be advised in order to avoid sexual transmission of the infections. The efficacy is 80–95%. There are no contraindications to the use of this form of contraception.

Pregnancy

Chronic liver disease

Evaluation before conception

Due to reduced fertility in advanced liver disease, pregnancy in the presence of cirrhosis and portal hypertension is rare. With advances in medical care, young people are surviving into adulthood with reproductive potential, with compensated cirrhosis. In general, the more advanced the liver disease, the higher the risk of complications to mother and child.

Pregnancy is not contraindicated in liver disease, but should be planned for a time when the disease is in a steady state. Drug therapy should be assessed for possible teratogens–e.g., in animal studies, spironolactone has caused genital abnormalities in males. It is essential that therapy with penicillamine or trientine for Wilson disease and prednisolone and azathioprine for autoimmune hepatitis (AIH) should be maintained. If the disease is advanced, liver transplantation should be considered first.

Neonatal immunization and immunoglobulin to prevent transmission of hepatitis B should be discussed with mothers who are carriers. Although there is no vaccination to prevent the transmission of hepatitis C, mothers will want to know about transmission rates and management for their infants (see Chapter 13). As there is no vaccination to prevent the transmission of hepatitis C, women wishing to become pregnant should have priority for interferon- and ribavirin-free treatment regimens (see Chapter 13).

Cirrhosis and portal hypertension

Although studies have not shown that pregnancy increases the risk of variceal bleeding, it is advisable that esophageal varices should be eradicated in advance of any pregnancy, by endoscopic banding, as bleeding during pregnancy is associated with fetal loss. The acute management is the same as in non-pregnant variceal bleeding, with hemodynamic stabilization, endoscopic banding, or sclerotherapy and octreotide. Vasopressin and terlipressin should be avoided if possible, as it causes decreased placental perfusion and potentiates placental abruption. It has also been associated with fetal digit necrosis and amputation [16].

Decompensated liver cirrhosis

In the presence of decompensated liver disease with ascites, encephalopathy, or liver failure, termination of pregnancy should be considered, because of the difficulties in bringing a normal infant to term. Twenty-four percent of women with cirrhosis will decompensate during pregnancy, requiring intensive care and possibly transplantation [17]. Coagulopathy may lead to massive hemorrhage at delivery. Splenic artery aneurysm rupture may occur in the third trimester, with high maternal and fetal mortality rates (70% and 80%, respectively).

Labor and delivery

Delivery should be precipitated if there is life-threatening deterioration in the mother; otherwise, the pregnancy should go to term. Cesarean section should be avoided in women with cirrhosis unless absolutely necessary, due to the presence of intra-abdominal collaterals, leading to an increased risk of bleeding and infection. Coagulation, fibrinogen, and platelet count should be assessed and blood product support should be available as required. In advanced cirrhosis, coagulation tests such as the international normalized ratio (INR) must be interpreted with caution since pro- and anticoagulation compounds are equally reduced. The INR thus overestimates the risk of bleeding. Epidural anesthesia may avoid the Valsalva maneuver, which may increase variceal pressure. However, if the platelet count is reduced, it may not be possible to provide this.

Postpartum

Thrombocytopenia and reduced synthetic clotting factors increase the risk of postpartum hemorrhage.

Fetal complications

Prematurity is increased in the setting of cirrhosis and requires the delivery to be in a setting in which neonatal intensive care facilities are available. β-blockers may cause growth restriction, and this should be monitored throughout the pregnancy. Fetal growth may also be reduced following a gastrointestinal bleed.

Pregnancy issues related to specific chronic liver diseases

Autoimmune hepatitis in pregnancy

Pregnancy tends to induce immune tolerance in AIH, with improving liver function on baseline dosages of immunosuppression [18]. This should not lead to reduction of maintenance dosages. Postpartum deterioration is common and should be anticipated. With cirrhosis, decompensation requiring transplantation may complicate pregnancy.

- *Steroids.* No teratogenic effects on the fetus have been reported in humans. In animal studies, high doses have led

Table 7.3 Immunosuppressant use in pregnancy and breastfeeding.

Drug	Side effects in pregnancy	Effects on fetus	Breastfeeding recommendations
Steroids	Poor wound healing, hypertension, weight gain, gastric ulceration, osteoporosis, glucose intolerance	In animal studies, growth retardation and premature rupture of membranes. At high doses, may cause cleft palate in animal studies	Less than 10% enters breast milk, so considered safe at maintenance corticosteroid doses
Tacrolimus (FK506)	Nephrotoxicity, neurotoxicity, glucose intolerance, hyperkalemia, diarrhea	Transient hyperkalemia	50% enters breast milk. Manufacturers recommend avoiding breastfeeding
Ciclosporin	Hypertension, nephrotoxicity, neurotoxicity, hyperkalemia, tremor, hirsutism, hypomagnesemia, glucose intolerance	Growth retardation Transient hyperkalemia	Secreted into breast milk, therefore not recommended
Mycophenolate mofetil	None known	Not recommended in pregnancy. Spontaneous abortion in up to 45% Malformations, cleft lip and palate, microtia, intrauterine growth retardation, intrauterine death	No information available regarding secretion into breast milk, but the manufacturer recommends avoiding breastfeeding
Azathioprine	Increased infections, alopecia, stomatitis, hepatotoxicity, fever, nausea and vomiting, pancreatitis, leukopenia, thrombocytopenia, macrocytic anemia	At high doses, growth retardation, preterm delivery, bone-marrow suppression if this has occurred in the mother; transient chromosomal abnormalities have been reported	Undetectable in breast milk, but potentially could be secreted, therefore avoid breastfeeding
Sirolimus	None known	Delayed fetal ossification, but no teratogenic effects	No data available
Basiliximab	None known	In animal studies, there were no teratogenic effects. No human data are available	–

to cleft lip and palate, premature rupture of membranes, and adrenal insufficiency.

- *Azathioprine.* In high doses, transient abnormalities in the neonate have been reported, including thymic atrophy, leukopenia, anemia, thrombocytopenia, reduced immunoglobulins, and transient chromosomal aberrations. No structural teratogenic effects have been reported. At dosages used for maintenance treatment in the mother, no adverse effects have been reported in the neonate.
- *Tacrolimus.* Transient hyperkalemia has been reported in the neonate, but no specific teratogenicity.
- *Ciclosporin.* No specific teratogenicity.
- *Mycophenolate mofetil* (MMF). There are concerns regarding the safety of MMF in the developing fetus, with an increased risk of spontaneous abortion and serious malformations. MMF should be avoided in pregnancy.

Wilson disease

Pregnancy is not contraindicated in Wilson disease. Fertility may be reduced in poorly controlled disease, with amenorrhea and frequent miscarriages.

Penicillamine as a chelating agent is well tolerated at conventional doses by both mother and child, with no increased incidence of fetal malformation. Unnecessarily high doses may lead to cutis laxa. Penicillamine has widely been replaced by trientine due to fewer side effects. Trientine showed some teratogenicity in laboratory animals, but human data are encouraging.

Zinc has also been used safely during pregnancy, with no teratogenic effect on the developing fetus, although one study reported malformations in 2 of 26 pregnancies.

Pregnancy following liver transplantation

Before conception

The menstrual irregularities that occur in chronic liver disease are reversed by liver transplantation, and fertility may return within 3 weeks of transplantation. It is generally advisable that pregnancy should be avoided within the first year after transplantation, and contraception should therefore be carefully addressed in those of child-bearing age [19].

Immunosuppressants in pregnancy

The largest experience of the use of immunosuppressive agents in pregnancy is with azathioprine and steroids. Information on the newer immunosuppressives in pregnancy is mainly from animal studies, as there are few data in humans (Table 7.3).

Pregnancy

Liver and renal function, CMV status, and hemoglobin should be closely monitored. Pregnancy should not alter graft function, so rejection and infection should be considered if liver function tests become abnormal (see Table 7.1) for the normal changes seen in liver function during

pregnancy. Liver biopsy has no increased risk in comparison with the non-pregnant woman, and adjustments to immunosuppressives in the face of acute rejection should be the same as in the non-pregnant. There is an increased risk of hypertension, with the risk being greater in those receiving ciclosporin and those with hypertension prior to conception.

PE, premature rupture of membranes, anemia, infection, and first-trimester abortion are all increased in pregnancies following transplantation.

Delivery

Normal vaginal delivery can occur. Up to 50% go into preterm labor. The risk of requiring a cesarean section is related to prolonged rupture of membranes or PE.

Effects on the fetus

Congenital malformations have not been reported. Fetal growth should be monitored by serial ultrasound scans, as there is an increased risk of intrauterine growth retardation from PE. Congenital CMV infection is the lead cause for neonatal death, and in one series all neonatal deaths in transplanted mothers were due to acute CMV infection. The risk of maternal and therefore fetal CMV infection is greatest with high-dose immunosuppression, e.g., in the early post-transplantation period, when pregnancy should be avoided.

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SECTION 5

Liver Disease in Infancy

CHAPTER 8

The Jaundiced Baby

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Key points

- Jaundice in a neonate at 14 days of age should undergo urgent investigation for liver disease.
- Biliary atresia should always be considered in infants with conjugated jaundice especially if there are pale stools.
- Early diagnosis of conjugated jaundice in infancy enables early medical and surgical management.
- Optimizing nutrition with high medium-chain triglyceride milk and supplementing with fat-soluble vitamins is essential.

Jaundice is common in babies, with physiological jaundice occurring in up to 60%. However physiological jaundice usually lasts 3–5 days and will have clinically resolved by 14 days of age (21 days in a pre-term baby) and hence babies who are jaundiced after this time require investigation to identify conjugated hyperbilirubinemia and liver disease (Figure 8.1).

Breast milk jaundice is unconjugated jaundice due to the high levels of maternal hormones within the milk. It is more common in males and multiple births when the hormone levels are highest. Infants may continue to be jaundiced for up to 3 months but do not usually require any phototherapy. The jaundice will reduce when breastfeeding is discontinued. If the level of bilirubin is below the phototherapy treatment line (350 μmol/L at 4 days of age or less prior to this; see NICE guidelines CG98) then there are no long-term complications.

Disorders of bilirubin conjugation

Persistence of jaundice beyond 14 days requires investigation to initially distinguish between unconjugated and conjugated bilirubin and then to identify pathological causes of unconjugated jaundice [1] (Table 8.1).

Gilbert syndrome and Crigler–Najjar syndrome

Both Gilbert syndrome (GS) and Crigler–Najjar (CN) are due to a reduction in the function of *UGT1A1* (OMIM*191740), which encodes the protein uridine diphosphate glucuronosyl transferase (UDPGT), a transmembrane protein of the smooth endoplasmic reticulum. After uptake of unconjugated bilirubin into the hepatocytes it is transferred to the endoplasmic reticulum where UGPGT catalyzes the conversion to bilirubin monoglucuronides and then diglucuronides.

The severity of the clinical symptoms correlates with the degree of residual function of UDPGT.

Gilbert syndrome

In GS the most common area to be affected is the promotor region of the gene, which reduces the function of the gene between 10 and 40%. GS is an autosomal recessive condition with a prevalence of approximately 8% of the population.

Clinical features and diagnosis

It usually presents with mild unconjugated jaundice (<70 μmol/L) in adolescents, and is associated with fatigue, dehydration, and intercurrent illness or mild right-sided abdominal pain. Often there is a history of prolonged jaundice or breast milk jaundice in the neonatal period. All clinical findings and liver biochemistry are otherwise normal. Genetic analysis is rarely required.

Treatment

It is a benign condition with an excellent prognosis, although patients should be warned that they may become more jaundiced with intercurrent illnesses. Reassurance for the child and family is required.

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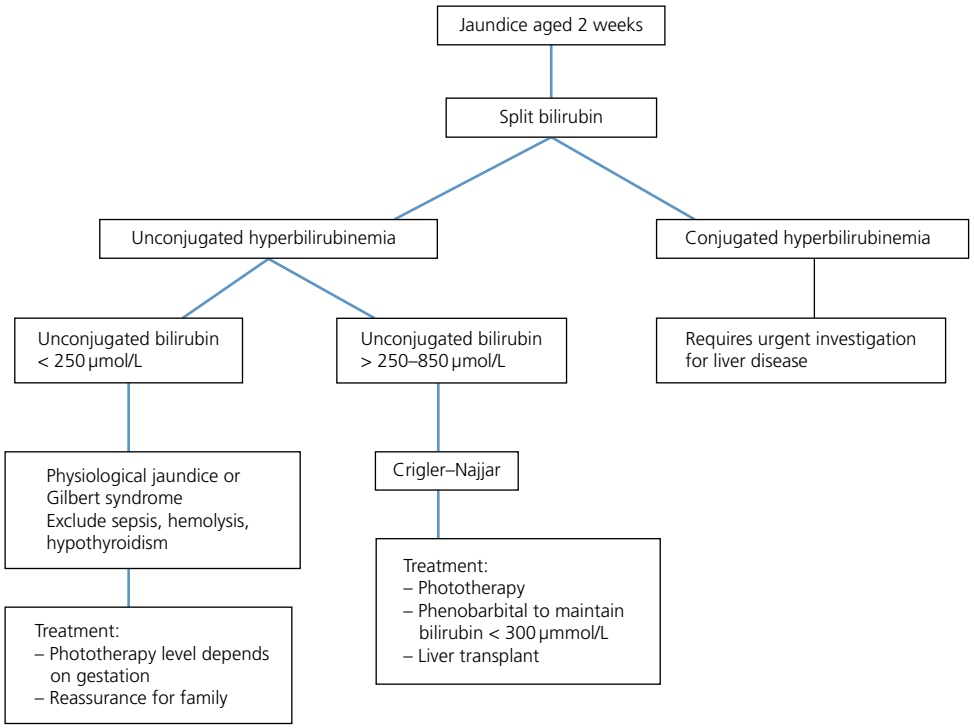


Figure 8.1 The investigation of infants who are jaundiced at 14 days to identify those infants with conjugated jaundice which requires urgent investigation of liver disease and those with pathological unconjugated jaundice.

Table 8.1 Causes of unconjugated jaundice in infants, clinical features, and diagnostic tests.

	Clinical features	Diagnostic tests
Increased heme breakdown	May occur within the first 24 h of life with a rapid rise in bilirubin levels	Full blood count
Hemolytic		
Rhesus or ABO incompatibility		Coombs test
Spherocytosis		Blood film
G6PD deficiency		G6PD level
Excessive bilirubin		
Extensive bruising		
Internal hemorrhage		
Polycythemia		
Sepsis	May occur at any time in the neonatal period	Urine culture Blood culture
Congenital hypothyroidism	Jaundice within the first month	Thyroid function tests
Pyloric stenosis or other upper gastrointestinal obstruction	Bilirubin increases with poor oral intake but resolves rapidly	Diagnosis is suspected from the history
Inherited disorders of conjugation Gilbert syndrome Crigler–Najjar syndrome	Persistent high levels of unconjugated hyperbilirubinemia	Mutations in *UGT1A1*

G6PD, glucose-6-phosphate dehydrogenase.

Crigler–Najjar type 1

CN type 1 is a rare autosomal recessive condition with a prevalence of 0.6/million.

There is a complete loss of function of *UGT1A1* and bilirubin levels are typically 350–750 µmol/L within the first week of life, hence there is a high risk of kernicterus.

Clinical features and diagnosis

There is a rapid rise in bilirubin levels in the early perinatal period. It may initially continue to rise despite phototherapy. There is no improvement when commencing phenobarbital. If there is a delay in instigating treatment or rapid increase in bilirubin which may occur with dehydration or sepsis, then

there is a risk of neurological damage from kernicterus. The suspected diagnosis is confirmed by genetic investigation for loss of function mutations in *UGT1A1*.

Treatment

The aim of treatment is to maintain the bilirubin to <150 µmol/L using exchange transfusion and at least 12 h/day of phototherapy. Liver transplant or auxiliary liver transplant is the best long-term option to prevent the lifelong risk of neurological damage as intercurrent illnesses or fasting can result in rapid increases in bilirubin levels.

Crigler–Najjar type 2

There is retention of some function of *UGT1A1* (approximately 10%) and serum bilirubin is typically between 100 and 400 µmol/L.

Clinical features and diagnosis

Unconjugated jaundice rises rapidly in the newborn period. The peak serum bilirubin is usually less than those with CN type 1. There is a good response with a reduction in bilirubin by 40–80% when commencing phenobarbital.

The suspected diagnosis is confirmed by genetic testing for mutations in *UGT1A1*.

Treatment

Bilirubin levels should be maintained at <250 µmol/L using phenobarbital which increases *UGT1A1* activity. Phenobarbital is a lifelong treatment as peaks in bilirubin levels can occur during intercurrent illness or fasting with a risk of kernicterus and permanent neurological damage.

Dubin–Johnson syndrome

This rare and benign condition is due to mutations in *ABCC2* (previously called *MRP2* and *MOAT*) which encodes the adenosine triphosphate (ATP)-dependent MRP2 transporter. Following diglucuronidation, bilirubin which has diffused to the canalicular pole is excreted into the bile by MRP2.

Clinical features and diagnosis

Clinical features are a mildly raised conjugated jaundice with no other clinical or biochemical abnormalities. In infants who are severely affected neonatal hepatitis may be seen on liver biopsy. In older children a liver biopsy typically shows a melanin-containing pigment in the centrilobular region. This pigment accumulates with time and is not seen in biopsies taken in infancy. Computed tomography (CT) scan will show high attenuation in the liver.

Management

In most cases no treatment is necessary except for reassurance. In those with neonatal hepatitis ursodeoxycholic acid (UDCA) may be beneficial.

Rotor syndrome

This is due to mutations in the *SLCO1B1* and *SLCO1B3* on chromosome 2, which encode organic anion transport proteins 1 and 3 (OATP1B1/3), respectively, disrupting hepatic reuptake of bilirubin conjugates.

Clinical features and diagnosis

Clinically there may be mildly raised conjugated jaundice whilst clinical examination and other liver biochemistry are completely normal.

Management

The condition is benign and no specific treatment is required. OATP1B1 is important in drug detoxification and hence there is a theoretical risk of drug toxicity (anticancer drugs, methotrexate, and statins) in Rotor syndrome.

Neonatal conjugated hyperbilirubinemia

In all infants with conjugated jaundice the most important condition to exclude is biliary atresia due to the timely need for operative intervention. Figure 8.2 provides a stepwise approach to investigating a child with conjugated hyperbilirubinemia.

In the presence of a raised conjugated bilirubin the GGT is most commonly raised. Biliary atresia is associated with a raised GGT except in very late presentations with end-stage liver disease. Other conditions are shown in Table 8.2.

A low or normal GGT when there is significant cholestasis is a useful test to guide further investigations. Conditions associated with conjugated jaundice and low GGT are:

- Progressive familial intrahepatic cholestasis types 1 and 2 and type 4 (*TJP2* mutations).
- Bile salt synthesis disorders.
- Arthrogryposis–renal–cholestasis (ARC) syndrome.

Biliary atresia

Biliary atresia is a progressive obliterative fibrosing cholangiopathy that exclusively presents in neonates. It has changed from being a universally fatal disease 30 years ago, to one in which palliative surgery and curative liver transplantation are available and it is the most common indication for liver transplantation in childhood. A high index of suspicion for biliary atresia is required in all jaundiced infants as a timely surgical Kasai portoenterostomy, to achieve bile flow, improves the outcome without the need for immediate liver transplant. It is an unpredictable disease which may progress despite timely surgery in an experienced center (see also Chapter 25).

Epidemiology

Biliary atresia occurs worldwide and is equally distributed between males and females. It is most common in East Asia with a reported incidence of 1 : 5000. The UK and France

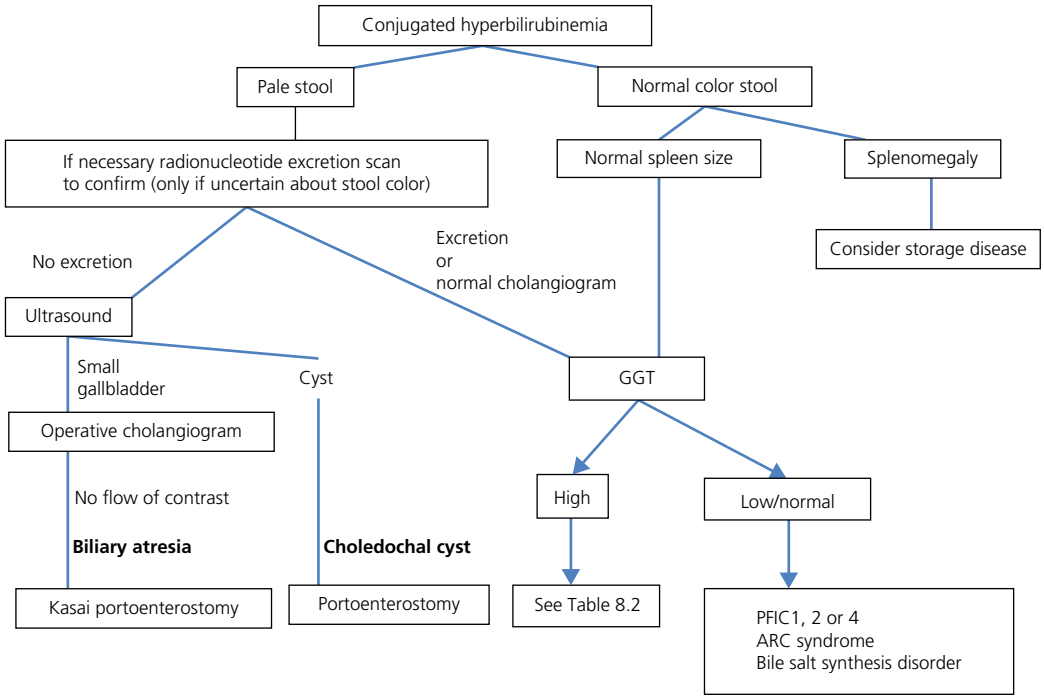


Figure 8.2 The initial investigation for infants at 2 weeks of age with conjugated jaundice. ARC, arthrogryposis–renal–cholestasis; GGT, γ-glutamyl transpeptidase; PFIC, progressive familial intrahepatic cholestasis.

Table 8.2 Causes of conjugated jaundice in infants associated with a raised γ-glutamyl transpeptidase (GGT) other than biliary atresia.

Differential diagnosis	Investigation	Results	Specific treatment
α1-Antitrypsin deficiency	Level and protein phenotype	Low α1-antitrypsin level PiZ phenotype	General management of cholestasis (see later)
Hypothyroidism	TFTs	Raised TSH Low T4	Thyroxine replacement
Hypopituitarism	TFT, cortisol, glucose	Low TSH, cortisol Hypoglycemia	Replace hormone deficiency
Galactosemia	Urine-reducing substances Plasma Gal-1-Put	Positive reducing substances Absent or reduced Gal-1-Put detected	Galactose-free diet
Tyrosinemia	Urine succinyl acetone DNA for mutations	High succinylacetone Mutations in *FAH*	NTBC, low tyrosine diet
Alagille syndrome	Echocardiogram Thoracic vertebrae X-ray Slit-lamp examination DNA for mutations	Triangular face Peripheral pulmonary stenosis Butterfly-shaped thoracic vertebrae, posterior embryotoxon, *JAG1* or *NOTCH2* mutations	Management of cholestasis (see later)
Congenital infection	Serology urine and blood, PCR for CMV, *Toxoplasma*	Positive testing	Ganciclovir may be beneficial for congenital CMV
Progressive familial intrahepatic cholestasis 3	GGT Liver biopsy DNA for mutations	High GGT cholestasis Specific findings on histology Mutation *ABCB4*	Management of cholestasis (see later)
Storage disease, e.g., Niemann–Pick disease type C	Liver biopsy Bone marrow biopsy Filipin staining DNA for mutations	Storage cells on bone marrow and liver biopsy (can be difficult to see in young children) Positive filipin staining of fibroblasts Mutation in *NPC1* and *-2*	Management of cholestasis (see later)
Citrin deficiency	Plasma and urine amino acids DNA	Increased plasma and urine citrulline and arginine Mutation in *SLC25A13*	Supportive with management of cholestasis
Peroxisomal disorders	Plasma very-long-chain fatty acids DNA	High levels of very-long-chain fatty acids Mutation in *PEX* genes	Palliation
Intestinal failure associated liver disease [2]	Liver biopsy	Specific findings on liver biopsy	Ursodeoxycholic acid, encourage enteral diet, prompt treatment of sepsis

CMV, cytomegalovirus; NTBC, nitisinone; PCR, polymerase chain reaction; TFT, thyroid function test; TSH, thyroid-stimulating hormone.

have most accurate national prevalence, ranging from 1 : 15,000 to 1 : 19,000. Estimates in the USA are 1 : 15,000.

It is seen in all racial groups however some are more susceptible. In a US study, black mothers were 2.5 times more likely to give birth to a child with biliary atresia then white mothers.

Inheritance

Biliary atresia does not appear to have a classical genetic inheritance pattern. It rarely occurs in families and in twins it is discordant. There are single case reports of affected dizygotic twins and vertical transmission.

Up to 20% of cases are termed syndromic as they are associated with other congenital anomalies. Biliary atresia splenic malformation (BASM) syndrome is the most common and is seen in 10% of cases in Europe and USA cases. The syndrome comprises biliary atresia with:

- Splenic malformation (polysplenia most commonly) (100%).
- Situs inversus (37%).
- Preduodenal portal vein (40%).
- Absent inferior vena cava (70%).
- Intestinal malrotation (60%).
- Cardiac anomalies (e.g., ventricular septal defect, atrial septal defect, hypoplastic left heart) (45%).
- Pancreatic anomalies (11%).

The remaining 80% are termed to have isolated biliary atresia (Figure 8.3).

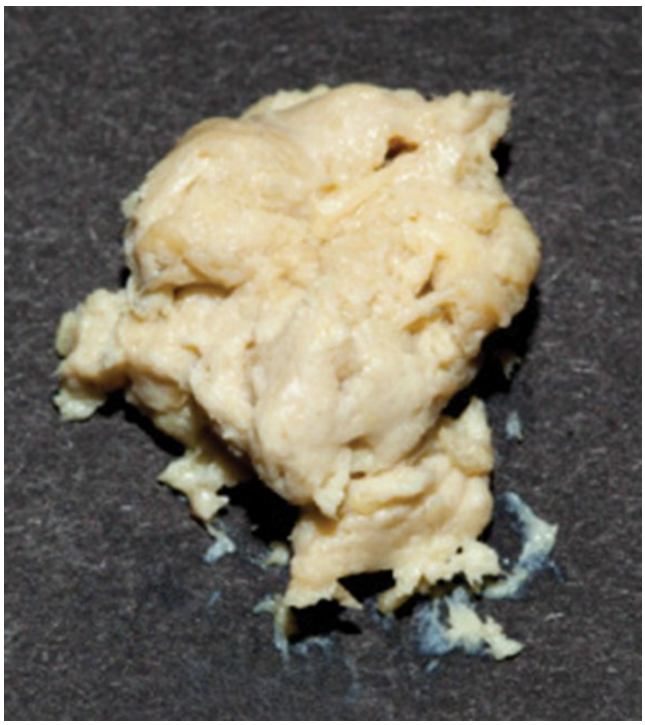


Figure 8.3 All infants with pale stool should be investigated for biliary atresia as a matter of urgency due to the timely need for operative intervention. If biliary atresia is excluded then other cause of cholestasis as shown in Table 8.2 can also cause pale stool if the cholestasis is severe.

Isolated biliary atresia is also associated with other genetic abnormalities such as trisomy 21 and 18 and may be associated with other developmental anomalies such as esophageal atresia, jejunal atresia, and anorectal malformations.

Etiology

Despite being the most common indication for liver transplant in childhood little is known about the precise etiology of biliary atresia such that it is thought to be multifactorial in nature [3].

There is no association between seasonal clustering, smoking, maternal age, education, income, alcohol or folic acid intake, gravidity, pre-term birth, or infant weight.

It is thought that the pathological process begins early during embryogenesis in those with syndromic biliary atresia whilst in those with isolated biliary atresia it may begin later or even postnatally. However some cases of isolated cystic type biliary atresia can be identified on antenatal ultrasound scan. When bile acids were measured in healthy newborn babies (using the Guthrie card) 77% of those with biliary atresia could be identified suggesting that biliary atresia is established prior to birth and is not a postnatal event.

Infection. The role of viruses in the development of biliary atresia has been extensively studied. Initial studies showed that mice inoculated with rotavirus strains RRV and SA11-FM developed jaundice with histological findings similar to that of biliary atresia in humans. In a study of 74 liver biopsies from children at diagnosis of biliary atresia, in only a third of cases was a virus identified and the detection rate increased with age suggesting the viruses are acquired. On long-term follow-up those who had viruses identified had similar outcomes to those who did not. In an independent study cytomegalovirus (CMV) reduced the clearance of jaundice following Kasai and increased mortality.

Genetics. The constant clinical features of the BASM syndrome form of biliary atresia suggest a crucial event in the developmental pathway during embryogenesis. The *INV* mouse model for biliary atresia develops situs inversus and biliary atresia with pathological changes in the ductal plate. However mutations in the *INV* gene are species specific and humans with BASM do not have mutations in *INV*. Situs inversus occurs in humans with mutations in *INV*.

It is possible that an inflammatory response with a periductal infiltrate of mononuclear cells is the mechanism for the damage to the bile ducts in biliary atresia although the trigger remains unknown. There is increased expression of HLA-DR, intracellular adhesion molecules (ICAM1 and E-selectin), increased soluble inflammatory molecules and cytokines (interleukin 2, interleukin 18, and tumor necrosis factor α). The development of fibrosis in biliary atresia is also much greater than in other neonatal-onset liver disease, even following a successful Kasai portoenterostomy, and may suggest a genetic polymorphism that increases susceptibility

to the development of fibrosis such as in the genes *CFC1*, *ICAM1*, macrophage migration inhibitory factor gene, CD14 endotoxin receptor gene, and hepcidin. Abnormalities in apoptosis due to a specific antigenic stimulation may also occur as there is an upregulation of Kupffer cells, natural killer cells, $CD3^+$ and $CD8^+$ T cells, and increased $CXCR3^+$ cells.

Cilia. Primary cilia are important for the flow of bile within bile ducts as well as normal placement of abdominal situs. The association of laterality abnormalities in BASM suggests cilia may be affected. Biliary cilia from infants with biliary atresia are reduced in number and abnormal in morphology compared to normal liver or other liver pathology.

Alloimmune response. The identification of maternal $CD8^+$ T cells in the bile ducts of babies with biliary atresia suggests there may be an alloimmune process akin to graft-versus-host disease, occurring in utero.

Toxins. An outbreak of biliary atresia in lambs born to ewes in New South Wales, Australia, occurred when the pregnant ewes ate the plant phytotoxin, biliatresone. In zebrafish, biliatresone also causes a biliary atresia like lesion which interferes with cholangiocyte polarity involving *SOX* and *NOTCH* genes. No human cases associated with toxins have been identified

Screening

The timely need for a successful Kasai portoenterostomy makes an early diagnosis mandatory. Therefore screening for biliary atresia in neonates may enable a prompt diagnosis. The use of stool color cards have been shown to be effective in Japan, Taiwan, and Canada. In Taiwan 5 years after the introduction of the stool color card, the rate of successful Kasai portoenterostomy has increased from 35% to 61% and 5-year survival with native liver from 27% to 64%.

In England the measurement of conjugated bile acids in the infant blood spot screening card (Guthrie card) was able to identify babies with biliary atresia in 77% of cases but it was not specific. Conjugated hyperbilirubinemia in the second week of life (measured by liquid blood screening samples) was both sensitive and specific, but is limited as a dry blood spot is used for infantile screening. Other screening techniques include serum Apo C II and III proteins, urinary sulfated bile acids and fecal conjugated bilirubin. These techniques require laboratory expertise and are expensive compared to the stool color chart.

Clinical features

Babies are usually born at term with a normal birthweight for gestational age. Jaundice is usually not evident at birth but develops over the first few days and all babies who are jaundiced at 14 days require a split bilirubin to identify conjugated jaundice. In those with conjugated jaundice further investigations are then necessary to identify biliary atresia and exclude other causes.

Biliary atresia is rare in pre-term infants but tends to become evident with pale stools and conjugated jaundice around the due date.

On examination most children with biliary atresia have few clinical signs except for jaundice and mild hepatomegaly. Splenomegaly and ascites are features of a late diagnosis when cirrhosis is established. It is important to identify comorbidities such as congenital heart disease and any cardiac murmur or signs require a detailed cardiology assessment.

The stool is pale due to the lack of bile pigment which becomes more obvious with time, becoming acholic by 6 weeks. The urine is generally dark due to the water-soluble bilirubin conjugates (in nappies the dark urine may cover the stool and be falsely reassuring).

Initially infants may gain weight as they are usually hyperphagic due to the poor absorption of long-chain fats but soon begin to fail to thrive without a medium-chain triglyceride (MCT) feed. Breast-fed babies maintain their growth for a longer time because of the fat content of breast milk [4].

Investigations

Blood tests. Typical biochemical variables are shown in Table 8.3. Conjugated bilirubin is typically >100 µmol/L however lower levels can be seen and should not be falsely reassuring. With the commencement of UDCA and adequate nutrition, the bilirubin may fall but this is not sustained and should not delay appropriate management.

Synthetic function (albumin and prothrombin time) are usually normal at presentation unless there is vitamin K deficiency or a late presentation with cirrhosis. Cholesterol may be raised but triglycerides are usually normal.

Radiology. These include:

- Ultrasound scan: there may be a mildly enlarged liver with no biliary dilatation and an absent or contracted gallbladder after a 4 h fast (20% of cases will have a normal gallbladder). A hyperechogenic liver hilum creates a triangular cord sign which is specific for biliary atresia but is operator dependent with sensitivity ranging from 49 to 73%. The anatomical anomalies associated with BASM syndrome may be seen on ultrasound (Figure 8.4).

Table 8.3 The typical biochemistry if a child with biliary atresia. Occasionally the bilirubin level may be lower but if it is associated with pale stools then there should be a high index of suspicion for biliary atresia.

	Typical concentration at presentation	Normal range
Conjugated bilirubin (µmol/L)	>100	<20
Alkaline phosphatase (IU/L)	>600	<500
γ-Glutamyl transferase (IU/L)	>100	20–40
Aspartate aminotransferase (U/L)	80–200	15–40
Alanine aminotransferase (U/L)	80–200	10–55
Albumin (g/L)	Normal at presentation	37–56
Prothrombin time (seconds)	Normal at presentation	9–13

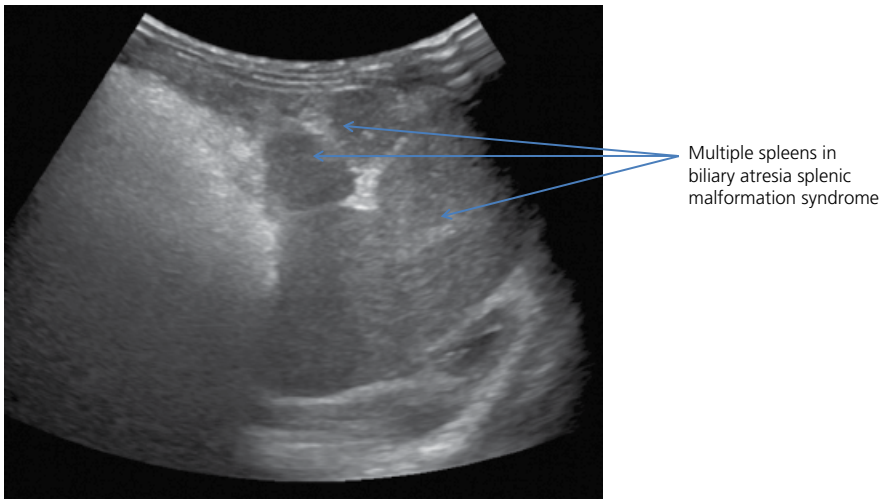


Figure 8.4 Ultrasound scan of a child with biliary atresia splenic malformation syndrome which accounts for 10–20% of cases of biliary atresia. The ultrasound demonstrates small multiple spleens.

- Radioisotope excretion scan: (TeBida or HIDA). The scan requires priming with 3–5 days of phenobarbital (5 mg/kg once daily). The injected radioisotope is taken up by the liver which should then be excreted into the intestine. In biliary atresia there is no excretion within 24 h. This test is not specific as severe intrahepatic cholestasis due to other causes of neonatal cholestasis may also not excrete the radioisotope (such as in Alagille syndrome (AGS) and α1-antitrypsin deficiency (A1ATD)).
- Bile aspiration: Japanese and Chinese groups have reported on continuous attempts to aspirate bile from the third part of the duodenum using a nasoduodenal tube. If there are no bile secretions over a 24-h period then it is strongly suggestive of biliary atresia.
- Endoscopic retrograde cholangiopancreatography (ERCP): can be used to visualize the biliary tract if diagnosis is uncertain but is technically difficult in infants.
- Magnetic resonance cholangiopancreatography (MRCP): currently this technology may not identify the luminal patency of the infant biliary tract that may only be 1 mm in diameter. With technical advances it may become a useful test.

Liver histology. A percutaneous liver biopsy provides information regarding extrahepatic biliary obstruction. Typically the findings are those of the following:

- Portal tract fibrosis.
- Edema.
- Ductular proliferation.
- Cholestasis with bile plugs.

If the biopsy is taken before 6 weeks the typical findings may not be present. Giant cell transformation can also be seen which can hinder differentiation from other causes of neonatal cholestasis.

Operative cholangiogram. This is the gold standard and definitive test. Dye is injected into the biliary tree under direct vision at laparotomy or laparoscopically, and the patency of the bile ducts assessed.

Clinical management

The infant should receive fat-soluble vitamins, UDCA, and an MCT-based feed before surgery and until clearance of jaundice following the operation.

Prior to the operative cholangiogram and Kasai procedure it is recommended to reduce the intestinal bacterial load using non-absorbable antibiotics. A suggested regime for the prior 48 h is as follows:

- Neomycin: 12.5 mg/kg/dose t.d.s. oral.
- Metronidazole: 7.5 mg/kg/dose t.d.s. oral.
- Lactulose: 5 mL b.d. oral.

Kasai portoenterostomy. This operative procedure was first described by Morio Kasai in 1950s and revolutionized the management of biliary atresia.

The procedure consists of complete excision of the extrahepatic biliary tree with transection of the porta hepatis at the liver capsule to expose the microscopic ductules. A jejunal loop is anastomosed to the cut surface to facilitate the drainage of bile from these ductules into the intestine.

A successful Kasai portoenterostomy is defined as normalization of bilirubin levels within 6 months of the procedure. The success of the procedure is dependent on the age at operation, extent of liver damage (fibrosis at time of Kasai portoenterostomy, ongoing inflammation, and episodes of cholangitis), and experience of the center. Fibrosis and cirrhosis are more likely to develop with longstanding obstruction and hence the hypothesis that the earlier the Kasai the better the outcome.

A UK study of 93 cases of biliary atresia in 15 centers showed a significant difference in success rate in those centers that operated on five or more cases each year (61% versus 14% 5-year survival with native liver) and led to

centralization of the service. At a 13-year review of this cohort 54% had a 13-year survival with native liver when operated on in those centers performing more than five Kasai portoenterostomies per year, compared to 27.3% in those who performed less than five per year.

Adjuvant treatment

A systematic review of 175 infants showed that those who received postoperative steroids had an improvement in the clearance of jaundice. This has not been seen in other smaller studies.

To prevent cholangitis some centers use low-dose rotating oral antibiotics for the first year following Kasai portoenterostomy (e.g., 3 months each of amoxicillin, cephalexin, and trimethoprim), oral probiotics may also be beneficial. UDCA (10 mg/kg twice daily) is used to aid bile flow for the first year and/or to treat pruritus.

All children will require adequate nutritional and fat-soluble vitamin supplements, particularly if the surgery has not been successful (see Chapter 25 and Table 8.5 later).

Complications

Cholangitis presents with acholic stools, abdominal pain and symptoms of sepsis. Treatment is with intravenous antibiotics for at least 10–14 days. Bacteria may be cultured from the blood or from a liver biopsy specimen. The development of hepatic bile lakes may occur at any time following Kasai portoenterostomy and are sources of recurrent infection.

It is thought that cholangitis occurs more commonly in the first 2 years following Kasai portoenterostomy however long term studies have shown that 63% had a diagnosis of cholangitis 5 years following Kasai portoenterostomy and hence vigilance for cholangitis should continue into adulthood. Recurrent or late cholangitis may suggest an obstruction in the roux loop requiring surgical reconstruction.

Fat-soluble vitamin deficiency is common and requires adequate supplementation. Bone fractures occur in up to 15% of patients which is six times higher than in the general population.

Even in children with a successful Kasai portoenterostomy, pruritus may be difficult to control but generally improves with age.

Portal hypertension with splenomegaly and/or hypersplenism develops in up to 50% of children within 5 years following Kasai portoenterostomy and hence surveillance and management of varices is important.

Hepatopulmonary syndrome due to abnormal shunting in the pulmonary vascular bed causes hypoxia which is reversible with liver transplantation.

Prognosis

The overall success for clearance of jaundice post Kasai portoenterostomy is approximately 60%, especially for those operated on in a timely manner. The outcome is variable and occasionally surgery as late as at 100 days will be successful.

Despite successful restoration of bile flow and clearance of jaundice, the inflammatory process continues following the Kasai portoenterostomy so fibrosis is progressive leading to cirrhosis and portal hypertension in the majority.

A large multicenter North American review of 219 patient with biliary atresia who were 5–18 years following a successful Kasai, showed that the majority of patients achieved normal growth and 75% had normal liver synthetic function however only 2% had no liver disease with no signs of portal hypertension, normal liver biochemistry and normal synthetic function. Despite 98% of patients continuing to have health concerns, over 50% had a positive health-related quality of life assessment [5].

Indications and timing for liver transplant

In the UK, 51% have a 4-year survival with their native liver following a Kasai. In infants in whom the Kasai portoenterostomy is unsuccessful (Figure 8.5) liver transplant is usually indicated within 6 months to 2 years of age. In those who have had a successful Kasai portoenterostomy, recurrent cholangitis, the development of cirrhosis with hepatic dysfunction and malnutrition, or the development of bleeding varices and ascites not responsive to endoscopic treatment are indications for transplantation.

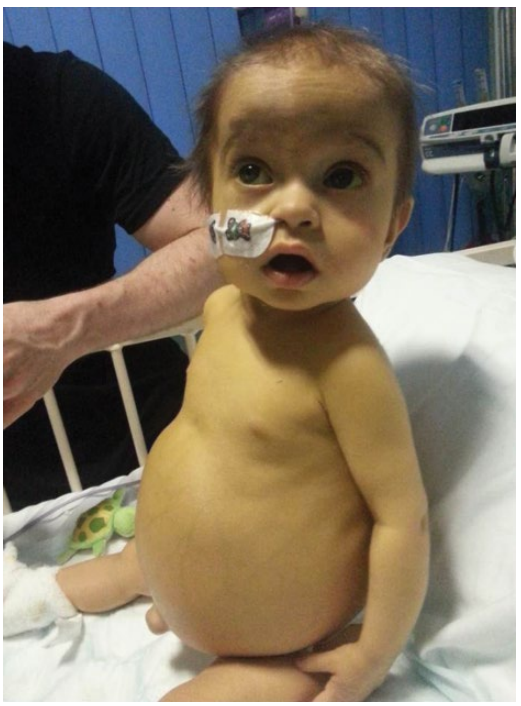


Figure 8.5 A child with biliary atresia with a failed Kasai procedure. The picture shows jaundice, dissented abdomen due to ascites with protrusion of the umbilicus and prominent abdominal veins. Despite a large abdomen her arms and legs show cachexia due to her severe liver disease despite receiving supplemental nasogastric tube feeding.

Alagille syndrome

AGS is a multisystem condition occurring with an estimated frequency of 1 in 30,000.

It has highly variable clinical features and penetrance. Patients range from being asymptomatic to those with severe neonatal cholestasis, cardiac or renal disease infancy [6].

Genetics

AGS (MIM118450) is an autosomal dominant condition due to mutations in either *JAG1* (95% of cases) or *NOTCH2* (5% of cases). *JAG1* encodes NOTCH signaling pathway ligand Jagged-1. Jagged/Notch interactions that occur at cell–cell contact points determine cell fate in early development. Interestingly, it was found that renal disease was more common in patients with *NOTCH2* than *JAG1* defects.

There is a lack of genotype–phenotype correlation in AGS and a range of phenotypes can be found in affected members of the same family suggesting additional genetic and/or environmental factors determine the final clinical phenotype.

Clinical features (Table 8.4)

Prior to molecular testing, AGS was a clinical diagnosis consisting of intralobular bile duct paucity on liver biopsy and at least three out of five other major clinical features as follows:

- Cholestasis.
- Cardiac disease with peripheral pulmonary stenosis.
- Skeletal anomalies with butterfly thoracic vertebrae (Figure 8.7).
- Posterior embryotoxon seen on slit-lamp examination of the eyes.
- Characteristic facies (Figure 8.6).

Hepatic investigations

Blood tests. The conjugated hyperbilirubinemia may be extremely high but improves with time. GGT can be extremely high, up to 20 times the upper limit of normal and usually remains elevated despite the cholestasis resolving. Hepatic transaminases may be up to 10 times the upper limit of normal and in general remain abnormal. Unless there is end-stage liver disease, synthetic function is maintained. Cholesterol and triglycerides are often elevated. Coagulation is usually normal.

Ultrasound of the liver. This is usually normal although occasionally a contracted gallbladder may be seen.

Radioisotope excretion. When cholestasis is severe there may be no excretion of isotope, which can make it difficult to distinguish from biliary atresia.

Histology. An adequate sample for assessment contains 6–20 portal tracts and a diagnosis of paucity of intralobular bile ducts is when the ratio is <0.5 (normal ratio 0.9–1.8). The paucity of bile ducts is progressive and hence in young infants the paucity may not be appreciated, particularly if there is neonatal hepatitis. In young infants ductular proliferation may also be seen suggesting large duct obstruction and making the differential diagnosis from biliary atresia difficult. In 15–20% of patients periportal and centrilobular fibrosis develop leading to biliary cirrhosis.

Cholangiogram (ERCP or surgically). This will demonstrate the patency of the extrahepatic biliary tree and exclude biliary atresia. This is only required if there is clinical uncertainty.

Genetics. A genetic diagnosis is made by identifying a mutation when sequencing of *JAG1* and *NOTCH2* genes. Although it is possible to perform antenatal genetic testing using DNA obtained from chorionic villous sampling or amniocentesis, it may be difficult to predict the severity of the disease in an affected fetus due to variable penetrance of the disease mutations.

Management of liver disease

See therapy for conjugated jaundice section.

Fat-soluble vitamins. Vitamin D deficiency can be especially difficult to treat in AGS and may require intramuscular injections.

Pruritus. Typically this is more severe than in other causes of cholestasis and may be difficult to control but does tend to improve with age. Partial external biliary diversion (in those without established hepatic fibrosis) may improve pruritus in some patients.

Xanthomas. These typically increase in number in the first few years but disappear when the cholestasis improves with a reduction in cholesterol. If the xanthomas are disfiguring or interfere with function (e.g., inner canthus of the eye) lipid-lowering medication, such as cholestyramine, may be beneficial. Partial external biliary diversion may also reduce cholesterol levels.

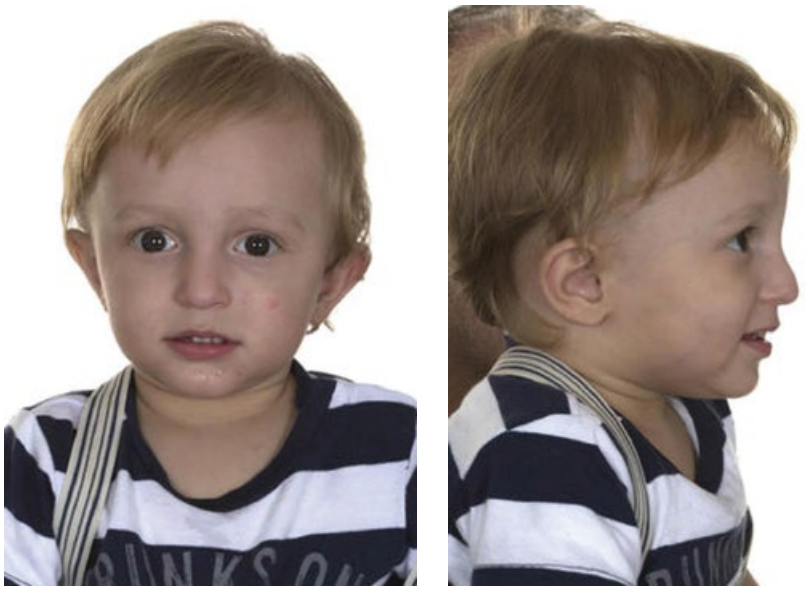
Nutrition. Poor growth and pubertal delay are common in AGS. Nutritional intake may be compromised in AGS and nasogastric or gastrostomy tube feeding may be required to achieve adequate calorie intake.

Indications for liver transplant

Liver transplant is rarely required for cirrhosis and decompensation of chronic liver disease. Other indications include marked deterioration in quality of life, such as intense refractory pruritus, or malnutrition with recurrent bone fractures despite optimal nutritional supplementation; however these indications are rarely seen with modern management.

Table 8.4 Alagille syndrome (AGS) is a multisystem disease. The more common clinical features are listed here.

Affected system	Clinical features	Initial investigations
Liver disease	Neonatal cholestasis is the presenting feature in 95% of cases. In others liver involvement may be mild and not clinically apparent. When cholestasis is severe it may be clinically difficult to distinguish from biliary atresia as the child may have acholic stool and dark urine. A Kasai procedure however is detrimental and may cause deterioration of liver function In early childhood liver disease is often associated with severe pruritus which significantly affects the child's quality of life and hypercholesterolemia with the development of xanthoma on the extensor surfaces. Xanthoma and pruritus may improve with time Hepatocellular cancer may develop in those with cirrhosis and should be screened for with regular abdominal ultrasound and α-fetoprotein measurement	Liver histology typically shows paucity of bile ducts
Facial features	Typically the features become more prominent with age and include triangular face with deep-set eyes, pointed chin, moderate hypertelorism, and prominent forehead. With age the protrusion of the mandible and chin becomes more prominent (Figure 8.6)	–
Cardiac disease	The typical congenital cardiac lesion is peripheral pulmonary stenosis however most other congenital cardiac lesions, particularly tetralogy of Fallot, have also been described. Cardiac disease is common and occurs in over 90% of AGS cases and is an important cause of early mortality	Echocardiogram is necessary to delineate the cardiac disease
Ophthalmology	Posterior embryotoxon may be supportive of the diagnosis of AGS, occurring in 90% of cases however it is not specific and can also be found in up to 15% of the normal population and in other syndromes such as 22q11.2 deletion. It has no long-term visual consequences Other ocular findings may be iris hypoplasia, abnormalities of the optic discs, abnormal retinal vessels, and pigmentary retinopathy	Slit-lamp examination
Skeletal	Abnormal fusion of the spine leading to a sagittal cleft creates the classical butterfly shape of the thoracic vertebrae, which occurs in 80% of cases. There are no long term clinical consequences of the vertebral changes (Figure 8.7). Other vertebral anomalies may occur such as pointed anterior process of C1, spina bifida occulta, fusion of adjacent vertebrae, hemivertebrae, and absence of 12th ribs Other skeletal manifestations include craniosynostosis and radioulnar synostosis with a curved little finger, shortening of the distal phalanges, and a fusiform appearance Fractures due to vitamin deficiency have been reported in up to 28% of AGS with the majority affecting the lower limb bones	Thoracic spinal X-ray
Renal disease	Renal disease is common in AGS and there are a wide range of manifestations Intrinsic renal diseases with tubulointerstitial nephropathy and mesangiolipidosis Structural changes with cysts are common ranging from simple benign cysts with no clinical consequences to multicystic dyplastic kidneys and renal failure Renal tubular acidosis, contribute to poor growth and chronic renal insufficiency Renal vascular disease which may require stenting	Ultrasound scan to identify structural lesions, biochemistry for renal function, and urinary analysis to identify renal tubular acidosis
Vascular disease	Intracranial vascular anomalies that are detectable on magnetic resonance angiography are seen in up to 15% of patients. They are often asymptomatic but may lead to intracranial bleeding accounting for 34% of mortality in AGS Other vessels may also be involved with intra-abdominal aneurysms, narrowing of the carotid artery and renal artery stenosis	Magnetic resonance imaging or magnetic resonance angiography will identify intracranial vascular anomalies if there are intracranial concerns although this is not a routine investigation
Benign intracranial hypertension	The incidence of benign intracranial hypertension, which may lead to optic atrophy and blindness may occur both pre- and post-liver transplant. Annual examination of the fundus is essential to look for early papilledema	



(A) (B)

Figure 8.6 (A,B) Typical facies of a child with Alagille syndrome with a triangular face, prominent forehead, deep-set eyes, hypertelorism, and small pointed chin. The features may not be prominent in the first few years of life but evolve with age.

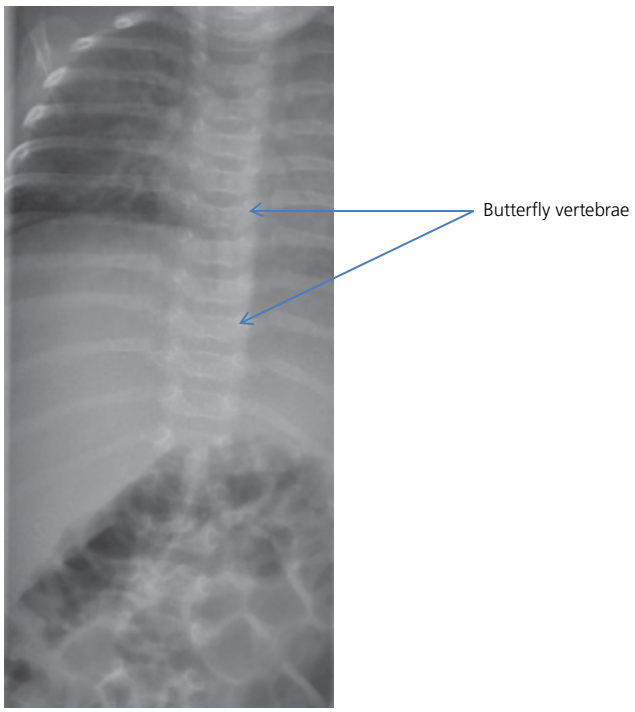


Figure 8.7 Butterfly vertebrae in a child with Alagille syndrome. Showing mild depression of the superior and inferior end plates of T6 and T9.

Occasionally early liver transplantation is indicated prior to the development of severe pulmonary hypertension and the need for complex cardiac surgery. Post-transplant survival rates are similar to other cholestatic indications, but few children achieve normal height post-transplant.

Prognosis

The natural history of the cholestasis is that it is typically worst in the first few years and then improves with relief of pruritus and xanthomas.

Gross motor delay occurs in 16% of children. Mental retardation is also thought to be highly prevalent but when confounding factors (liver disease, nutrition) are eliminated they may be no more prevalent than in the general population.

Hepatocellular carcinoma may develop at any age and may be difficult to differentiate from focal nodular hyperplasia (see Chapter 28).

The estimated overall mortality is 20–30%, due to cardiac disease, intracranial bleeding, or progressive liver disease. The largest study of outcome showed that in 163 children with AGS and liver involvement, 44 (33%) required liver transplantation, with those presenting with neonatal cholestasis more likely to result in a worse outcome. With improvements in nutritional support however this may reduce. Actuarial survival rates with native liver were 51% and 38% at 10 and 20 years, respectively, and overall survival rates were 68% and 62%, respectively.

Genetic counseling. This is necessary to identify other family members with AGS who would benefit from screening and to provide accurate information for further children including prenatal diagnostic investigations.

Non-syndromic bile duct paucity

The main cause of bile duct paucity on liver histology if AGS has been excluded. Other causes of histological bile duct paucity are shown in Table 8.5.

Table 8.5 Causes of non-syndromic intrahepatic bile duct paucity.

Prematurity	
Infection	Cytomegalovirus
	Rubella
	Syphilis
Metabolic	α1-Antitrypsin deficiency
	Cystic fibrosis
	Zellweger syndrome
	Progressive familial intrahepatic cholestasis type 1
	Ivemark syndrome
	Prune belly syndrome
	Hypopituitarism
	HNF-1 β mutations
Chromosomal/genetic	Cystic fibrosis
	Trisomy 18, 21
	Partial trisomy 11
	Monosomy X
	Neonatal ichthyosis sclerosing cholangitis
Immune mediated	Graft-versus-host disease
Severe idiopathic neonatal hepatitis	
Idiopathic bile duct paucity	

CMV infection is the most common cause for bile duct paucity and the histology may support this diagnosis with the identification of viral inclusions. Bile duct paucity in metabolic disease such as A1ATD is a poor prognostic sign with severe liver disease.

Bile duct paucity is the main finding in graft-versus-host disease of the liver following bone marrow or stem cell transplant.

If no other cause is identified the condition is known as isolated bile duct paucity. It has a poorer prognosis than AGS with persistent severe cholestasis and progressive liver damage.

Alpha-1-antitrypsin deficiency

A1ATD (MIM613490) is the most common inherited metabolic disorder causing liver disease in infants in the white population with an estimated incidence of 1 in 2500. It is an autosomal recessive disorder, caused by mutations in *SERPINA1*. Diagnosis is made by studying the protein phenotype as little is known about the penetrance of mutations in *SERPINA1* in the general population. It is a clinically heterogeneous condition manifesting as liver disease in infancy in 10% of cases or early-onset lung disease in adulthood. It also causes relapsing severe panniculitis and vasculitis.

A1AT is a protease inhibitor made in the endoplasmic reticulum of hepatocytes. In the disease state (PiZ or PiSZ) the protein is abnormally folded and is retained within the endoplasmic reticulum. This is seen as periodic acid–Schiff (PAS) positive granules in liver biopsy. Some of the abnormal protein undergoes proteolysis whilst cells that have a large amount of protein undergo apoptosis and compensatory hepatocyte proliferation, hence the development of fibrosis and the risk of hepatocellular carcinoma. In children, the retention of the abnormal protein leads to liver disease whilst in adults it is the lack of protease inhibitor which results in lung damage and hence adult lung disease is amenable to supplementation.

Clinical features

Babies with A1ATD are often born small for gestational age, which may distinguish them from biliary atresia.

The liver disease can present at any time:

- Most commonly in the neonatal period with conjugated hyperbilirubinemia:
  - often born small for gestational age (as opposed to the well-grown babies with biliary atresia)
  - the stools may be pale but this may be variable (as opposed to biliary atresia when the stools are progressively pale)
  - there is hepatomegaly, but splenomegaly is unusual unless there is significant fibrosis.
- Of those who present in infancy 5% have late hemorrhagic disease of the newborn with an intracranial bleed. This can be avoided by the administration of intramuscular vitamin K.
- Incidental finding of raised transaminases in older children.
- Signs of portal hypertension develop in a third of children and may be the presenting feature if jaundice was not prominent in the newborn period.

Investigations

Biochemistry. There is conjugated hyperbilirubinemia with raised GGT, hepatic transaminases, and alkaline phosphatase. Albumin is usually normal and unless there is an associated vitamin K deficiency, coagulation is also normal.

A1AT protease inhibitor (Pi) phenotype. This is assessed by isoelectric focussing on polyacrylamide gels. The normal phenotype is PiMM and the commonest homozygote form leading to A1ATD is PiZZ. Other forms may also result in liver disease, including the PiSZ phenotype. The heterozygous state of PiMZ does not cause liver disease, but may be associated with transient neonatal jaundice. It is essential that the test be performed in an experienced laboratory. CMV infection may cause a spurious Z band.

A1AT serum level. The serum level in patients with PiZZ phenotype is often reduced to <0.6 g/L (normal range 0.8–1.8 g/L). However, as A1AT is an acute phase reactant and therefore may be artificially elevated in liver inflammation, these levels cannot be relied upon and phenotype must be obtained in all patients with cholestasis.

Genotype. This is only available in reference laboratories and is not a commonly used diagnostic tool as it is not known what factors determine penetrance of the phenotype.

Liver biopsy. In early infancy there is an acute hepatitis of varying severity. This may resemble idiopathic neonatal hepatitis, but giant cells are rarely prominent. If there is severe cholestasis, liver histology may also mimic biliary

atresia, with marked ductular reaction in the portal tracts. Fatty infiltration may be seen around portal tracts. There is hepatocellular necrosis and inflammatory cell infiltrate. Fibrosis may be present, with or without portal bridging

PAS-positive diastase-resistant granules are positive in hepatocytes. These are 2–20 nm in diameter and correspond to amorphous material within the endoplasmic reticulum, seen on electron microscopy. However, they may not be prominent in early biopsies, and only become marked after 3 months of age.

With increasing age, the cholestasis, inflammation, and hepatocellular necrosis resolve. By 1–2 years old inflammation becomes limited to expanded portal tracts and adjacent hepatocytes.

Management

Neonates presenting with A1ATD benefit from the general management of their cholestatic liver disease with nutritional support with MCT feed and fat-soluble vitamin supplementation.

Prognosis

The conjugated hyperbilirubinemia and abnormal biochemistry usually improves with good nutritional and supportive management. The raised hepatic transaminases may return to completely normal levels with resolution of liver disease. Others continue to have raised enzymes (up to 5–10 times the upper limit of normal) with a risk of progressive fibrosis, cirrhosis, and the development of portal hypertension.

Close follow-up throughout childhood at a specialist center is mandatory, in order to detect signs of progressive liver disease and the possible need for management of portal hypertension or the need for transplantation. Children who develop jaundice after the neonatal period should be followed up closely as deterioration in liver function may progress rapidly.

Genetic counseling. If wished, other members of the family can be tested for A1AT. Prenatal diagnosis is available, but in view of the variable outcome, termination is not necessary. The family should be counseled about avoiding alcohol and cigarette smoke and the potential early development of emphysema.

Progressive familial intrahepatic cholestasis

Progressive familial intrahepatic cholestasis (PFIC) are rare diseases with an incidence of 1 in 50,000 to 1 in 100,000, worldwide occurrence and equal sex distribution. They are a heterogeneous group of inherited cholestatic diseases, caused by mutations in the hepatoceullar transport system genes involved in bile synthesis. Four genes have been identified to date. They have characteristic clinical, biochemical, and histopathological features. In PFIC types 1, 2, and 4 mutations are characterized by low-normal GGT despite cholestasis. PFIC3 is associated with a high GGT [7].

Genetics and nomenclature

PFIC is autosomal recessively inherited. Interaction between the PFIC genes and the modifier genes has a role in the severity of the clinical phenotype. Modifier genes include the apical sodium-dependent bile acid transporter (ASBT) and the farsenoid X receptor (FXR), a bile acid activated transcription factor that mediates transcriptional repression of genes important in bile acid and cholesterol homeostasis.

PFIC1 (MIM211600) is also known as FIC1 deficiency or Byler disease and is caused by mutations in *ATP8B1*. Benign recurrent intrahepatic cholestasis type 1 (BRIC1; MIM243300) is also caused by mutations in *ATP8B1* and is an allelic condition to PFIC1. ATP8B1 protein translocates phospholipids, such as phosphatidylserine, from the outer to the inner canalicular membrane leaflets.

PFIC2 (MIM601847) is also known as BSEP deficiency (bile salt export pump deficiency). It is caused by mutations in *ABCB11*. Benign recurrent intrahepatic cholestasis type 2 (BRIC2; MIM605479) is also caused by mutations in *ABCB11* and is an allelic condition to PFIC2. BSEP is the major canalicular bile salt export pump in humans, which extracts bile salts from hepatocytes into canaliculi.

PFIC3, also known as MDR3 (class III multidrug resistance P-glycoprotein) deficiency, is caused by mutations in *ABCB4*. MDR3 is an ATP-binding cassette (ABC) transporter located in the canalicular membrane of hepatocytes. There it flops the phospholipids of the phosphatidylcholine (PC) family from the inner to the outer leaflet for biliary excretion where it is required for the formation of mixed micelles in bile.

PFIC4 (MIM615878). In 2014 truncating protein mutations in tight junction protein 2 (*TJP2*) on chromosome 9q21.11, were identified in 12 children from eight families, with the phenotype of PFIC with low GGT cholestasis. Truncation of the protein causes disruption of the integrity of the cholangiocyte membrane. It is probably only localized to the liver in humans [8].

Clinical features

PFIC1. Presentation is in the first months of life with recurrent episodes of jaundice which becomes persistent. Pruritus may be severe with very high serum bile acid levels. Due to the extrahepatic expression of *ATP8B1* other clinical features include pancreatitis, diarrhea, sensorineural deafness, and short stature. The extrahepatic manifestations may become more evident or severe following liver transplantation.

PFIC2. Cholestasis is usually permanent from the time of presentation in infancy. It may present with coagulopathy secondary to fat-soluble vitamin K deficiency. Pruritus is a

major clinical feature. Due to the expression of ABCB11 in the liver only, there are no extrahepatic manifestations. Hepatocellular carcinoma has been reported in childhood and should be monitored with α-fetoprotein levels and serial ultrasound scans.

PFIC3. Cholestasis is variable with only one-third of patients having cholestasis in infancy. It may present at any time during childhood or even as an adult. Late presentation is with complications of chronic liver disease such as portal hypertension and liver failure. Pruritus is mild compared to other forms of PFIC. Treatment with UDCA normalizes biochemistry in up to half of patients (in whom the *ABCB4* mutation retains some function). In those with complete loss of function the liver disease is progressive and liver transplant will be necessary.

Low phospholipid-associated cholelithiasis syndrome (LPAC). This is due to mutations in MDR3 and is characterized by cholesterol gallstones and intrahepatic microlithiasis, along with recurrent biliary symptoms which can persist despite cholecystectomy. Intrahepatic brown pigment stones may also occur. UDCA may improve biliary symptoms even before the dissolution of stones occurs.

PFIC4. Cholestasis develops early in infancy (1 week to 4 months). The liver disease is progressive with nine of 12 described patients requiring transplantation in childhood (range 1.5–10 years). Two other children who have not been transplanted have developed portal hypertension. Extrahepatic manifestations have included subdural hematomas despite a normal clotting profile and chronic respiratory disease. It is not known if these are related to mutations in *TJP2* or incidental. In the Amish population a single mutation in *TJP2* has been identified to cause hypercholanemia with severe fat-soluble vitamin deficiency and severe pruritus without cholestasis or progressive liver disease.

Intrahepatic cholestasis of pregnancy. This is associated with mutations in *ATP8B1*, *ABCB11*, and *ABCB4*. To date, it has not been described in *TJP2*. There is severe pruritus usually commencing in the third trimester and when severe steatorrhea may occur. There is a high risk for prematurity and stillbirths. The definitive treatment is delivery of the baby. In the interim, limited fat intake, fat-soluble vitamin supplementation, and UDCA can provide symptomatic relief (see Chapter 7).

Benign recurrent intrahepatic cholestasis. Mutations in *ATP8B1*, *ABCB11*, and *ABCB4* can be associated with sudden-onset pruritus and cholestasis usually in adolescence or the third decade. There may be a trigger such as the oral contraceptive pill. It resolves with time (see Chapters 7 and 36).

Diagnosis

Biochemistry. Transaminases can be up to 5 times normal and GGT up to 10 times normal in PFIC3 otherwise it is normal or low. The cholesterol tends to be low. Synthetic function is maintained until there is liver failure. The prothrombin time is abnormal at presentation due to poor absorption of vitamin K but resolves with treatment.

Ultrasound scan. It is important to exclude an obstructive cause for the cholestasis. There are no specific findings associated with PFIC although cholelithiasis can be the presenting feature of PFIC3.

Histology. This is as follows:

- PFIC1: there is a bland canalicular cholestasis with no bile duct paucity or proliferation. There is minimal inflammation. Biopsies taken later in childhood may show a more marked giant cell change with fibrosis. Electron microscopy shows paucity of canalicular microvilli and coarse granular bile (known as Byler's bile).
- PFIC2: canalicular cholestasis with hepatocellular necrosis and giant cell transformation. There is portal fibrosis and inflammation from infancy. Bile duct loss can occur. On electron microscopy there is amorphous or filamentous bile. Immunohistochemical staining shows lack of BSEP expression.
- PFIC3: liver biopsy shows portal fibrosis and ductular proliferation with a mixed inflammatory infiltrate. There may be bile plugs in the lobule and some giant cell changes can be seen. Later in the disease, biopsies will demonstrate biliary cirrhosis.
- PFIC4: there is a bland cholestasis with no specific bile duct loss or proliferation (Figure 8.8).

Genetics

Identification of mutations in *ATP8B1* (PFIC1), *ABCB11* (PFIC2), or *TJP2* (PFIC4) can aid clinical diagnosis in this group of conditions. Due to some clinical overlap, particularly between the three genes, some laboratories offer sequencing of all genes simultaneously. Screening of large phenotype-specific gene panels has become easier with the advent of new generation sequencing technologies.

Management

- Cholestasis: for all children UDCA may be helpful in improving bile flow and reducing pruritus.
- Pruritus: antipruritic medication will be necessary. For those with intractable pruritus and without fibrosis nasobiliary drainage or partial biliary diversion can be effective although the extrahepatic manifestations of PFIC1 may be exacerbated. Bile adsorptive resins such as cholestyramine are the most effective in reducing pruritus, but are unpalatable.
- Nutritional management will be essential whilst the patient is cholestatic. It is essential to supplement with fat-soluble vitamins as deficiency is common.

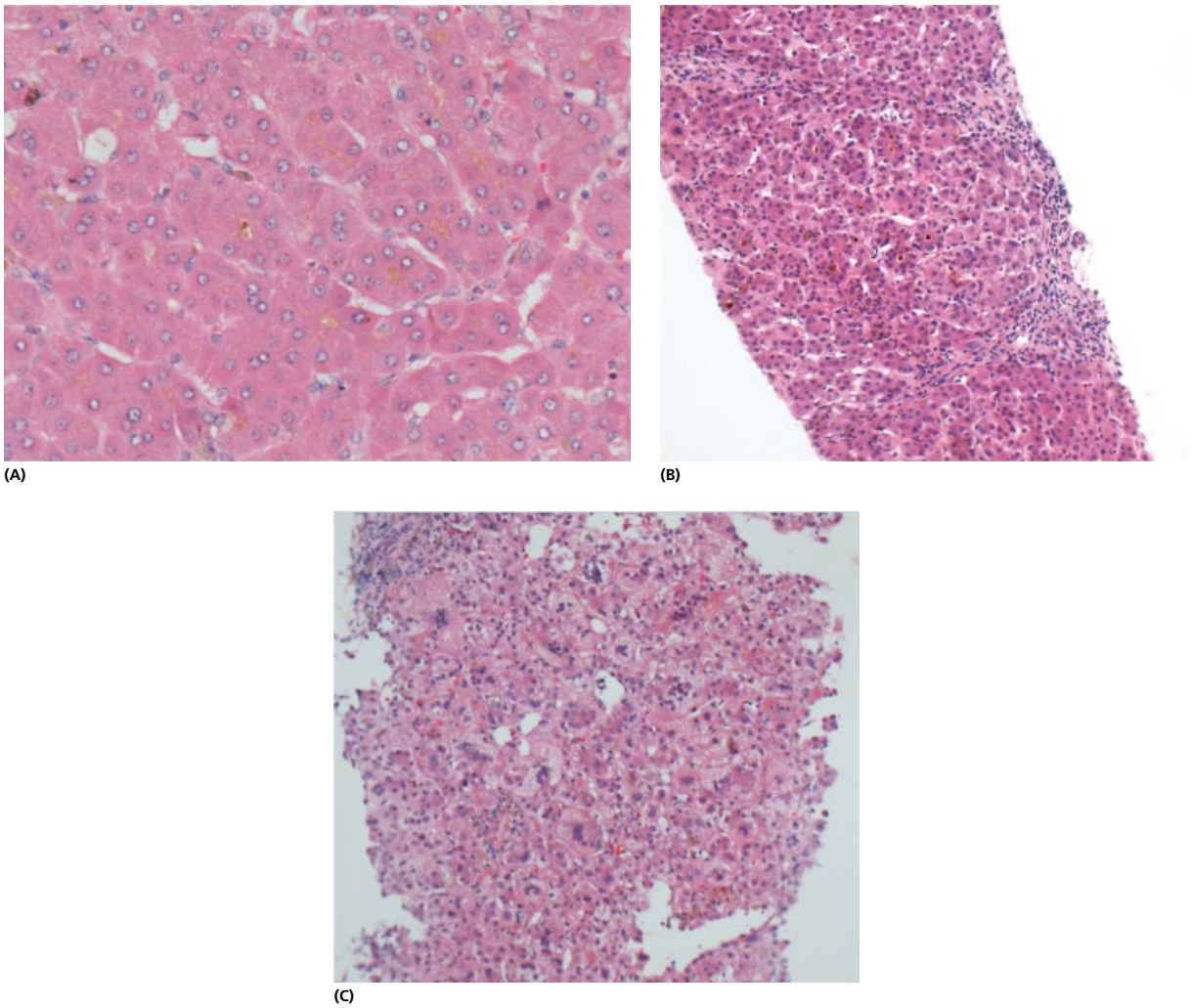


Figure 8.8 The typical histological findings of progressive familial intrahepatic cholestasis (PFIC) type 4. (H&E, × 200.) Neonatal hepatitis – disarray, giant cell change of hepatocytes and cholestasis. (A) Bland cholestasis of PFIC1. (H&E, × 400.) (B) Bland cholestasis of PFIC4. Bridging fibrosis. (H&E, × 200.) (C) PFIC2. Indistinguishable from NNH of any cause. (H&E, × 200.)

Indications for liver transplantation

Most patients will require liver transplant in childhood for progressive liver disease. For those with PFIC2, -3, and -4 this will be curative. For those with PFIC1 the extrahepatic manifestations may worsen especially the diarrhea. Graft steatosis leading to cirrhosis and the need for re-transplant occurs with PFIC1.

Recurrence of disease (jaundice, pruritus) has been recognized in children who have been transplanted for PFIC2. These patients developed anti-BSEP antibodies presumably because the acquired transporter is acting as a neoantigen.

Cholelithiasis

Gallstones in children are usually identified on ultrasound scan and may not be evident on abdominal X-ray. Gallstones may be more common in children with trisomy 21. In asymptomatic children with no underlying medical diagnoses, complete resolution within 6 months is likely and no medical intervention is required.

Stones may be identified in the fetus during antenatal scans. Children should be scanned postnatally; however there is usually spontaneous early resolution and no long-term complications.

In those with biliary obstruction then UDCA (20 mg/kg/day) may help resolution of stones but if this is unsuccessful or cholangitis develops then surgical intervention (cholecystectomy or percutaneous transhepatic cholangiography and lavage of the bile ducts, with or without a simultaneous sphincterotomy) will be required following treatment of the infection (see Chapter 25).

Inspissated bile

This is identified on ultrasound scan and usually develops following severe hemolysis such as in sickle cell disease, rhesus, or ABO incompatibility. Prematurity and sepsis may also be risk factors. Viscous bile in cystic fibrosis (CF) may also cause similar features.

UDCA may aid resolution; however if there is prolonged biliary obstruction fibrosis will develop and hence surgical intervention or percutaneous lavage may be required.

Spontaneous perforation of the common bile duct

This may occur antenatally or postnatally. The infant may be unwell with biliary peritonitis. There is usually biliary ascites, jaundice, abdominal pain, and fever. The diagnosis is made by measuring high levels of bilirubin in the ascites fluid. The management is usually by drain insertion if there is no distal duct obstruction. If there is obstruction within the bile duct a surgical repair and removal of the obstruction will be necessary.

Neonatal ichthyosis sclerosing cholangitis

This is a rare disorder with only 10 cases described. Infants with this condition have severe ichthyosis, decreased scalp hair, and scaring alopecia. The infants always have sclerosing cholangitis with jaundice, pruritus, and hepatomegaly. The natural history for the liver disease is variable with some resolving whilst in others it is progressive with extensive fibrosis, biliary proliferation, and chronic liver failure with portal hypertension. Liver transplant is the only management option for progressive liver disease. It is an autosomal recessive condition with mutations identified in the *claudin-1* gene that causes tight junctions to become leaky with regurgitation of paracellular bile resulting in liver injury.

Idiopathic neonatal hepatitis syndrome

This is a diagnosis of exclusion of other conditions and characteristic biopsy findings of giant cell transformation of the hepatocytes, lobular cholestasis, and little inflammation. The bile ducts are usually normal. It has an excellent prognosis if idiopathic, with full resolution. MCT-based feed and fat-soluble vitamin supplementation whilst jaundiced may be necessary (Figure 8.9).

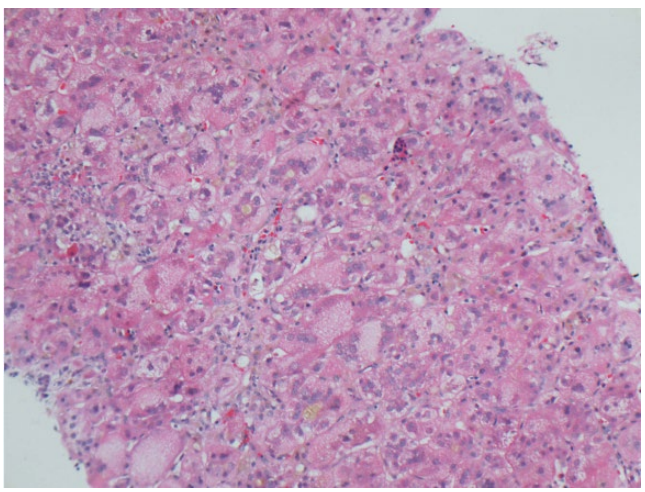


Figure 8.9 The typical histological appearance of idiopathic neonatal hepatitis showing giant hepatocytes, disarray of the parenchyma, and lobular inflammatory cells.

Multiple intestinal atresias. Intestinal atresias, abdominal wall defects, and associated immunodeficiencies may be associated with neonatal hepatitis syndrome where the prognosis is guarded.

Neonatal hepatitis in pre-term infants

Pre-term infants are at risk of developing cholestasis. The cause is usually multifactorial with hypotheses being difficult in establishing enteral feeding, the immature biliary tree, the need for parenteral nutrition, and sepsis. Supplementation with fat-soluble vitamins and UDCA whilst jaundiced, usually leads to complete resolution and an excellent prognosis.

Biliary atresia is rare in pre-term infants and has an atypical presentation presenting near to term and hence the development of pale stools requires further investigations to avoid missing the diagnosis.

Neonatal asphyxia

Perinatal asphyxia causes systemic hypoxia that may also include the liver. The liver involvement is variable and may occur despite no apparent effect on other organs (i.e., no hypoxic ischemic encephalopathy or renal asphyxia). The liver enzymes tend to increase from 24 h of birth with a peak in levels at up to 72 h of age. The decline in liver biochemistry to baseline can take up to 2 weeks. The liver enzymes trend up to two times the upper limit of normal. Higher levels may be seen in infants who have undergone cardiac surgery requiring bypass for more than 2 h. Small for gestational age babies are no more at risk than those who are term and fully grown infants. Understanding the trend in biochemistry aids the recognition of hypoxia as the cause for the transaminitis. Raised liver biochemistry which follows a different pattern requires further investigation.

Rises in neonatal bilirubin is caused by many factors and although it rises in hepatic hypoxia it has a more variable course without a specific trend.

There is no specific treatment for the hepatic component of birth asphyxia. Controlled induced hypothermia as a systemic treatment to protect against cell necrosis has been shown in small studies to also be beneficial to the liver.

Bile acid synthesis defects

Bile acid synthesis disorders are treatable. They typically cause cholestatic liver disease in early childhood with progressive neurological disease in later childhood and adulthood due to the deposition of cholesterol metabolites in the central nervous system. The neurological manifestations may arise without liver disease being apparent [9].

There are 16 enzymes involved in converting cholesterol to the primary bile acids cholic and chenodeoxycholic acid and a defect in any could cause disease.

The cholestasis is typically associated with a low GGT. There is often marked fat-soluble vitamin deficiency (especially vitamin D and A) due to poor absorption [10].

Clinical features

Four of the more common deficiencies are as follows:

- *3β-Hydroxy Δ^5-C_{27}-steroid dehydrogenase (3βHSD) deficiency*. There is a wide range of presenting clinical features. It may present in the neonatal period with neonatal hepatitis and cholestasis which when severe leads to acholic stool. In milder cases the liver manifestations may not be detectable and the presentation can be at any age (including adulthood) with failure to thrive, steatorrhea, or fat-soluble vitamin deficiency, with vitamin D deficient rickets being the most common. Pruritus may also be a feature even when liver disease is not overt.
- *Δ^4-3-Oxosteroid 3β-reductase deficiency*. There is heterogeneous clinical presentation similar to 3βHSD deficiency. There may be life-threatening cholestasis in infancy or it may present in later childhood with features of chronic liver disease.

  Sterol 27-hydroxylase deficiency (cerebrotendinous xanthomatosis, CTX). CTX can manifest in a wide variety of ways. The cholestasis in infancy is variable ranging from spontaneous resolution to death. In those who spontaneously resolve the diagnosis may not become apparent until later in childhood when chronic diarrhea and cataracts may develop. Neurological illness predominates in adulthood with dementia, ataxia, spastic paraparesis, seizures, or peripheral neuropathy.
- Tendon xanthomas develop, and there is a high risk of premature atherosclerosis with heart disease.
- Bile acid-CoA: amino acid *N*-acyltransferase deficiency (BAAT deficiency).

The last step in the production of bile acid glycine and taurine conjugates is catalyzed by amino acid *N*-acyltransferase. BAAT deficiency causes mild jaundice, steatorrhea, and fat-soluble vitamin deficiency. Mutations in BAAT are also associated with hypercholanemia in the Amish population.

Investigations

Biochemistry. GGT is low despite cholestasis in all cases. Fat-soluble vitamin deficiency can be profound and may be the presenting feature.

Urine. The diagnosis is made by identifying the abnormal bile acid composition in serum and urine with lack of primary bile acids and high concentration of intermediary metabolites. This is usually identified using fast atom bombardment mass spectroscopy (FAB-MS) of urine. This can be masked by the use of UDCA which gives a false negative result.

Histology. Neonatal hepatitis and giant cell changes. Δ^4-3 oxosteroid 3β-reductase deficiency can also show histological features of iron overload similar to those seen in neonatal hemochromatosis and also steatosis.

Genetics. All disorders are autosomal recessive:

- 3βHSD deficiency: *HSD3B7* mutations.
- Δ^4-3 oxosteroid 3β-reductase deficiency: *AKR1D1* mutations.
- Sterol 27-hydroxylase deficiency: *CYP27A1* mutations.
- Bile acid-CoA: amino acid *N*-acyltransferase deficiency: *BAAT mutations*.

Management

Replacement of the deficient primary bile acid reduces the formation of toxic intermediary compounds. Cholic acid (15 mg/kg once daily) and ursodeoxycholic (15 mg/kg twice daily) acid is beneficial. Fat-soluble vitamin supplementation will be required.

Prognosis

If the liver damage prior to diagnosis is not too advanced then bile acid replacement can lead to normalization of liver biochemistry, fat-soluble vitamin deficiency, and histology. Without treatment there is progressive liver disease due to the formation of toxic metabolites. This results in the need for liver transplantation which can be avoided if diagnosed and treated promptly.

Bile acid replacement can also reverse the neurological manifestations of CTX.

Arthrogryposis–renal dysfunction–cholestasis syndrome

ARC syndrome is an autosomal recessive multisystem disease. With the availability of molecular testing it is appreciated that there are an increasing number of phenotypes in a spectrum of disease. At the cellular level there is impairment of endosomal maturation, or fusion [11].

Clinical features

The classical features are arthrogryposis (skeletal contractures), Fanconi-like renal tubular dysfunction, and cholestasis (Figure 8.10).

Clinical features which are newly described include:

- Platelet diathesis (abnormal platelet aggregation) leading to bleeding following invasive procedures. Therefore liver biopsies are contraindicated as there is a high mortality rate secondary to bleeding.
- Icthyosis.
- Failure to thrive (not accounted for by the degree of liver or renal involvement).
- Hypotonia.
- Developmental delay.
- Chronic diarrhea.
- Hypothyroidism.
- Recurrent sepsis.
- Deafness.
- Congenital cardiovascular anomalies.
- Agenesis of corpus callosum.
- Nephrogenic diabetes insipidus.

Investigations

Biochemistry. There is conjugated hyperbilirubinemia with low GGT at presentation. A single case report has described a patient with high GGT despite the molecular diagnosis of

Figure 8.10 Infant with arthrogryposis–renal–cholestasis syndrome. Note the joint deformity, malnutrition, and low tone.

ARC syndrome. Despite a normal platelet count the platelet function is abnormal (abnormal protein trafficking and impairment in mature type II multivesicular bodies maturation in megakaryocytes underlie the α-granule deficiency) and liver biopsy is contraindicated due to the high mortality rate from biopsy.

Histology. Historical biopsies showed bile duct paucity with neonatal hepatitis.

Genetic. ARC syndrome is due to mutations in vacuolar protein sorting 33 homologue B (*VPS33B*) and VPS33B interacting protein (*VIPAR*), an apical–basolateral polarity regulator. There is a phenotype–genotype correlation, with those who retain some protein function having milder disease.

Treatment

Treatment is supportive with optimal nutritional support for both the liver and renal disease. Fat-soluble vitamins and support for the Fanconi syndrome will be necessary. This is a multisystem disease and there is no role for liver transplant.

Prognosis

Most infants die within the first year of life from renal and liver disease, although milder variants have been described but long-term survival is rare.

Jaundice in multisystem disorders

Congenital infection

Cytomegalovirus

CMV is the most common cause of congenital infection, affecting 1–2% of newborns, most of whom are asymptomatic. Primary maternal infection in the second or third trimester is associated with more severe fetal disease. CMV infection can be acquired postnatally via breast milk.

Clinical features

- Intrauterine growth retardation and prematurity.
- Jaundice (60–80%).
- Petechial rash.
- Hepatosplenomegaly.
- Central nervous system involvement (microcephaly, intracranial calcification, and chorioretinitis). Progressive hearing loss and cerebral palsy may become evident.

Investigations

The diagnosis is made from a positive CMV PCR, culture of virus from the urine (in the first 4 weeks), and positive CMV IgM (although this does not distinguish congenital from early acquired CMV).

Liver biopsy demonstrates giant cell hepatitis; the classical inclusion bodies are rarely seen in neonatal infection. Occasionally it causes intrahepatic bile duct paucity.

Management

Liver disease usually resolves with no treatment necessary. Treatment with ganciclovir or valganciclovir for at least 6 weeks improves the long-term neurological and hearing function.

Prognosis

Most children have mild liver disease which resolves with no treatment. However there are case reports of:

- Hepatic fibrosis.
- Non-cirrhotic portal hypertension.
- Intrahepatic calcification.
- Cirrhosis and chronic cholestasis requiring liver transplant.

Persisting neurodevelopmental, visual and hearing abnormalities are the main problem in the majority of patients.

Toxoplasmosis
Congenital toxoplasmosis is comparatively rare. Maternal infection in the third trimester is more likely to cause fetal infection than infection earlier in pregnancy.

Clinical features
Neonatal hepatitis with hepatosplenomegaly (which can be severe and life threatening) is an important feature, but may be less obvious than central nervous system involvement with chorioretinitis (with large pigmented scars), hydrocephaly, or microcephaly. Intracranial calcification is usually prominent, leading to convulsions, nystagmus, and evidence of increased intracranial pressure. Pneumonitis may be severe.

Investigations
Toxoplasma serology identifies infection. Liver biopsy may demonstrate a non-specific hepatitis or portal fibrosis with bile duct reaction.

Treatment
If a mother in the first trimester develops toxoplasmosis then prevention of transfer to the fetus is with spiramycin. If toxoplasmosis develops after 18 weeks' gestation or the fetus is known to have contracted toxoplasmosis (through amniocentesis) then pyrimethamine, sulfadiazine, and folinic acid are recommended. Spiramycin therapy after birth may prevent progression of central nervous system and liver disease. Prognosis depends on the extent of neurological or optic disease.

Rubella
Congenital infection with rubella virus is now rare because of immunization.

Clinical features
It may cause intrauterine growth retardation, anemia/thrombocytopenia, congenital heart disease (patent ductus arteriosus or pulmonary artery stenosis), cataracts, chorioretinitis ("salt-and-pepper" appearance), mental retardation, and sensorineural deafness. Hepatosplenomegaly is usual.

Investigations
The diagnosis is made by serological testing. A liver biopsy is rarely required but if done shows a typical giant cell hepatitis.

Prognosis
The disease may be self-limited or progress to cirrhosis.

Syphilis
Congenital syphilis is increasing in frequency. It causes a multisystem illness and may be identified on antenatal ultrasound scans.

Clinical features
- Intrauterine growth retardation and subsequent failure to thrive.
- Severe anemia and thrombocytopenia.
- Nephrotic syndrome.
- Periostitis.
- Nasal discharge ("snuffles").
- Skin rash.
- Fever.
- Diffuse lymphadenopathy.
- Hepatomegaly.
- Jaundice may be present within 24 h of birth or develop after treatment. Jaundice may be severe. Some babies with congenital syphilis never develop jaundice but present with a typical rash on the palms and soles or only with fever, as well as prominent hepatomegaly. Central nervous system involvement occurs in up to 30% of infants.

Investigations
Diagnosis involves serological testing, including the Venereal Disease Research Laboratory (VDRL) test and confirmatory testing for specific antitreponemal antibodies. Radiographs of long bones may show typical bony abnormalities in the first 24 h of life and aid rapid diagnosis.

Liver histology in untreated congenital syphilis may reveal numerous treponemes in hepatic tissue, but after treatment with penicillin, giant cell hepatitis without detectable treponemes is the usual finding. Bile duct paucity may develop.

Management
Penicillin is an effective treatment.

Human immunodeficiency virus infection
Although infants with congenital human immunodeficiency virus (HIV) infection may present with hepatosplenomegaly, conjugated hyperbilirubinemia in the neonatal period is rare. There is an increased incidence of congenital CMV infection in HIV-infected infants. Congenital HIV infection may present clinically as hepatitis with jaundice although later than in the neonatal period, typically at around 6 months of age.

Erythrovirus (parvovirus) B19 infection
Congenital parvovirus B19 infection may cause profound anemia, leading to hydrops and fetal death which is more common if infection occurs before 20 weeks' gestation.

Clinical features
The spectrum includes conjugated hyperbilirubinemia, hepatomegaly, severe coagulopathy, dermal erythropoiesis ("blueberry muffin" rash), anemia, and perinatal distress.

Investigations
Liver biopsy showed diffuse sinusoidal fibrosis, siderosis, and little giant cell transformation of hepatocytes but excessive extramedullary hemopoiesis. Despite features of early hepatic insufficiency, serum aminotransferases may be low or near normal. The diagnosis is made by PCR for the presence of

parvovirus B19, although placental histology may suggest prenatal parvovirus infection. The outcome depends on the severity of the infection.

Listeriosis

Congenital infection with *Listeria monocytogenes* typically involves the liver.

Clinical features

Although meningitis is the predominant clinical feature of the systemic disease, infants have hepatosplenomegaly and are sometimes jaundiced. Pneumonia is usually present. A history of maternal illness is common.

Investigations

The diagnosis is made by isolating the organism from blood, cerebrospinal fluid, or liver. Liver biopsy may reveal simply a diffuse hepatitis or, more commonly, diffuse areas of focal necrosis.

Management

Treatment is with penicillin.

Tuberculosis

Congenital tuberculosis is rare, but since the prevalence of tuberculosis in women of child-bearing age has risen in the past few years, tuberculosis in infants may become more common. Newborn infants may be infected by aspirating infected amniotic fluid or cervical secretions at the time of delivery.

Investigations

Practical criteria for diagnosis are a proven tuberculous infection in a newborn baby with at least one of the following: lesions in the first week of life; primary hepatic complex or caseating granulomas in the liver; tuberculous infection of the placenta or maternal genital organs; and exclusion of postnatal infection by investigation of contacts.

Clinical features

Hepatomegaly is common in infants with tuberculosis, but jaundice is rare and indicates severe disease. Respiratory distress, poor feeding, and fever are frequent. The mortality approaches 30%.

Management

Quadruple antitubercular antibiotic regimen *excluding* ethambutol is recommended. A high index of suspicion appears to be required for diagnosis, as tuberculosis in this age group often has atypical clinical features and delays in commencing therapy increase the mortality rate.

Acquired infection

Neonatal sepsis

In the neonatal period hepatobiliary dysfunction secondary to sepsis, especially Gram-negative sepsis is common. The bilirubin tends to rise by day 3 of sepsis with a peak at 10 days and resolution by 60 days. The hepatic enzymes tend to rise more slowly with a peak at day 38 and resolution by day 60. There are no hepatic long-term complications [12].

Galactosemia, which can present with Gram-negative sepsis and liver dysfunction should be excluded.

Herpes simplex virus

In the neonatal period both herpes simplex virus (HSV) type 1 and 2 can cause severe liver failure, disseminated infection, and multiorgan failure.

Clinical features

It usually presents in the first 2 weeks of life with non-specific symptoms. Vesicles may or may not be present on the skin. HSV type 2 is usually acquired from active genital infection hence a cesarean section is recommended.

Investigations

HSV may be identified from scrapings of skin vesicles or blood PCR. Herpes identified in the eye or EEG recording support the diagnosis.

Liver biopsy, which is rarely carried out due to profound coagulopathy, shows areas of necrosis with viral inclusions in intact hepatocytes.

Management

Early treatment with aciclovir may modify the clinical course and hence all newborns with suspected sepsis should be treated with aciclovir and broad-spectrum antibiotics.

Liver transplant for infants in whom only the liver is affected may be successful; however rapid dissemination with multiorgan failure is more common. There is a very high mortality rate (see Chapters 9 and 18).

Enteric viral sepsis (echovirus, coxsackieviruses, adenoviruses)

These cause disseminated viral sepsis with multiorgan involvement and severe acute liver failure usually within the first 5 weeks of life.

Clinical features

The incidence is greatest at the seasonal peak of echovirus infections (late summer to early autumn). The infant's mother may report that abdominal pain developed just prior to the onset of labor. Vertical infection near the time of birth is associated with more severe disease in the infant. Echovirus serotype 11 appears to be most virulent for newborns although other serotypes have been implicated.

If there is myocarditis or heart failure then coxsackie A and B viruses are more likely.

Adenoviruses causes the same non-specific clinical symptoms with liver failure.

Investigations

Identification of the virus is either from stool culture, throat swab, or blood PCR.

Management

Antiviral treatment may improve the dismal prognosis of neonatal enteroviral hepatitis if commenced early in the disease course. Pleconaril has been used successfully in some enteroviral infections and cidofovir treats adenovirus. Mesenchymal stem cells, interferon, and immunomodulation are areas of research for coxsackievirus infection.

Prognosis

The mortality with acute pattern liver failure is of the order of 85–90%. Meticulous supportive care is essential. Infants who recover may initially develop severe cholestatic jaundice but subsequent hepatic function appears entirely normal.

Varicella zoster

Varicella may occur in newborn infants if maternal infection occurs within 14 days of delivery. It tends to be more severe in premature infants and is mild in term infants after 10 days of age. Early presentation or protracted disease in an infant of any gestational age may lead to a fatal outcome. This severe disease is characterized by jaundice and extensive skin and multisystem involvement, especially pneumonia. In severe or fatal cases, hepatic parenchymal involvement can be demonstrated. Treatment with aciclovir may attenuate the infection or be curative.

Hepatotropic viruses: hepatitis A, B, C, and E

Infection with hepatotropic viruses in neonates does not cause jaundice unless there is acute liver failure or severe hepatitis.

Hepatitis A

Hepatitis A is rare in the neonate, but congenital infection may occur if the mother is infected 1–2 weeks before delivery. The typical picture in the early neonatal period is a nonspecific diarrheal illness.

Hepatitis B

Vertical hepatitis B infection is subclinical in the neonatal period; prompt administration of both hepatitis B immunoglobulin and hepatitis B immunization provides protection against chronic infection in 93% of infants at risk. Infants in whom this regimen fails may have been infected transplacentally. Without immunoprophylaxis, infants may become chronic carriers or develop acute hepatitis B or fulminant hepatic failure after a 3–4 month incubation period.

Hepatitis C

Hepatitis C is not a cause of neonatal hepatitis syndrome.

Hepatitis E

This virus is endemic and is usually self-limiting but does not cause neonatal jaundice.

However in pregnant women it causes liver failure with at least a 10% mortality rate. Loss of the fetus or newborn occurs in over half of infants born to mothers with hepatitis E due to:

- Stillbirth or miscarriage.
- Prematurity.
- Infant viral infection with hepatitis, jaundice, and, in some, overwhelming liver failure.

If the infant survives the neonatal period there is a good prognosis and no long-term liver complications.

Human herpesvirus 6 infection

Human herpesvirus 6 (HHV-6) causes exanthema subitum, a common but usually benign febrile illness in infants; other HHV-6 infections are common and self-limited without a rash. Acute liver failure has been reported.

Paramyxovirus infection (syncytial giant cell hepatitis)

Clinical features

In neonates, syncytial giant cell hepatitis is associated with a severe hepatitis, which does not meet the criteria for fulminant liver failure.

In children, fulminant hepatic failure is common, while rapidly progressive chronic hepatitis occurs in adults.

Older infants may have features of a chronic active hepatitis or autoimmune hemolytic anemia.

Hepatitis with moderately elevated serum aminotransferases frequently progresses to chronic cholestasis and decompensated cirrhosis over 6–12 months.

Investigations

Liver biopsy. Liver histology and electron microscopy show both the characteristic syncytial-type giant cells and viral inclusions consistent with the morphology of paramyxoviruses. Formation of giant multinucleated hepatocytes is a characteristic response of infantile hepatocytes to injury, which is not often seen in hepatitis in adults. Syncytial giant cells differ from the giant cells of neonatal hepatitis because the outline of the liver cell plates remains evident, with indistinct, "smudged" borders between the cells. They may form because of cell fusion secondary to paramyxovirus, in the same way as with other viruses such as respiratory syncytial virus and measles virus.

Management

Spontaneous recovery is uncommon. Treatment with the antiviral agent ribavirin appeared efficacious in one infantile case and in a few adults. Most babies require liver transplantation before the end of the first year of life.

Endocrine disorders

Hypothyroidism

This is more commonly associated with unconjugated jaundice; however neonatal hepatitis may occur.

Hypopituitarism

Clinical features

- Failure to thrive, hypoglycemia, and jaundice which typically present in the neonatal period [13].
- In males there is micropenis and low testosterone levels.
- Septo-optic dysplasia.

Investigations

The diagnosis is made by identifying the deficiency in pituitary hormones.

The transaminses are mildly elevated but the GGT is variable and can be normal.

Radionucleotide excretion scan can show no excretion into the gastrointestinal tract if severe.

Liver histology is that of canalicular cholestasis and eosinophilic infiltrate.

It is important to look specifically for septo-optic dysplasia which is associated with absent corpus callosum and septum pellucidum, and hypoplasia of the optic nerves.

Management

The cholestasis is thought to be due to the central adrenal insufficiency, and may improve spontaneously but this is accelerated by hormone replacement. There is no treatment for septo-optic dysplasia. Without hormone replacement, progression to cirrhosis has been reported

McCune–Albright syndrome

Genetics

This broad spectrum of manifestation is due to a somatic mutation during embroyogenesis of the α-subunit of Gs which is the G protein that stimulates cyclic adenosine monophosphate (cAMP). Patients' somatic cells are mosaic for this mutation and hence the clinical features are determined by the distribution of affected cells.

Clinical features

The classical clinical triad is polyostotic fibrous dysplasia, skin hyperpigmentation, and endocrine dysfunction. If the liver is affected the symptoms include neonatal jaundice with histology which ranges from normal to giant cell hepatitis [14].

Management

Supplementation of the deficient hormones. There is no specific liver therapy required.

Prognosis

The natural history is resolution of the cholestasis but persisting elevated transaminases despite normal histology, but no progression of liver fibrosis unless there are other comorbidities such as viral hepatitis. Hepatoblastoma has been reported.

Metabolic disease

Many metabolic diseases cause jaundice with liver failure in the newborn period (see Chapter 9).

Niemann–Pick disease type A

This is due to lysosomal sphingomyelinase deficiency. Clinical features include hepatosplenomegaly, failure to thrive, and progressive neurological deterioration. Jaundice is unusual. Fetal ascites has been reported.

Niemann–Pick disease type C

This rare (1 in 120,000) neurovisceral disease is due to mutations in *NPC1* or *NPC2*. Mutations lead to impaired intracellular trafficking of lipid and hence accumulation of cholesterol and glycosphingolipids in organs such as the brain, liver, and spleen. Its clinical presentation is extremely heterogeneous as the neurological signs arise at different ages (from neonatal period through to adulthood) [15].

Clinical features (Figure 8.11)

The systemic symptoms which tend to precede the onset of neurological symptoms may include:

- Hepatosplenomegaly with a specifically prominent spleen.
- Fetal hydrops.
- Ascites.
- Two-thirds present with neonatal cholestasis.
- Chronic liver failure.

The systemic symptoms are also variable. Some have overwhelming liver failure in infancy whilst in others the hepatosplenomegaly is not appreciated until the onset of neurological symptoms. In adults isolated splenomegaly occurs. In those who have severe liver disease but survive

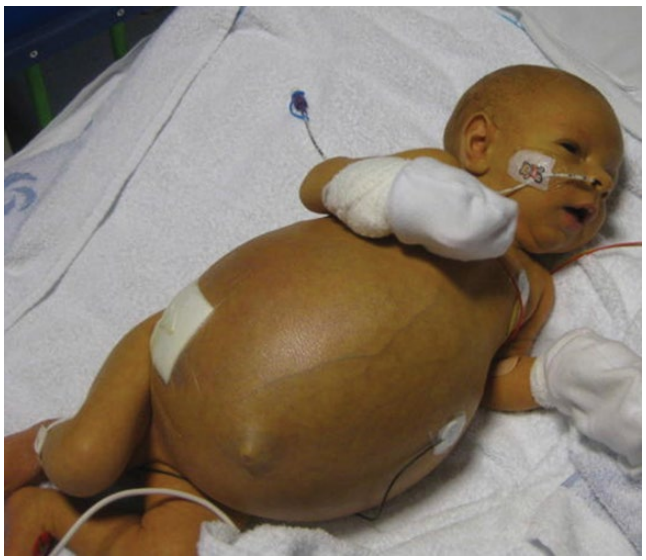


Figure 8.11 Niemann–Pick disease type C presents in many different ways including signs of chronic liver disease. This child has jaundice, ascites, distended abdominal veins, palmer erythema, and hepatosplenomegaly.

infancy, fibrosis with the development of portal hypertension can occur. Monitoring for portal hypertension is necessary using endoscopy as the spleen size is an unreliable indicator.

The neurological manifestations have insidious onset including:

- Low muscle tone with frequent falls.
- Motor developmental delay.
- Arrested or delayed speech.
- Cerebellar signs.
- Dystonia.
- Dysphagia – silent aspiration is detected by videofluoroscopy.
- Abnormal vertical and then horizontal eye movements progressing to complete supranuclear gaze palsy.
- Psychiatric illness is associated with adult-onset disease.

All patients who survive infancy will develop neurological signs that are progressive and life limiting.

Diagnosis

Biochemical. These include:

- Mild thrombocytopenia.
- Mildly elevated ALT and AST.
- Raised conjugated bilirubin in the neonatal period (but rare after infancy).
- Plasma low-density lipoprotein and high-density lipoprotein cholesterol may be decreased and triglycerides increased.
- Chitotriosidase is used in a screening test for Gaucher disease but may also be positive in Niemann–Pick disease. It is however absent in 6% of the normal population and has a low sensitivity and specificity.
- Oxysterols – sensitive and specific markers for Niemann–Pick disease. They may also correlate with disease severity and time of neurological symptom onset.
- Filipin stain of bone marrow, liver tissue, or fibroblasts (demonstrates impaired cellular cholesterol transport) if positive is a definitive diagnosis.
- Electron microscopy of the skin or liver may also be diagnostic.

Genetic. These include:

- Autosomal recessive inheritance of either of two genes *NPC1* (chromosome 18, q11-q12) or *NPC2* (chromosome 14, q24.3).
- 95% have mutations in *NPC1* and 4% in *NPC2*, with 1% not having an identifiable mutation suggesting other genes are involved.
- Genetic testing enables a specific diagnosis, prenatal diagnosis for subsequent pregnancies, efficient identification of affected siblings, and identifies those with *NPC2* mutations which may be amenable to stem cell transplant.
- Variant phenotype occurs when there is one known pathogenic mutation and one unknown mutation. *NPC1* p.P1007A in which a missense mutation on codon 992 is most frequently seen.

Histology. Liver biopsy shows a histologically severe neonatal hepatitis, pericellular fibrosis, and pseudoacinar formation. The diagnosis is confirmed by identifying the characteristic PAS-D-resistant material (foam) in Kupffer cells and hepatocytes, which may be difficult to identify in neonates.

Bone marrow. Bone marrow aspiration may show foam cells and can be used for filipin staining. In early disease foam cells may not be present.

Management

The mainstay of treatment is supportive management of liver failure, cholestasis symptom control of seizures, dystonia, and dysphagia.

Miglustat (*N*-butyldeoxynojirimycin, Oxford GlycoSciences, UK) is an iminosugar which is a competitive inhibitor of the enzyme glucosylceramide synthase which catalyzes the first step in glycosphingolipid synthesis. It crosses the blood–brain barrier and reduces the glycosphingolipid accumulation. It reduces the progression of neurological symptoms.

In up to 50% of patients it has adverse effects with the most common being diarrhea, flatulence, weight loss, and tremor. The side effects reduce with time. It can take 6 months to a year to see any clinical benefit. Miglustat is not offered at the time of diagnosis due to the side effects as well as the uncertainty of neurological manifestations.

Prognosis

In most infants, liver disease resolves and jaundice disappears within the first year of life. Long-term complications of portal hypertension may occur. Neurological symptoms become obvious by 5 years of age. Most children develop loss of upward gaze due to vertical supranuclear ophthalmoplegia, which is regarded as a pathognomonic sign. Most children die in early adolescence from bronchial pneumonia rather than liver failure.

Citrin deficiency (citrullinemia type II)

Citrin is a mitochondrial aspartate glutamate carrier associated with the urea cycle, encoded by *SLC25A13*, found on chromosome 7q21. It is expressed mainly in the liver; also in the kidney and heart. Lack of citrin leads to liver-specific dysfunction of argininosuccinate synthetase. It plays an important role in reduced nicotinamide adenine dinucleotide (NADH) disposition, because it transports reducing equivalents into mitochondria as part of the malate aspartate shuttle, which is critical for aerobic glycolysis.

In adults, citrullinemia type II presents with fatty liver, hepatitis, and iron accumulation; episodic neurological symptoms associated with hyperammonemia may also occur.

In infants it has been termed "neonatal intrahepatic cholestasis with citrin deficiency" (NICCD).

Clinical and biochemical features

The diagnosis should be suspected in an infant who has neonatal hepatitis with hypoglycemia or hypergalactosemia.

Jaundice is common but failure to thrive without jaundice may occur. Features of compromised liver synthetic function such as ascites and coagulopathy may be found; poor growth or outright failure to thrive is frequent. Cataracts have been found in some infants.

Investigations

Biochemistry. A diverse range of metabolic abnormalities may occur including defects in gluconeogenesis (hypoglycemia can be pronounced); aerobic glycolysis; urea synthesis and possibly in fatty acid synthesis; galactose metabolism is abnormal due to decreased UDP-galactose epimerase activity and increased plasma levels of galactose; and plasma levels of citrulline, tyrosine, threonine, arginine, and methionine are elevated.

Liver biopsy. Most specimens showed varying degrees of fibrosis. The degree of inflammation varied among the specimens, with half showing moderate or severe inflammatory changes. Fat deposition in hepatocytes, which may be severe, was observed in almost all of the specimens. There was a mixture of two types of hepatocytes with macrovesicular or microvesicular fat droplets, and cholestasis was observed at a rate of 77%. Hemosiderin deposition, mostly mild and localized in periportal hepatocytes and macrophages in portal areas, was observed in 57% of the specimens.

Genetics. Autosomal recessive inheritance of mutations in *SLC25A13* results in citrin deficiency. The highest incidence is in East Asians (1 in 17,000), but it has been found throughout the world. Numerous pathogenic mutations have been found.

Management

A lactose-free formula is preferable, since lactose may be cytotoxic while the infant still has neonatal hepatitis. MCTs and fat-soluble vitamins should be supplied when cholestatic. Curiously, older children and adults have peculiar food preferences: they avoid sweets and rice, and they prefer peanuts and beans.

Prognosis and indications for liver transplantation

The hepatitis associated with citrin deficiency appears to resolve spontaneously in most affected infants, usually within the first year of life; occasionally, the neonatal liver disease is severe and progresses to liver failure, requiring liver transplantation. Living-donor transplants with a heterozygote donor appear to be successful.

In those with resolution of their neonatal liver disease the clinical features of citrullinemia may develop in adolescence and social drinking may provoke hepatic deterioration.

North American Indian childhood cirrhosis

Severe chronic cholestatic liver disease was described in 14 North American Indians living in northwestern Quebec, Canada.

Clinical features

- Conjugated hyperbilirubinemia which resolved within the first year of life disappeared during the first year of life.
- Hepatosplenomegaly.
- Pruritus.
- Facial telangiectasia.
- Portal hypertension.

Investigations

Biochemistry. Serum aminotransferases, GGT, alkaline phosphatase, and bile acids are elevated. Serum cholesterol may be normal or raised in most patients.

Electron microscopy of the liver shows widening of the pericanalicular microfilament cuff.

Genetics. All patients are homozygous for a missense mutation (R565W) in *FLJ14728*, conventionally called *CIRH1A*, on chromosome 16q22. The gene product, cirhin, is a nucleolar protein of ribosomal biogenesis that may play a role in biliary development.

Prognosis

The liver disease typically progresses to biliary cirrhosis in this disorder, although liver transplantation is often not required in the first decade of life.

Zellweger syndrome

Zellweger syndrome is a rare disorder, with an incidence of 1 in 100,000. The sexes are affected equally. It is the prototype of the peroxisomal biogenesis disorders, characterized by multiple abnormalities of peroxisome function. The molecular and cell biology of these disorders is complex, involving multiple *PEX* genes, which encode peroxins – proteins required for peroxisome assembly. Zellweger syndrome is most often associated with mutations in *PEX1* and *PEX6*. Bile acid synthesis is abnormal because of selective or generalized deficiency of the peroxisomal enzymes involved in side-chain modification. In Zellweger syndrome, C_{27} bile acids accumulate; these are principally trihydroxycoprostanic acid (THCA) and dihydroxycoprostanic acid (DHCA). These would ordinarily undergo side-chain modification in the peroxisome to chenodeoxycholic acid and cholic acid.

Clinical features

Zellweger syndrome is a multisystem disorder.

Liver symptoms. In the first 3 months of life, hepatic involvement may not be prominent, although some babies

have persistent conjugated hyperbilirubinemia. Fifty per cent of infants are not jaundiced but have hepatosplenomegaly with evidence of poor hepatic synthetic function. Cirrhosis may develop.

Other features. Profound hypotonia, facial dysmorphism with a high forehead and large fontanelles, developmental delay, seizures, bony abnormalities such as epiphyseal calcifications, adrenal insufficiency, and cystic malformations in the brain and kidneys. Failure to thrive and feeding difficulties are common.

Investigations

The diagnosis is confirmed by demonstrating abnormal bile salt metabolites using FAB-MS, or by the detection of very-long-chain fatty acids in serum. Hepatic histology may be normal, although there may be excess iron deposition. Hepatic fibrosis is typical. Paucity of the small (portal) bile ducts may be found. Electron-microscopic studies of the liver reveal an absence of peroxisomes in the hepatocytes. Mitochondria may appear abnormal and muscle biopsies may be suggestive of a mitochondrial myopathy.

Prognosis

Treatment is supportive, as death is inevitable. Liver transplantation is contraindicated because of the multisystem disease. Primary bolus therapy with cholic and chenodeoxycholic acid may produce some initial improvement, but does not prolong life.

Lysosomal acid lipase deficiency

Lysosomal acid lipase (LAL) is a serine hydrolase which hydrolyzes cholesteryl ester and triglycerides which have been delivered to the lysosomes, into free cholesterol and free fatty acids. It results in the accumulation of triglycerides and cholesterol esters in various tissues of the body, leading to pathological conditions such as Wolman disease with complete loss of LAL function and cholesteryl ester storage disease (CESD) with partial loss of LAL function.

Clinical features

Infants with Wolman disease present in the first few weeks with persistent vomiting, failure to thrive, hepatosplenomegaly, liver dysfunction (without jaundice), and hepatic failure. Adrenal calcification is a striking feature but is present in only about 50% of cases. Inheritance is autosomal recessive. The majority die in early infancy.

In CESD children develop an enlarged liver, leading to cirrhosis and chronic liver failure before adulthood. Children may also develop calcium deposits in the adrenal glands and jaundice. Onset varies, and the disorder may not be diagnosed until adulthood where it can be mistaken for fatty liver disease.

Management

Enzyme replacement therapy for both Wolman disease and CESD is available from Synageva. Certain drugs may be given to help with adrenal gland production, and children may need to be fed intravenously. A low-cholesterol diet may be beneficial in CESD.

Gaucher disease

Although this is rarely associated with neonatal hepatitis syndrome, an infant has been described with neonatal Gaucher disease and cholestasis, as well as prominent thrombocytopenia.

Mucopolysaccharidoses

Neonatal hepatitis syndrome is unusual with most forms of mucopolysaccharidosis but has been reported with type VI mucopolysaccharidosis, Maroteaux–Lamy disease, in which the lysosomal enzyme arylsulfatase B is abnormal. The infant had typical facies and a cherry-red spot on the retina. Type VII mucopolysaccharidosis (β-glucuronidase deficiency) has also been reported with neonatal hepatitis.

Respiratory causes

Cystic fibrosis (see Chapter 16)

The incidence of liver disease in children with is 27–35% [16]. In these children cirrhosis develops in the first decade of life followed by the development of symptoms and signs of portal hypertension related complications. Liver cirrhosis is the most common non-pulmonary cause of death, accounting for 2.5% of CF mortality.

In screened neonates only 5.7% developed cholestasis. Those who have meconium ileus are more likely to develop cholestasis in infancy. This does not increase the risk of developing CF-related liver disease in childhood.

Clinical features

The spectrum of infant hepatic disorder is highly variable, but the clinical presentation is with jaundice, hepatomegaly, and failure to thrive.

- Some infants have giant cell hepatitis.
- Extrahepatic bile duct obstruction may be due to inspissated bile actually plugging the common bile duct requiring choledochotomy.
- Some infants have a lesion resembling that of biliary atresia, where the common hepatic and common bile ducts are apparently damaged by the abnormal bile so that portoenterostomy is required to restore bile flow.
- Paucity of intrahepatic bile ducts ("non-syndromic duct paucity").
- Severe hepatic steatosis has been reported in infants with CF who are typically not jaundiced.

Management

Early studies suggested that infants with severe liver disease had meconium ileus, and this is supported by more recent data obtained at autopsy in patients similar with respect to pulmonary function, nutritional status, and Shwachman score. Children with cirrhosis had a statistically significant relationship between the incidence of mucous plugs in liver tissue histologically and meconium ileus in infancy or distal intestinal obstruction syndrome later in life. Occurrence of neonatal hepatitis syndrome in itself does not necessarily predict early development of cirrhosis.

Chromosomal disorders

Cytogenetic abnormalities, including trisomy 13, cat-eye syndrome, deletion of the short arm of chromosome 18, and 49 XXXXY, have been reported in association with biliary atresia.

Trisomy 18

Trisomy 18 is associated with growth retardation, skeletal abnormalities, and complex congenital heart disease.

The liver manifestations include:

- Giant cell hepatitis.
- Extrahepatic biliary atresia.
- Paucity of bile ducts.

Trisomy 21

Liver manifestations of trisomy 21 include [17]:

- Neonatal cholestasis.
- Extrahepatic biliary atresia.
- Severe hepatic fibrosis associated with transient myeloproliferative disorder raising the possibility that hepatic fibrogenesis might be due to high concentrations of growth factors derived from megakaryocytes. Infants with this transient leukemia have a poorer prognosis overall when there is jaundice and hepatic dysfunction.

Cardiac disorders

The role of chronic passive congestion, or functional hepatic venous obstruction, in neonatal hepatitis syndrome is difficult to assess. Babies with severe chronic congestive heart failure may develop moderate hepatomegaly or hepatosplenomegaly, as well as ascites [18].

In those who have had a Fontan procedure in infancy that resulted in a chronically elevated central venous pressure, cirrhosis with related complications may develop. Encephalopathy has not been described. Focal nodular hyperplasia due to changes in vascular flow through the liver may develop.

Infants with acute circulatory failure associated with severe congenital heart disease or shock may develop elevated serum aminotransferases, coagulopathy, and jaundice with mild to moderate conjugated hyperbilirubinemia, which resolves rapidly once hepatic perfusion is restored.

Autoimmune disease

Neonatal lupus erythematosus

Neonatal lupus erythematosus is due to passage of maternal anti-Ro and anti-La antibodies across the placenta, leading to damage to fetal tissues, which express Ro and La antigenic determinants. Other susceptibility factors remain undefined.

Clinical features

The heart, skin, and liver are most likely to be involved, rarely with thrombocytopenia and leukopenia. Congenital heart block is the most dramatic cardiac manifestation. A rash resembling discoid lupus erythematosus may be present in the newborn period or may develop some weeks later. Hepatic involvement, evident in approximately 10% of cases, is often limited to elevated serum aminotransferases, but neonatal hepatitis syndrome is found. Occasionally this is severe enough to mimic extrahepatic biliary tract obstruction, with acholic stools and non-draining hepatobiliary scan. In severe cases, a clinical phenotype of neonatal hemochromatosis may be found. Transient unexplained isolated conjugated hyperbilirubinemia in the perinatal period, and later presentation at 2–3 months of age with transient elevations of serum aminotransferases, are other possible clinical presentations. In most infants, the liver disease resolves completely between 6 and 12 months of age, as the maternal antibodies are degraded. Mild fibrosis was found in one child on repeat liver biopsy.

Diagnosis

Deposits of associated antibodies (anti-Ro and/or anti-La) may be found in affected liver tissue on immunofluorescence.

The diagnosis of neonatal lupus erythematosus is difficult in the child who does not have congenital heart block, a typical skin rash, or a history of maternal systemic lupus erythematosus or Sjögren syndrome. The risk of neonatal lupus erythematosus in subsequent pregnancies appears variable, estimated at 10–50%.

Giant cell hepatitis with autoimmune hemolytic anemia

This is a rare progressive disease that is often fatal. It leads to liver and multiorgan failure. The cause is unknown but it is postulated to be an autoimmune disease [19].

Clinical features

The symptoms often develop at 6–24 months year of age with jaundice, hepatitis, hepatosplenomegaly, and with Coombs positive hemolysis.

Investigations

Liver biopsy shows giant multinucleated cells not typical of autoimmune disease.

Management

Some patients have responded to treatment with prednisolone and azathioprine, but the disease has frequently been refractory to immunosuppressive treatment and may

recur following liver transplantation. Rituximab may be beneficial.

Prognosis

Mortality relates to the high dose immunosuppression and relapse including after transplantation.

Hemophagocytic lymphohistiocytosis

This is a life-threatening disorder of uncontrolled macrophage cells resulting in excessive hemophagocytosis, immune dysregulation, and cytokine storm [20]. It may be familial or secondary to viruses, malignancy or autoimmune (macrophage activation syndrome). Due to the non-specific nature of presentation it can be difficult to diagnose in the initial stages when early treatment allows for improved survival.

Clinical features

Large splenomegaly and hepatomegaly occur due to the infiltration of the organs with lymphocytes and histiocytes. Liver failure is often present due to the infiltration and jaundice is a common feature. The coagulopathy which can be profound is a result of the hypofibrinogenemia and liver failure.

Diagnosis is based on molecular diagnosis or five of the following criteria:

- Fever.
- Splenomegaly.
- Cytopenias (two or more cell lines).
- Hypertriglyceridemia.
- High ferritin.
- Hemophagocytosis on bone marrow or lymph node.
- Low or absent natural killer cell activity.
- Elevated soluble CD25.

Management and prognosis

Without treatment it is a 100% fatal disease. Early treatment is important to increase survival with treatment. Treatment regimens include steroids, etoposide, and ciclosporin. Stem cell transplant in those who survive the initial period can be curative.

Standard therapy for conjugated hyperbilirubinemia

The care of a child with liver disease is complex and requires multidisciplinary input to achieve the ideal management. The medical and surgical teams need to work closely to facilitate a timely Kasai portoenterostomy and to decide on timing for transplant if necessary.

Expert nutritional in the cholestatic child is essential. Not only can it improve the liver disease but also ensure that the infant is in an optimal condition for surgery. Many of the infants will have spent considerable time unwell in hospital therefore physiotherapy is important for rehabilitation and to aid progression of normal childhood development.

For many families the diagnosis of a cholestatic disease in infancy means lifelong medication, hospital appointments, and the possibility of transplantation. They may also have to come to terms with a genetic disease. Psychological support for these families and for the child when they are older is essential.

Nutrition

Optimizing nutrition and recognizing specific nutritional needs are essential in infants with liver disease. Good nutrition is an important part of clinical management. Close monitoring of weight gain, with changes in nutritional management instigated promptly if weight gain is poor, may help stabilize neonatal cholestasis and facilitate hepatic regeneration. A dietitian working with the family and clinician is essential to achieve this (see Chapter 6).

Difficulties in providing effective nutrition include:

- Increased calorific requirement in children with liver disease. Most infants will require at least 30% more than their normal nutritional requirements. Often jaundiced babies initially appear hungry due to their increased nutritional needs and poor triglyceride absorption
- Poor absorption of long-chain triglycerides due to reduced or absent bile salt micelles. This also reduces fat-soluble vitamin absorption. A change of feed from primarily long-chain triglycerides to MCTs enables absorption of fats without the need for bile salt micelles. This means the infant will effectively absorb more calories. Most prescription MCT feeds have 60–65% MCT.
- Unwell and may not feed well. The introduction of supplemental feeding via a nasogastric tube in children who are not able to take the full amount of feed will ensure optimal nutrition is being received. This may be in the form of top-up feeds to increase the volume at each feed or if the child is not thriving, an overnight feed for 12 h will increase the calories provided within a 24 h period.

Vitamins

All children with conjugated jaundice require supplemental fat-soluble vitamins as they are not absorbed effectively in the absence of bile micelles. It is important to monitor the vitamin levels as supplemental vitamins are absorbed at different rates. Recommended vitamin doses are provided in Table 8.6.

Ursodeoxycholic acid

UDCA is a bile acid, used to improve the flow of bile in children with cholestasis. It may also be beneficial in improving pruritus.

Pruritus

Pruritus may be a distressing symptom that is difficult to control. It can lead to mutilation of the skin through excess scratching, secondary infection of the skin, poor sleep (and

Table 8.6 Recommended fat-soluble vitamin and ursodeoxycholic acid (UDCA) doses. Alfacalcidol and α-tocopheryl are first-line supplements for vitamin D and vitamin E, respectively. In severe malabsorption intramuscular vitamin D and E may be required.

Vitamin A	5000 units/day
Ergocalciferol (vitamin D)	1200 IU/day
Alfacalcidol (aid calcium absorption)	50 ng/kg/day
Vitamin E:	
α-Tocopheryl acetate or	50 mg/day
Polyethylene glycol (Vedrops)	25 IU/kg/day
Vitamin K	2.5 mg/day
Ursodeoxycholic acid	10 mg/kg twice daily

Table 8.7 Medication used for pruritus, with recommended doses.

Drug	Dose
Cholestyramine	1–4 g daily
Phenobarbital	3–5 mg/kg/day
Rifampicin	5–10 mg/kg/day
Ondansetron	2–4 mg twice daily
Naltrexone	6–20 mg/kg/day
Antihistamines	These are ineffective but may cause sedation at night to provide symptomatic relief

hence development), and irritability. It is most common in AGS, PFIC, and biliary atresia. The cause is unknown, but in some cases it is associated with elevated plasma bile salts

Treatment

The general advice is to keep the child cool as heat exacerbates pruritus; to keep nails short and body and limbs covered. Cotton eczema body suits may be useful. The skin is often excessively dry which compounds the itching. Regular moisturizing with emollients will provide symptomatic relief.

Medical management is provided in Table 8.7. Antihistamines are often used at night to provide a sedative effect but otherwise are not effective against the pruritus associated with cholestasis. Cholestyramine, which is a bile salt resin is an effective but unpalatable medication which also reduces elevated cholesterol. It is recommended that other medication is given 1 h prior to cholestyramine or 4 h after, due to it binding other fat-soluble medication such as vitamins medication. Due to the sedative effects phenobarbital may not be tolerated outside the neonatal period.

Rifampicin is very effective and is the initial drug of choice after cholestyramine. Tolerance to any of these medications is common requiring drug holidays to provide long-term relief.

Clinical trials with apical sodium dependent bile acid transporter inhibitors (ASBTi) which reduce the enterohepatic bile salt cycle are in progress with variable results in AGS and PFIC1.

The pruritus may improve with age in patients with AGS.

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CHAPTER 9

The Acutely Ill Baby

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Key points

- A structured diagnostic approach to the acutely unwell infant is crucial.
- In infantile liver failure galactose containing feed should be stopped and intravenous aciclovir commenced immediately.
- Rapid molecular genetic diagnosis is feasible for an increasing range of disorders.
- Universal newborn screening has transformed the natural history of some disorders including tyrosinemia type 1.

The majority of patients presenting with liver disease in infancy will have either cholestatic liver disease (see Chapter 8) or acute liver failure with multisystem disease. Recent advances in laboratory techniques and molecular genetics have dramatically improved both the speed and accuracy of diagnosis for many of these conditions whilst therapeutic developments have altered outcomes. This chapter outlines the clinical presentation and approach to the diagnosis and management of infants presenting with an acute illness.

Approach to diagnosis and management

Infants and neonates have limited responses to severe illness irrespective of etiology, but important diagnostic information may be obtained from simple clinical and laboratory observations obtained at the time of presentation.

Age at presentation is important, with three risk periods recognizable: at birth, in the neonatal period, and later in infancy. Conditions presenting at birth imply an intrauterine process, while early neonatal presentation may imply a toxic process, often infective or metabolic in origin (Box 9.1).

In establishing etiology, the clinical history should enquire about preceding symptoms and precipitants. In infants presenting soon after birth, information about pregnancy and delivery is of obvious relevance. In all age groups, a dietary history is crucial, with particular attention being paid to symptoms on weaning or on fasting, and recent changes in diet. In neonates the first sign of altered consciousness may be difficulty in feeding, whereas in older infants vomiting is a frequent accompaniment of encephalopathy. A history of consanguinity or of any previously affected siblings should be sought.

Clinical assessment should first concentrate on the airway–breathing–circulation (ABC) of resuscitation, followed by a complete physical examination. Particular attention should be paid to the presence of hepatomegaly, neurological abnormalities, tachypnea, and unusual odors. It is important to recognize intercurrent illnesses such as pneumonia, septicemia or congenital heart disease, which may have precipitated the acute episode and will require specific treatment.

Box 9.2 lists the investigations that are required immediately. Additional samples of each biological fluid should be separated and frozen, with subsequent analysis depending on the results of the initial screen. An algorithm for diagnosis of the acutely ill infant is given in Figure 9.1, with appropriate second-line investigations outlined in Table 9.1.

Until the diagnosis is established, management is supportive with correction of hypoglycemia, acid–base imbalance, electrolyte imbalance, and coagulopathy as required (see Chapter 18). It is prudent to exclude lactose, fat, and protein from the diet during the first 24 h of acute illness while awaiting specific diagnostic information, but restriction should be for as short a time as possible. Parenteral empirical broad-spectrum antibiotic treatment should be commenced once initial samples have been obtained and continued until sepsis has been excluded.

* The work relating to this chapter was carried out while the author was based at Birmingham Children's Hospital NHS Foundation Trust, UK.

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Box 9.1 Differential diagnosis of acutely ill infants.

Conditions presenting at birth

- Hydrops fetalis
- Lysosomal storage disease
- Niemann–Pick disease types C1 and C2

Conditions presenting in neonatal period

- Infection:
  - coxsackie A or B
  - herpes simplex
  - adenovirus
  - cytomegalovirus
  - parvovirus
  - echovirus
- Neonatal hemochromatosis (gestational alloimmune liver disease)
- Mitochondrial cytopathy
- Galactosemia
- Organic acidemias
- Urea cycle defects
- Disorder of fatty acid oxidation
- Wolman disease

Conditions presenting later in infancy

- Tyrosinemia type 1
- Glycogen storage disease
- Hereditary fructose intolerance
- Recurrent acute liver failure syndrome
- Transaldolase deficiency

Box 9.2 Initial investigations in the acutely ill infant.

Blood*

- Bacterial culture
- Prothrombin time, partial thromboplastin time, fibrinogen, D-dimers
- Bilirubin, alkaline phosphatase, transaminases, albumin, γ-glutamyl transferase
- Acid–base balance
- Glucose
- Lactate
- Ammonia, amino acids
- Full blood count and film
- Urea, sodium, potassium, calcium
- Acylcarnitine profile
- DNA for mutation analysis

Urine*

- pH
- Microscopy and culture
- Reducing substances
- Ketones
- Organic acids
- Amino acids

CSF (if coagulation and neurological state allow)

- Gram stain and culture
- Protein, glucose
- Lactate

Radiology

- Chest and wrist X-ray
- Echocardiography

* Store plasma, serum, and urine samples for further investigations (see Table 9.1).

Infection

Intrauterine or postnatal infections are important and common causes of acute illness in the neonate; these are fully discussed in Chapters 8 and 13 (see also Boxes 9.1 and 9.2).

Fetal and neonatal ascites

Fetal or neonatal ascites are rare presentations that occur in about 1 in 3000 pregnancies and are associated with intrauterine infection, inborn errors of metabolism, or rhesus hemolytic disease. As the rate of fetal loss is high, few infants present with this complication. The use of anti-D immunoglobulin has reduced the incidence of hemolytic disease and the majority of cases are now secondary to disorders of cardiac structure or rhythm, hematological, gastrointestinal, or genitourinary disease. Hepatic and metabolic causes account for about 4% of cases, with lysosomal storage disorders being the commonest of this group which may even include mitochondrial disorders (Box 9.3). Metabolic causes are particularly important to recognize as they may recur and early prenatal diagnosis may be possible [1].

A number of pathogenic mechanisms may contribute to the development of ascites: cardiac failure; anemia due to infiltration of the reticuloendothelial system; hepatic infiltration or insufficiency producing hypoalbuminemia; or mass effects resulting in vascular and lymphatic obstruction.

The diagnosis may be suspected on antenatal ultrasound or at birth because of abdominal distension. Hepatosplenomegaly is usually present.

Investigation

If the child dies, the placenta and fetus should be examined comprehensively. It is necessary to exclude the commoner infective, cardiovascular and hematological causes, and search for storage disorders as follows:

- Blood film for vacuolated lymphocytes.
- Bone marrow aspirate for storage cells.

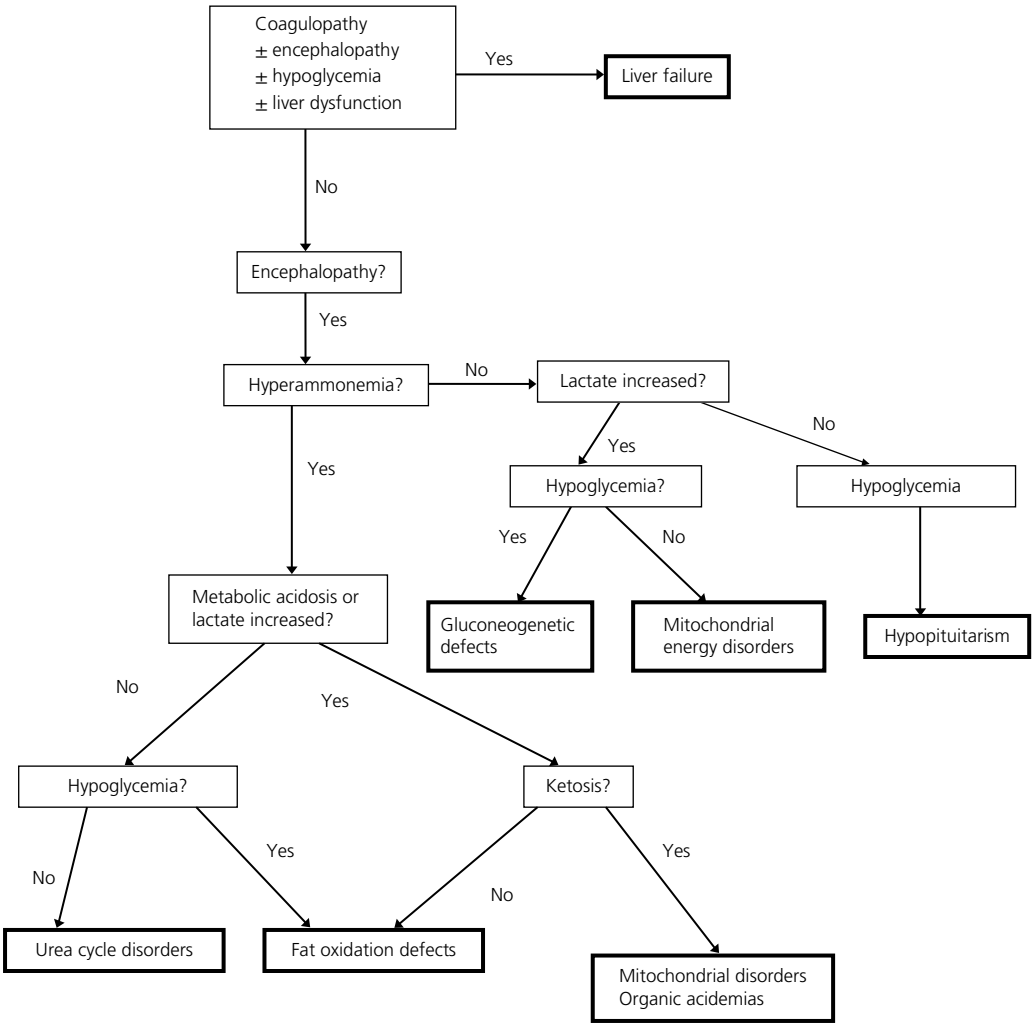

Figure 9.1 Algorithm for the initial investigation/diagnosis of the acutely ill infant. The bold boxes represent phenotypic groups. For each phenotypic group second-line investigations are summarized in Table 9.1.

- Plasma transferrin electrophoresis for carbohydrate-deficient glycoprotein syndrome.
- Urinary oligosaccharidases and glycosaminoglycans.
- White cell or fibroblast culture or placental cell line for lysosomal enzyme studies.

Treatment

This should be started prenatally, if possible, with close obstetrical liaison regarding the mode and place of delivery. Resuscitation may require immediate paracentesis and transfusion in the labor suite. Subsequent management and prognosis depend on the etiology. The prognosis is poor for storage diseases presenting in this manner. Symptomatic relief may be gained by the use of spironolactone (3 mg/kg/day), fluid restriction (50–75% maintenance), and 4.5% albumin transfusion (5–10 mL/kg).

Galactosemia

This autosomal recessive disorder is caused by deficiency of galactose-1-phosphate uridyl transferase (GALT) and has an incidence of 1 in 45,000. Several different allelic variants with varying degrees of residual activity have been recognized. The commonest mutation (Q188R) accounts for >70% of abnormal alleles while N314D is associated with a milder form, the Duarte variant. Much more rarely, a defect of the epimerase enzyme can occur with similar clinical presentation. Galactose, galactitol, and galactose-1-phosphate (Gal-1-P) accumulate following feeding. While accumulation of galactitol causes cataract it is unclear which metabolites cause liver dysfunction.

Clinical presentation and diagnosis

Infants may present with collapse, hypoglycemia, and encephalopathy in the first few days of life, or with progressive jaundice and liver failure. Vomiting, diarrhea, jaundice,

Table 9.1 Second-line investigations following initial assessment.

Phenotypic group	Second-line investigations
Liver failure	Fe, transferrin, ferritin Cholesterol and triglycerides Galactose-1-phosphate uridyl transferase Plasma amino acids Urinary organic acids and succinyl acetone Specific urine and stool viral culture DNA for common mutations in *POLG, DGUOK*, MPV17, and *TRMU* Urinary polyols
Mitochondrial energy disorders*	Pre-/postprandial plasma lactate, glucose, FFA and 3-OH Plasma carnitine, acylcarnitine Plasma amino acids, creatine kinase Urinary organic acids CSF lactate DNA for common mutations in *POLG, DGUOK*, MPV17, and *TRMU*
Muscle biopsy for RCE/ mtDNA depletion	Brain MRI EEG and visual evoked potentials
Urea cycle disorders	Plasma amino acids Carnitine and acylcarnitines Lactate, glucose, FFA, and 3-OH Creatine kinase Urinary amino, organic and orotic acids
Fat oxidation defects*	Carnitine and acylcarnitines; lactate, glucose, FFA, 3-OH Creatine kinase Urinary organic acids
Gluconeogenetic defects	Lactate, glucose FFA, 3-OH Creatine kinase, urate, cholesterol, triglycerides Urinary organic acids and oligosaccharides Specific enzyme assays (see text)
Organic acidemias*	Plasma amino acids Carnitine and acylcarnitines Urinary organic acids
Hypopituitarism	09.00 h cortisol, thyroid function tests, growth hormone, IGF-1

CSF, cerebrospinal fluid; EEG, electroencephalogram; Fe, iron; FFA, free fatty acids; IGF-1, insulin growth factor 1; MRI, magnetic resonance imaging; 3-OH, 3-hydroxybutyrate; RCE, respiratory chain enzyme.
* Fibroblast culture for specific enzymatic diagnosis.

Box 9.3 Causes of neonatal ascites.

Congenital infection

- Cytomegalovirus
- Toxoplasmosis
- Syphilis

Metabolic

- Lysosomal storage disorders:
  - Salla disease
  - Sialidosis type II
  - Niemann–Pick disease type A and C
  - GMI gangliosidosis
  - Mucopolysaccharidosis VII
  - Wolman disease
- Gaucher disease
- Tyrosinemia type 1
- Neonatal hemochromatosis (gestational alloimmune liver disease)
- Carbohydrate-deficient glycoprotein syndrome
- Mitcohcondial DNA mutation
- Transaldolase deficiency

Other

- Cardiac disease
- Hepatoblastoma
- Hemangioendothelioma
- Mesenchymal hamartoma

and poor weight gain are common in early infancy. Cataracts (characteristically "oil-drop") are present shortly after birth and may be associated with intraocular hemorrhage and retinal detachment. There is a high incidence of Gram-negative sepsis, which is usually associated with a severe coagulopathy. Renal tubular dysfunction is common.

The diagnosis is classically suggested by the detection of urinary reducing substances without glycosuria, but urinary tests are neither sensitive nor specific. An associated proximal renal tubular defect may result in aminoaciduria and glycosuria while reducing substances in the urine are a frequent nonspecific finding in other forms of neonatal liver disease. Alternatively, galactosuria may not be detected in an infant with galactosemia who is severely ill and no longer taking a lactose-containing formula.

The diagnosis should be confirmed by demonstration of reduced GALT activity in blood. Misleading results may be obtained if the baby has been transfused (false negative) or in glucose-6-phosphate dehydrogenase deficiency (false positive). Where there is confusion, other diagnostic options include measuring erythrocyte Gal-1-P, testing for the Q188R mutation or measuring parental-erythrocyte GALT.

Hepatic pathology initially demonstrates fatty change, periportal bile duct proliferation and iron deposition with extramedullary hematopoiesis (Figure 9.2). If galactose ingestion persists, hepatic fibrosis and cirrhosis may develop, although cirrhosis may be present at birth.

Management and prognosis

A lactose-free diet should be started immediately in any infant suspected of having galactosemia and continued until galactosemia has been definitely excluded. Liver function improves within days following exclusion of galactose from the diet unless liver failure or cirrhosis is already established. Progressive liver disease is very rare. Cataracts may improve if treatment is started early enough. Galactose elimination should be lifelong but long-term complications such as cognitive impairment, speech defects, hypergonadotropic hypogonadism, and motor abnormalities are common despite

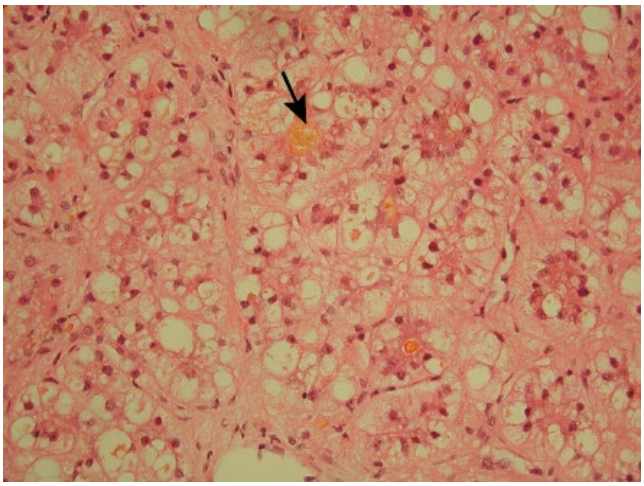

Figure 9.2 Galactosemia may present with acute liver failure or cholestasis with a giant cell hepatitis. This 6-week-old baby presented with both, as she developed cholestasis from biliary obstruction due to biliary sludge. Note bile plugs (arrow).

dietary treatment. This probably results from ongoing endogenous synthesis of Gal-1-P and generalized glycoprotein hypoglycosylation rather than dietary indiscretion [2].

Neonatal screening

Neonatal screening for galactosemia is not universally available but leads to early detection except in those babies who present with fulminant hepatitis. Antenatal diagnosis is possible by mutation detection via chorionic villus sampling.

Infantile liver failure

Acute liver failure in infancy usually presents with multisystem involvement. The diagnosis may initially be difficult as jaundice may be a late feature. Infants may be small for gestational dates or have intrauterine growth retardation. The clinical presentation includes hypotonia, hypoglycemia, and hypotension (Figure 9.3). Coagulopathy is invariable in association with liver dysfunction and moderate hyperammonemia. Encephalopathy is frequent but not inevitable in infants and may be difficult to recognize (see Chapter 18). Neurological problems such as nystagmus and convulsions may be secondary to cerebral disease or encephalopathy. Renal tubular acidosis or lactic acidosis are common.

Physical examination and investigations should be directed at identifying and excluding multiorgan disease and pathology.

General measures include intravenous dextrose to maintain blood glucose at 4–8 mmol/L, prophylactic antibiotics and intravenous aciclovir, antifungal therapy, intravenous ranitidine to prevent gastric bleeding, correction of coagulopathy if appropriate, and/or exchange transfusions (see Chapter 10). Once baseline and essential investigations have been conducted (see Box 9.2) galactose and protein should be excluded from the diet until the underlying diagnosis is confirmed or galactosemia specifically excluded. As spontaneous recovery is unlikely, all children should be assessed for liver transplantation unless there is irreversible multiorgan disease.

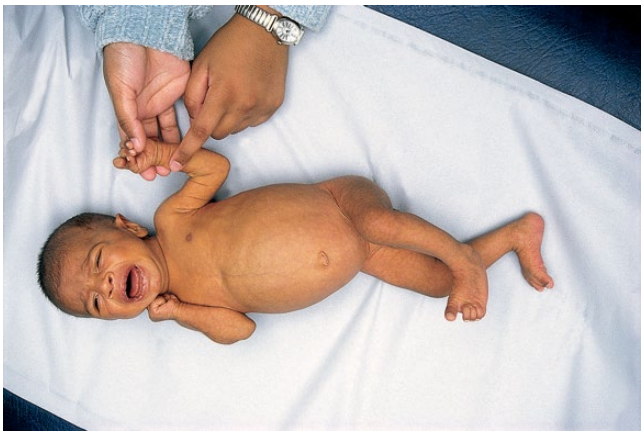

Figure 9.3 Infants with acute liver failure present with multiorgan failure, coagulopathy, encephalopathy, and jaundice.

Neonatal hemochromatosis

This disorder, also known as gestational alloimmune liver disease, is the commonest cause of acute liver failure in the neonate. The disease is characterized by prenatal liver disease combined with extrahepatic siderosis with sparing of the reticuloendothelial system (see Chapter 10).

Disorders of mitochondrial energy metabolism

This rare group of disorders may present with acute liver failure, multiorgan disease, and Alpers syndrome. There is a wide range of clinical phenotypes with any mode of inheritance – autosomal recessive, autosomal dominant, or transmission through maternal DNA. The pathological effects are secondary to dysfunction of the electron transport chain resulting in cellular adenosine triphosphate deficiency, impaired fat oxidation, and the generation of toxic free radicals. Clinical symptoms are variable depending on the nature of the primary defect, its tissue distribution and abundance, and the importance of aerobic metabolism in the affected tissue [3].

Mitochondrial DNA (mtDNA) is a separate 16.6-kb genome of maternal origin, which encodes two ribosomal RNAs, 22 transfer RNAs, and 13 subunits of the respiratory chain. Nuclear DNA (nDNA) contains approximately 1000

mitochondrial genes that encode most subunits of the respiratory chain and all of complex 2 subunits. In addition, they encode the proteins that control transcription, translation, salvage, and repair of mtDNA. In the acutely ill infant, three entities are most relevant: isolated deficiencies of the electron transport chain enzymes, mtDNA depletion syndromes, and Alpers syndrome.

Deficiencies of the electron transport chain enzymes

The most common isolated defects involve complexes IV and I. A large number of causative nuclear gene defects have been recognized, but in individual cases the diagnostic yield is still relatively low [3].

Mitochondrial DNA depletion syndrome

Mitochondria normally contain multiple copies of mtDNA. Replication of mtDNA is regulated by a number of factors encoded by nuclear genes. Mutations in these nuclear genes lead to reduction in copy numbers of mtDNA resulting in mitochondrial DNA depletion with autosomal recessive inheritance. Pathogenic mutations causing mtDNA depletion have been described in 10 genes to date, of which at least four result in liver disease (DGUOK, POLG, MPV17, and Twinkle) [3, 4].

Alpers syndrome

Alpers syndrome is an autosomal recessive, developmental mtDNA depletion disorder caused by mutations in *POLG*. It is characterized by degenerative brain and liver disease which may be precipitated by valproate treatment [4]. The liver disease may present with infantile liver failure but may evolve from asymptomatic biochemical liver dysfunction. Seizures, which are focal and refractory, usually precede liver disease but in infancy, liver disease may be the presenting symptom. Also, where presentation is in infancy the developmental regression may be more difficult to appreciate.

Clinical presentation

The clinical presentation is varied, and the onset may be prenatal with structural brain abnormalities. Non-specific dysmorphic features are not uncommon. Neurological features are prominent, and lethargy and hypotonia are almost invariable. Cardiac involvement includes hypertrophic cardiomyopathy, and there may be proximal renal tubulopathy. Hepatic involvement is unpredictable and includes isolated hepatomegaly, neonatal cholestasis, and acute liver failure.

Diagnosis

A structured, tiered, diagnostic evaluation should be used (Box 9.4). Persistently elevated plasma lactate is still the most sensitive test for mitochondrial cytopathies. Definitive diagnosis is now by genetic analysis. As a minimum children presenting with a suspected hepatic mitochondrial cytopathy should have common mutations in *POLG*, *DGUOK*, *MPV17*, and *TRMU* excluded [4].

If pathogenic mutations are not detected, definitive diagnosis requires demonstrating dysfunction of electron transport chain function in affected tissue and/or proving reduced mtDNA copy number (<30% of control) [4]. The most useful tissue to sample is usually muscle, as it is easily accessible with well-established normal ranges. In the presence of liver failure, demonstration of extrahepatic involvement will preclude liver transplantation and abnormalities in muscle correlate highly with neurological involvement. The exception is Alpers syndrome where muscle biopsy is usually normal at presentation and lactic acidosis is infrequent. In this disorder visual evoked responses (VER) and brain MRI are often abnormal and the EEG may be characteristic.

Box 9.4 Hierarchical investigation schema in suspected mitochondrial liver disease.

Tier 1

- Pre-/postprandial plasma lactate, glucose, free fatty acid, and 3-OH
- Plasma carnitine, acylcarnitines
- Plasma amino acids, creatine kinase, thymidine
- Urinary organic acids, amino acids, tubular resorption phosphate, albumen/creatinine ratio
- Cerebrospinal fluid lactate/protein (if feasible)
- Electrocardiography and echocardiography
- Electroencephalography and visual-evoked potentials
- DNA for common mutations in *POLG, DGUOK*, MPV17, and *TRMU*

Tier 2

- Tissue analysis:
  - Liver biopsy: (if feasible) tissue for light microscopy, electron microscopy, and oil red O stain. Frozen tissue for respiratory chain enzyme activity analysis and mtDNA copy number
  - Muscle biopsy: tissue for light microscopy, electron microscopy, oil red O stain, and histochemistry for respiratory chain complexes. Frozen tissue for respiratory chain enzyme activity analysis and mtDNA copy number
  - Skin biopsy: set up for fibroblast culture

Tier 3

- Cranial magnetic resonance imaging/spectroscopy

Tier 4

- Extended molecular screening: this will be guided by the clinical phenotype, results of the tissue analysis, and local facilities. Currently suggested genes should include *SUCLG1*, *BCS1L*, *SOC1*, *TFSM*, *TWINKLE*, *ACAD9*, *EARS2*, *GFM1*, *RRM2B*, *TK2*, and *SUCLA2*

Liver biopsy, if feasible, usually reveals both microvesicular and macrovesicular steatosis, with hepatocyte degeneration and micronodular cirrhosis (Figure 9.5). Electron microscopy may reveal abnormal shape or number of mitochondria. Respiratory chain enzymes and mtDNA copy numbers should be measured in liver tissue but interpretation can be problematic because abnormalities may be secondary to the liver disease rather than the primary cause of it [5].

Cranial MRI and magnetic resonance spectroscopy (MRS) are increasingly useful in confirming central nervous system involvement, but in general share the lack of specificity of many investigations for suspected mitochondrial cytopathies.

Management and prognosis

Supportive management for acute liver failure may be the only therapeutic option. If valproate has been used it should be immediately discontinued and *N*-acetylcysteine and carnitine commenced. Dichloroacetate may provide symptomatic benefit by lowering lactate concentrations, but clinical benefit has not been established and there are concerns about side effects. Liver transplantation may be successful if the defect is confined to the liver [6] which is highly unusual if the presentation is with infantile liver failure. Transplantation is usually contraindicated in infantile liver failure due to mitochondrial disease if multisystem involvement is demonstrated, as in Alpers syndrome, and all forms of mtDNA depletion with the possible exception of selected cases due to mutations in *MPV17*. Disease due to mutations in *TRMU* is important to recognise as there is a real chance of spontaneous recovery with an excellent long-term prognosis [4].

Antenatal diagnosis

Inheritance is most commonly autosomal recessive, but antenatal diagnosis is possible only if the underlying mutation is known.

Tyrosinemia type I

Tyrosinemia type I is an autosomal recessive disorder due to a defect of fumaryl acetoacetase (FAA), which is the terminal enzyme in tyrosine degradation. Intermediate metabolites such as maleyl- and fumaryl-acetoacetate are highly reactive compounds that are locally toxic and mutagenic within the liver. The secondary metabolite succinylacetone (SA) has local and systemic effects (Figure 9.4) including inhibition of porphobilinogen synthase, accounting for the porphyria-like neurological crises seen.

The gene for FAA is on the short arm of chromosome 15. More than 90 mutations have been described to date [7], although in some populations a single mutation may be prevalent. A hallmark of the condition is the extremely high lifetime risk of developing hepatocellular carcinoma (HCC), which historically is at least 40%.

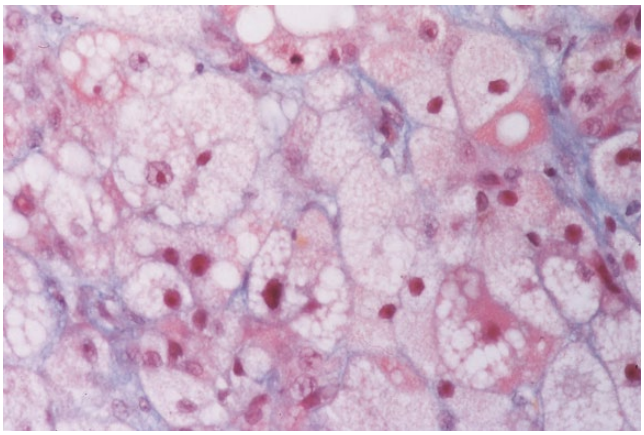

Figure 9.4 Mitochondrial deletions or depletions. These rare inherited defects present with acute liver failure with multisystem involvement. Hepatic histology may demonstrate microvesicular steatosis.

Clinical presentation

The disease is found worldwide but is particularly common in the Saguenay–Lac-Saint-Jean region of Canada (incidence 1 in 500) and in parts of Pakistan and northern Europe. In Birmingham, the incidence is 1 in 20,000, reflecting the mixed ethnic population.

Clinical presentation is heterogeneous even within the same family. Acute liver failure is the most common presentation usually in infants between 1 and 6 months of age; these patients present with mild jaundice, coagulopathy, encephalopathy, and ascites with inguinal hernias. Hypoglycemia is common and may be secondary to liver dysfunction or hyperinsulinism due to pancreatic islet cell hyperplasia [7].

In older infants, failure to thrive, coagulopathy, hepatosplenomegaly, hypotonia, and rickets are common. Older children may present with chronic liver disease, cardiomyopathy, renal failure, or a porphyria-like syndrome with self-mutilation. Neurological symptoms include muscle weakness, particularly respiratory muscle weakness. Renal tubular dysfunction is almost invariable and hypophosphatemic rickets may occur at any age.

Diagnosis

Investigations and diagnostic findings include:

- Mildly elevated bilirubin.
- Mildly abnormal transaminases (100–200 IU/L).
- Elevated alkaline phosphatase (>600 IU/L).
- Low albumin (<30 g/L).
- Prolonged prothrombin time (>20 s).
- Grossly elevated α-fetoprotein levels (40,000–200,000 mg/L).
- Increased plasma tyrosine, phenylalanine, and methionine (three times normal depending on age).
- Significant urinary SA is a pathognomonic but not invariable finding.

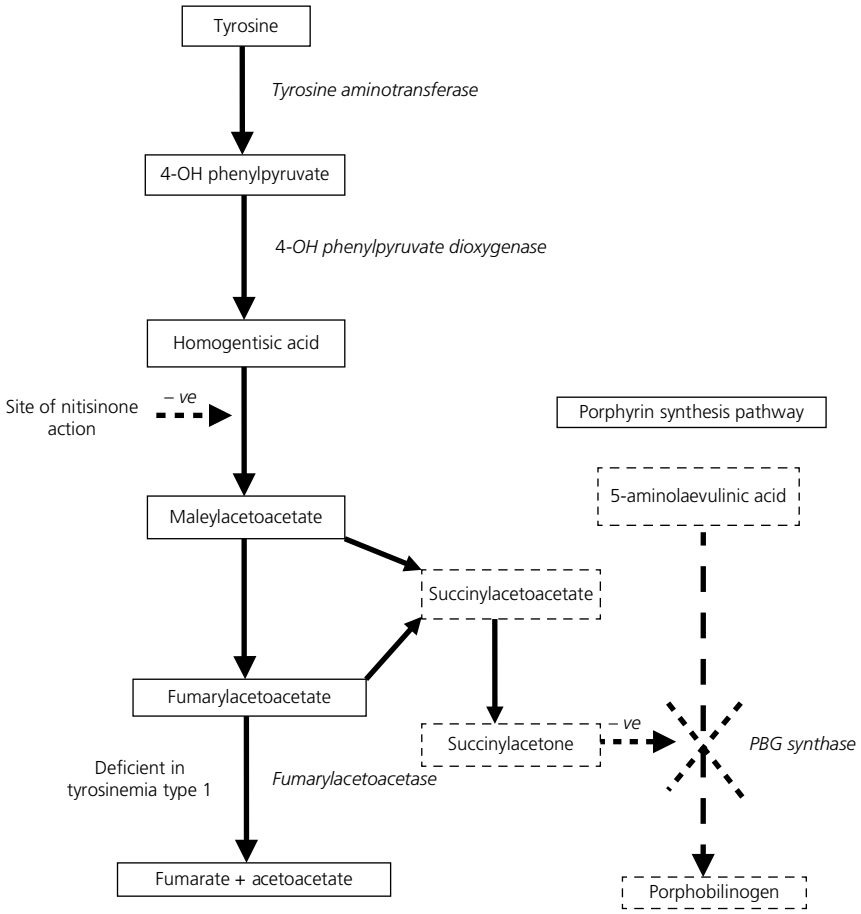

Figure 9.5 Metabolic pathway for tyrosine metabolism. 4-OH, 4-hydroxybutyrate.

- Increased urinary δ-aminolaevulinic acid.
- Proximal tubular dysfunction with phosphaturia and aminoaciduria is invariable.
- Echocardiography may reveal a hypertrophic cardiomyopathy.
- Radiological evidence of rickets.

Hepatic histology is non-specific with steatosis and siderosis. Cirrhosis is almost always established at the time of clinical presentation.

The diagnosis is usually confirmed by mutation analysis but FAA activity can be measured in fibroblasts or lymphocytes.

Management

The introduction of nitisinone, which prevents the formation of toxic metabolites (Figure 9.4), has transformed the natural history of this disease.

Treatment with nitisinone (1 mg/kg/day) in addition to a phenylalanine- and tyrosine-restricted diet leads to rapid reduction of toxic metabolites within hours, and disappearance within 1 month. Over 500 patients worldwide have been treated with nitisinone, with normalization of renal tubular function, complete control of porphyria-like crises, and improvement in both nutritional status and liver function [8, 9]. It has recently been appreciated that some patients with tyrosinemia type 1 appear to have significant learning difficulties. Potential causes for these cognitive deficits include nitisinone treatment, high tyrosine levels, low phenylalanine levels, a consequence of liver failure, or an intrinsic feature of tyrosinemia 1. At present it appears most likely these are related to the amino acid abnormalities [7].

Nitisinone therapy requires close monitoring of plasma amino acids. Tyrosine levels should be kept <400 μmol/L, with the phenylananine level within normal range. Nitisinone protects against the development of HCC, but this is related to the age at which treatment is started. Compared to starting in the first month of life the relative risk of HCC is 2.5 at 1–6 months, 6.3 at 6–12 months, and 12.7 if started >1 year of age [9].

Due to the risk of HCC α-fetoprotein levels should be monitored every 3 months, abdominal ultrasound every 6 months and hepatic MRI annually (unless ultrasound is abnormal). A sustained rise of α-fetoprotein, or a failure to fall with treatment, is an indication to consider liver transplantation. Liver transplantation remains a highly effective

treatment for tyrosinemia but has become second line since the introduction of nitisinone.

Current indications for liver transplantation include:

- Unresponsive acute liver failure (failure to improve after 1 week of treatment).
- Established HCC.
- Chronic liver disease with future risk of HCC:
  - at development of first nodule
  - if abnormal α-fetoprotein evolution.

Prognosis

More than 90% of children presenting with acute liver failure respond to nitisinone. Most of these children will have chronic liver disease and hence some future risk of developing HCC. However if treatment is started before 6 months >80% will remain well on treatment at 10 years.

Children treated following newborn screening have an excellent outcome. They remain clinically normal with no biochemical or radiological evidence of liver dysfunction up to the age of 15 [10].

For those who require rescue, liver transplantation has a >80% 5-year survival with good quality of life [8]. It results in a functional "cure" although renal production of SA persists.

Screening

Neonatal screening programs have been established in populations with relatively high incidences of tyrosinemia. The improved outcome and prevention of HCC following preemptive nitisinone treatment makes the case for universal screening compelling. This should be based on the detection of SA, which is highly specific for HT1, and is feasible in screening programs [9].

Antenatal diagnosis

Antenatal diagnosis is possible either by chorionic villus sampling to measure FAA directly or from mutation analysis if the genotype is known. Alternatively, SA can be measured in amniotic fluid.

Familial hemophagocytic lymphohistiocytosis

This rare disorder is a clinical syndrome due to a variety of underlying inherited disorders. It is characterized by uncontrolled proliferation of activated lymphocytes and histiocytes secreting inflammatory cytokines. The disease is classified depending on the location of the primary genetic defect: FHL1, unknown; FHL2, *PRF1*; FHL3, *UNC13D*; FHL4, *STX11*; and FHL5, *STXBP*. These are all autosomal recessive. If the disease presents in later infancy or in older childhood the distinction between familial and infection associated hemophagocytic syndrome may be extremely difficult to make.

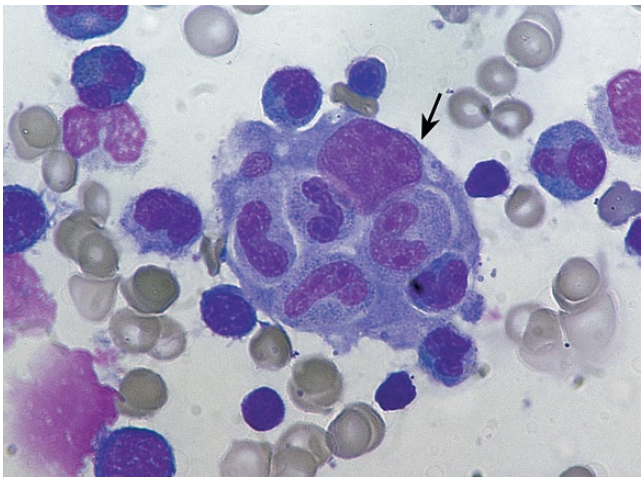

Figure 9.6 Familial erythrophagocytic syndrome presents with jaundice, liver failure, and pancytopenia. The diagnosis is made by demonstrating erytrophagocytosis in liver or bone marrow aspirate.

Clinical presentation and diagnosis

Infants present with malaise, jaundice, hepatosplenomegaly, relapsing fever, and skin rash.

Laboratory investigations show:

- Consumptive coagulopathy with hypofibrinogenemia.
- Cytopenia (≥2 cell lines).
- Elevated plasma triglycerides.
- Increased serum ferritin and lactate dehydrogenase (LDH).
- Low albumin and sodium.
- Decreased perforin levels by flow cytometry.
- Impaired natural killer and cytotoxic T-cell function.
- Erythrophagocytosis in bone marrow or liver (Figure 9.6).
- Cerebrospinal fluid lymphocytic infiltration and/or increased protein.

Treatment and prognosis

The condition is fatal without treatment, but 50% may recover with current management [11]. Etoposide, ciclosporin, and steroids are used to induce remission with maintenance ciclosporin to maintain remission. Relapse is frequent and stem cell transplant is necessary for long-term survival. Matched unrelated donor transplants are as effective as matched sibling transplants and offer 70% 3-year survival. Liver transplantation is contraindicated and inappropriate.

Recurrent acute liver failure syndrome

The recently described recurrent acute liver failure (RALF) syndrome is caused by mutations in *NBAS* which encodes for neuroblastoma amplified sequence protein which is involved in retrograde transport between endoplasmic reticulum and Golgi apparatus [12].

Clinical features and diagnosis

Children present with recurrent bouts of liver failure following a febrile illness, severe coagulopathy and encephalopathy starting after 6 months and before 2 years of age.

- Very high transaminases (>2000).
- Severe coagulopathy (INR 2–10).
- Minimal jaundice.
- Liver biopsy shows steatosis in the acute phase, normal between bouts.

Bouts are self-limiting and recovery may be hastened by antipyresis, aggressive support and the use of intralipid infusion (2 g/kg/day). There is complete recovery between episodes which persist throughout childhood but diminish in adulthood. Liver transplantation has been successfully used in some cases prior to recognition of the underlying disorder.

Inborn errors of metabolism associated with hepatic enzyme deficiency

There are a number of inborn errors of metabolism associated with defective hepatic enzymes, which are characterized by profound acidosis, hyperammonemia or disorders of fatty acid oxidation. The relevance of these disorders is that presentation is usually in the neonatal period with an acute illness, which may be associated with abnormal liver function tests, hepatomegaly and rarely liver failure [13]. A brief overview of these disorders is provided.

Urea cycle disorders

There are four main disorders in which defects in enzymes of the urea cycle lead to neurotoxicity from an accumulation of ammonia and glutamine. These defective enzymes are:

- Carbamyl phosphate synthetase (CPS).
- Ornithine transcarbamylase (OTC).
- Arginosuccinic acid synthetase (citrullinemia).
- Arginosuccinate lyase (ASA).

These disorders are autosomal recessive apart from OTC, which is X-linked. Females with OTC have varying degrees of deficiency and clinical involvement.

Clinical features and diagnosis

There is a wide range of clinical features, which are associated with increased plasma ammonia. Hepatomegaly and abnormal transaminases are usual. Neonates present shortly after birth and are extremely ill, with vomiting, lethargy, seizures, and coma. The diagnosis may be suspected if there is a low urea and alkalosis.

Females with OTC may present later in infancy or even in adult life. Clinical features include episodic vomiting with neurological dysfunction, which may be precipitated by intercurrent infection or ingestion of protein. A history of natural avoidance of high-protein foods is common.

Investigations include:

- Hyperammonemia.
- Plasma amino acids, which demonstrate abnormal citrulline levels: elevated in citrullinemia, low in ASA, and undetectable in OTC and CPS.
- Argininosuccinic acid is also high in ASA.
- Orotic aciduria is present in OTC classically and also in ASA and citrullinemia.
- Glutamine and alanine concentrations may also be elevated.
- Prothrombin time may be increased.

The biochemical features are diagnostic in citrullinemia and ASA. The diagnosis is confirmed by finding pathogenic mutations or by measuring specific enzyme activity in liver tissue for CPS and OTC.

The main differential diagnoses for an elevated ammonia in the newborn periods include poor sampling, organic acidemia, liver dysfunction including acute liver failure (see also Chapters 10, 18, and 19), or transient hyperammonemia of the newborn.

Treatment

Emergency management of hyperammonemia. The initial aim of treatment is to reduce ammonia as quickly as possible to prevent further neurotoxicity. Dietary protein should be withdrawn. Initial management is pharmacological (Box 9.5), with dialysis if hyperammonemia is severe. Intravenous dextrose, in combination with insulin, is provided to minimize catabolism. Phenylbutyrate and sodium benzoate form conjugates with glutamine and glycine, respectively, which are excreted in the urine and provide an alternative route for elimination of nitrogenous compounds. Arginine, which corrects the arginine deficiency resulting from the block in the urea cycle, also acts as a source of ornithine for reconstitution of the cycle if there is residual activity.

Box 9.5 Emergency management of hyperammonemia.

Stop all dietary protein

- *If ammonia >200 mmol/L*
  - IV 0.18% saline/10% glucose
  - Sodium benzoate:
    - loading dose 250 mg/kg
    - continuous infusion 250 mg/kg/day
  - Sodium phenylbutyrate
    - Loading dose 250 mg/kg:
    - continuous infusion 250 mg/kg/day
  - Arginine in 10%:
    - loading dose 350 mg/kg over 2 h
    - continuous infusion 350 mg/kg/day
- *If ammonia >400 mmol/L or rising despite treatment*
  - Dialysis
  - Increase doses of benzoate and phenylbutyrate to 500 mg/kg/day

Continuous venovenous hemodialysis is more rapidly effective than hemofiltration or peritoneal dialysis, especially in the newborn period. Dialysis should be continued until ammonia is <150 µmol/L. The intravenous dextrose and insulin is continued until the ammonia is <100 µmol/L and the child is neurologically normal. Protein can slowly be reintroduced at this stage.

Maintenance treatment. This consists of dietary restriction of protein (0.7 g/kg) supplemented with essential amino acids and a high-calorie diet sufficient to allow normal growth. Oral sodium benzoate (0.1–0.25 g/kg/day) and/or phenylbutyrate (0.25–0.6 g/kg/day) are given. Frequent nutritional assessment and monitoring of plasma ammonia and amino acids are required. Plasma glutamine should be maintained at <800 µmol/L to minimize risk of acute decompensation. The need for protein restriction may be less severe in children with ASA and citrullinemia, and arginine rather than benzoate is used for maintenance treatment.

Due to the poor prognosis and severity of the nutritional regimen, liver transplantation is often indicated and has been life transforming [14]. The indications for liver transplantation in urea cycle disorders are patients with:

- Very severe disease with a poor prognosis.
- Progressive liver disease that will ultimately result in liver failure.
- Major complications that are life threatening and cannot be controlled satisfactorily by other means [14].

Liver transplantation results in complete functional correction of the defect. Although some residual biochemical abnormalities may persist [14], they do not appear to have any functional consequences.

Liver transplantation should not be performed as emergency rescue in infants with neonatal coma, but hepatocyte transplantation may be a useful bridge to liver transplantation [15]. The potential applications of hepatocyte transplantation are discussed below.

Prognosis and outcome

Without treatment the disease is usually fatal and most children die during the acute neonatal illness. The most important determinant of outcome is the degree of hyperammonemia and duration of neonatal coma. Coma lasting longer than 5 days is associated with severe neurological abnormality. Children remain at lifelong risk of hyperammonemic coma, either during intercurrent illness or following dietary indiscretion. Families and patients should be taught an emergency regimen and have open access to hospital treatment.

Defects in fatty acid oxidation

Fatty acid oxidation provides an important source of energy during fasting, especially in childhood when glycogen stores are limited. Hepatic fatty acid oxidation produces ketone bodies, which are an important secondary energy source for many tissues, including the brain. Defects in any of the proteins in this pathway may lead to disease, and more than 20 individual fatty acid oxidation defects (FAD) have been recognized to date (Table 9.2). All have autosomal recessive inheritance.

Table 9.2 Recognized fatty acid oxidation defects.

Defect	Clinical phenotype	Other features	Metabolic abnormalities	Diagnosis confirmation
CTD	H, C, M	–	Low free carnitine	EM
CPT1	H, C,R, M	RTA	High free carnitine	EM
CACT	H, C, M	Early death	C16 and C18 species	EM
CPT2	H	RTA	C16 and C18	493C>T
VLCAD	H, C, R, M	–	C14, C16, and C18	–
MCAD	H, C, R, M	–	C8 DCA	985A>G in 90% symptomatic cases
LCHAD/MTP	H	Retinopathy, neuropathy	C16 and C18 species DCA	1528G>C
SCHAD	H	Hyperinsulinism	C4-OH Urinary ethymalonic acid	EM
MAD	H, C, M	Congenital malformations Renal cysts RTA	C6, C8, C10, and C12 Urinary ethylmalonic, glutaric and adipic acids DCA	EM

Cn, acylcarnitine species; C, cardiomyopathy or arrhythmia; CACT, carnitine/acyl carnitine translocase; CTD, carnitine transporter deficiency; CPT, carnitine palmitoyltransferase; DCA, dicarboxylic aciduria; EM, enzyme measurement in cultured fibroblast; H, acute hepatic presentation; LCHAD, long-chain 3-hydroxyacyl- CoA dehydrogenase; M, myopathic presentation; MAD, multiple acyl- CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; MTP, mitochondrial trifunctional protein; R, rhabdomyolysis; RTA, renal tubular acidosis; SCHAD, short-chain 3-hydroxyacyl- CoA dehydrogenase; VLCAD, very-long-chain acyl-CoA dehydrogenase.

Pathophysiology

The first step in fatty acid metabolism is lipolysis in response to fasting, resulting in circulating free fatty acids (FFA). Fatty acids are then transported across the plasma membrane and are transformed to coenzyme A (CoA) esters in the cytosol before entry to the mitochondria for further metabolism. Long-chain acyl-CoA esters are transported into the mitochondrion by a three-step carnitine-dependent pathway. Defects in each of these steps and in carnitine uptake have been recognized. Within the mitochondria acyl-CoA esters undergo β-oxidation. This is a four-step cyclical process where fatty acids are sequentially degraded to acetyl-CoA, with a molecule of acetyl-CoA being released at each step. β-oxidation results in a continuous flow of electrons to the electron transport chain by electron transfer flavoprotein (ETF) and its dehydrogenase (ETF-DH). The first two cycles of β-oxidation of long-chain fatty acids take place at the inner mitochondrial membrane using very-long-chain acyl-CoA dehydrogenase (VLCAD) and the associated trifunctional protein (which contains the other three enzymes needed to complete a cycle, including long-chain 3-hydroxyacyl-CoA dehydrogenase, LCHAD). Shorter chain fatty acids are oxidized within the mitochondrial matrix by length-specific enzymes. Within the liver acetyl-CoA is used for ketone body synthesis.

Defects at any stage in the pathway will result in failure of energy production and inadequate ketone body production. Moreover, when β-oxidation is defective, FFA undergo ω-oxidation in microsomes producing dicarboxylic acids.

Clinical features and diagnosis

The commonest presentation in yalopathy which may be provoked by weaning or intercurrent infection. Hepatomegaly, elevated transaminases, modest hyperammonemia occur in >80% of cases with cholestasis in up to one-third [16]. In severe cases there may be a "Reyes-like syndrome" illness which can be fatal or even cause sudden infant death syndrome.

Other clinical features include:

- Hypotonia.
- Cardiomyopathy.
- Rhabdomyolysis.
- Metabolic acidosis.
- Maternal fatty liver of pregnancy or HELLP (hemolysis, elevated liver enzymes, and low platelets).

These disorders have not been thought of as common causes of liver failure. The best diagnostic yield will be from blood acylcarnitine profile and urinary organic analysis at the time of decompensation.

Biochemical investigations reveal:

- Elevated aminotransferases.
- Low plasma carnitine.
- Abnormal acyl carnitine profile, especially during decompensation or following a controlled fast.
- Elevated ratio of FFAs : 3-hydroxybutyrate.
- Increased urinary ratio of dicarboxylic acids: 3-hydroxybutyrate.
- Mild to moderate hyperammonemia and hyperlactidemia.
- Metabolic acidosis.
- Elevated plasma creatinine kinase.

Confirmation of specific defects may be difficult due to the variety of potential enzyme defects. In selected patients, loading tests with medium-chain or long-chain triglyceride may be helpful. In medium-chain acyl-CoA dehydrogenase deficiency and LCHAD common single mutations make the diagnosis simpler [16]. All recognized defects are expressed in skin fibroblasts. In-vitro analysis with labelled myristate, palmitate, and oleate are useful for diagnostic screening and in-vitro acylcarnitine profiling can provide very specific diagnostic information.

There remain a group of patients who have a phenotype suggestive of FAD, but in whom no specific defect can be demonstrated. The concept of synergistic heterozygosity has been proposed as an explanation, where two heterozygous defects at different steps in FAD might result in functional effects [17]. A similar mechanism could account for some of the significant phenotypic variability seen in recognized defects.

A fascinating aspect of this group of disorders is the association with maternal illness during pregnancy (see also Chapter 7). This association was first noted when a mother with acute fatty liver of pregnancy (AFLP) delivered an infant with LCHAD deficiency. Recently it has become clear that this association is not limited to LCHAD deficiency but may occur in any fatty acid oxidation defect including medium- and short-chain defects [18]. The mechanism of the maternal illness is unclear but presumably results from limited ability of an obligate heterozygote mother to detoxify metabolites produced by an affected fetus. At most, FAD account for 20% of AFLP and probably rather less. Given the implications for the child and future pregnancies it is crucial that all children born after a pregnancy affected by AFLP are systemically screened for FAD and if necessary prospectively treated until results are available.

Carnitine palmitoyl transferase 1 deficiency

This disorder is due to a defect in the carnitine palmitoyl transferase enzyme at the outer mitochondrial membrane. This defect prevents mitochondrial uptake of long-chain fatty acyl-CoA, the rate-limiting step in fat oxidation. Presentation is usually in the first year of life with acute hypoketotic hypoglycemia following an episode of fasting or intercurrent illness. Hepatomegaly, renal tubular acidosis, convulsions, and coma are reported.

Investigations demonstrate the typical features of fat oxidation defect (see earlier) except:

- Plasma carnitine may be normal or increased.
- Acylcarnitine profile is normal.
- Liver histology demonstrates microvesicular and macrovesicular steatosis.
- Muscle biopsy may show accumulation of glycogen and lipid.

The diagnosis is confirmed by demonstrating impaired oxidation of palmitate in fibroblast culture.

Treatment consists of the avoidance of fasting and provision of a low-fat diet with medium-chain triglyceride supplements. The prognosis is good and normal growth and development can be achieved.

Medium-chain acyl-coenzyme dehydrogenase deficiency

This is the commonest FAD in white people. The incidence in the UK is approximately 1 in 10,000 with a single mutation (c.985A > G) accounting for 90% of abnormal alleles in symptomatic cases. The presentation is usually a hepatic phenotype in infancy, but asymptomatic cases are common especially since the advent of newborn screening. De-novo presentation in later childhood or as an adult is rare but can be fatal.

Universal newborn screening was introduced in the UK in 2009. Historically up to 25% died in their acute illness with some survivors suffering neurological sequelae [16]. Dietary treatment is unnecessary but children should avoid medium-chain tryglyceride (MCT). Standard management consisting of avoiding unnecessary fasting and using an "emergency regime" results in an excellent outlook.

Multiple acyl-coenzyme A dehydrogenase defect (glutaric acidemia type 2)

This rare disease is due to deficiency of either the α- or β-chain of ETF or of its dehydrogenase (ETF-DH). This then prevents energy production by all acyl-CoA dehydrogenases. This is a very heterogeneous disorder with a number of phenotypes including:

- Severe neonatal illness with profound metabolic acidosis.
- Congenital malformations, including polycystic kidneys, defects in the anterior abdominal wall, and genital abnormalities.
- Dysmorphic features, including low-set ears, high forehead, rocker-bottom feet, and single palmar creases.
- A later myopathic presentation which may be associated with spasticity or extrapyramidal features.

Biochemical investigations and diagnostic findings include:

- Increased urinary organic acids, including ethylmalonic, glutaric, and adipic acids.
- Plasma lactate is usually elevated.

Treatment consists of a high-carbohydrate, low-protein, and low-fat (without MCT) diet.

Patients who survive or present later should be assessed for riboflavin responsiveness, and if responsive treated with 100–300 mg/day riboflavin. Synthetic ketone bodies have been successfully used in three children with significant improvement in muscle and cardiac function.

Many infants with structural abnormalities die within the first week of life. Some respond to initial treatment but often succumb to cardiomyopathy in infancy.

Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency

The enzyme LCHAD is a component of the trifunctional protein of the inner mitochondrial membrane and has optimal activity for C12 to C16 chain length fatty acids. A common mutation (G1528C) has been recognized, accounting for 70% of abnormal alleles.

The clinical presentation is similar to other disorders of fatty acid oxidation with infantile hypoketotic hypoglycemia, sudden infant death syndrome or a Reyes-like illness.

Distinguishing features include:

- Severe cardiomyopathy.
- Marked hypotonia.
- Diarrhea and failure to thrive.
- Peripheral neuropathy and retinitis pigmentosa.
- Neonatal cholestasis or acute liver failure.
- Hepatic histology demonstrates microvesicular steatosis but progression to cirrhosis is frequent.

Diagnosis is confirmed either by mutation detection or by specific enzymatic diagnosis from cultured fibroblasts.

Maternal AFLP may develop when a heterozygote mother carries a fetus with LCHAD deficiency, particularly if the fetus carries the G1528C mutation (see also Chapter 1).

Treatment and prognosis

The primary aim of treatment is to avoid excess fasting and suppress lipolysis, especially in the first 6 months of life. This may require the occasional use of nasogastric tube feeds or nocturnal uncooked cornstarch, but in general these should not be necessary. All children should be provided with written individualized emergency regimes for use during intercurrent illnesses.

In disorders of long-chain fat oxidation, dietary LCT should be restricted with MCT supplementation. Essential fatty acid supplementation should be provided with walnut oil [19].

LCT restriction can be relatively modest in MAD and VLCAD (25–30% of calorie intake). In LCHAD/MTP LCT intake should be as low as possible. This precludes breastfeeding and requires the use of specific MCT-based formula feed.

Even after weaning, LCT intake should be kept to <10% of calories. Docosahexanoic acid supplementation is recommended; although it does not prevent eventual progression of retinopathy, its use is associated with retention of retinal function and visual acuity.

All those with muscle symptoms should be encouraged to maintain activity levels with the use of a pre-exercise MCT supplement where necessary.

Routine carnitine supplementation is no longer recommended except for carnitine transporter deficiency and in some children with MAD.

The prognosis for those who present clinically, especially if it is in the neonatal period, is poor. Overall mortality in the first episode as high as 60%, whereas once a patient has been diagnosed and their family provided with strategies the risk of future decompensation is low. A comprehensive national study has shown an overall mortality of 50% with 60% mortality in LCHAD and VLCAD deficiency where the presentation is clinical [16]. Between 20 and 30% of those who present clinically with MCAD and survive are left with significant developmental sequelae [16]. In contrast, the outlook following diagnosis by newborn screening is much more encouraging. Mortality and morbidity from MCAD, LCHAD, and VLCAD have decreased in screened population, albeit at the cost of recognizing many cases who would have remained asymptomatic [20]. The impact of screening on rarer disorders is still uncertain, but appears generally positive.

Organic acidemias

Inborn errors of organic acid metabolism produce life-threatening illness early in life. They should be suspected in any patient with metabolic acidosis. Propionic acidemia (PA) and methylmalonic acidemia (MMA) are the commonest of the organic acidemias.

Propionic acidemia

PA is due to an autosomal recessive defect in the enzyme propionyl-CoA carboxylase. The genes are *PCCA* and *PCCB* which encode the α- and β-subunit, respectively. A number of mutations have been defined with some genotype–phenotype correlation. Most propionate is derived from catabolism of the essential amino acids valine and isoleucine, and to a lesser extent from threonine and methionine. Odd-chain fatty acids are synthesized from the 3-carbon propionyl-CoA and subsequently act as a propionate source when they are oxidized during catabolism or fasting. Anaerobic bacterial metabolism contributes approximately 20% of propionate turnover. Biotin is a coenzyme for this pathway.

Methylmalonic acidemia

MMA is part of a group of disorders with abnormal metabolism of branch-chain amino acids due to defective activity of methylmalonyl-CoA mutase. The gene has been localized to chromosome 6q12-21.2, and more than 20 mutations have been identified. The methylmalonyl-CoA mutase enzyme has a vitamin B_{12}-derived cofactor, 5′-deoxyadenosylcobalamin.

There is considerable genetic heterogeneity as some patients are B_{12}-responsive due to defects in the synthesis of the cofactor, while unresponsive patients have defects in the mutase enzyme itself.

Maple syrup urine disease

Maple syrup urine disease (MSUD) is caused by deficiency of branch-chain amino acid (BCAA) 2-ketoacid dehydrogenase complex.

When this is severe the only way to decrease BCAA levels is via protein synthesis, as renal excretion is minimal. Over 100 pathogenic mutations have been described with reasonable genotype–phenotype correlation. The incidence is very high in the Amish population in Pennsylvania.

Clinical presentation and diagnosis

These disorders have a wide clinical spectrum of severity. The most severely affected present in the newborn period with encephalopathy, hypotonia, hepatomegaly, and subsequent coma. Some children present later with recurrent illness characterized by bouts of lethargy, abnormal behavior and altered consciousness, or occasionally with a slowly progressive form with failure to thrive and developmental delay. In general patients are more susceptible to infection.

First-line investigations demonstrate:

- Metabolic acidosis and ketosis (arterial pH <6.9; serum bicarbonate <10 mmol/L).
- Neutropenia and thrombocytopenia.
- Hyperammonemia, which may be profound.
- Plasma amino acids show hyperglycinemia (>600 mmol/L)
- Urinary organic acids reveal either the characteristic propionyl-CoA derivatives, including glycine and carnitine conjugates, methyl citrate and 3-hydroxypropionate, or urinary methylmalonate.

In MSUD the secondary abnormalities are less severe. There is the characteristic sweet odor and the plasma amino acid pattern is usually diagnostic.

Definitive diagnosis is achieved by demonstrating pathogenic mutations or the relevant enzyme defect in cultured fibroblasts. Liver histology may show fatty change or mild biliary changes.

Management and prognosis

Initial management of the acute crisis involves intravenous 10% dextrose and sodium bicarbonate and dietary protein restriction. Carnitine (200 mg/kg/day) and metronidazole (20 mg/kg/day) help to detoxify and decrease production of propionate, respectively. Insulin may be useful, especially if hyperglycemia occurs. Hyperammonemia requires specific treatment with sodium benzoate or dialysis (see Urea cycle disorders earlier).

In MMA higher fluid intakes are necessary and all patients should have a trial of pharmacological doses of vitamin B_{12} (1 mg/day).

Maintenance treatment consists of an individually titrated low-protein high-calorie diet with overnight nasogastric tube feeding. Amino acid supplementation may be necessary. Carnitine supplementation is continued. Metronidazole may be used continuously or intermittently as required. Sodium benzoate is useful in those with a recurrent hyperammonemia.

In B_{12}-responsive MMA treatment should be lifelong with little need for dietary restriction. With the exception of those patients with MMA who respond to B_{12}, patients remain at risk of recurrent metabolic decompensation, often in association with intercurrent illness. The outlook for patients with neonatal presentation is poor. Neurological abnormalities are common, with severe hypotonia, progressive neurodevelopmental delay and learning difficulties. Basal ganglia damage and stroke-like symptoms are common in patients who presented early, but all affected patients are vulnerable to neurological damage [21]. Nutritional progress is difficult and systemic complications such as pancreatitis, cardiomyopathy, and osteoporosis occur. Tubulointerstitial nephritis with progressive renal impairment occurs in MMA and may lead to renal failure in adolescence.

In MSUD acute management may require dialysis to lower leucine levels quickly. Enteral BCAA-free amino acid mixture should be introduced as quickly as possible. Subsequently, valine and isovaline supplements should be titrated to plasma amino acid levels.

Maintenance treatment for MSUD requires close dietary monitoring using a mixture of natural protein and BCAA-free amino acid supplement.

The best outlook is in those children who received preemptive treatment following neonatal screening. However even in this group, psychological and developmental difficulties are common [22].

Appreciation of this poor outlook has led to consideration of early liver transplantation before the onset of systemic complications. Initial experience demonstrates that liver transplantation results in useful partial correction of PA and MSUD. Diet is normalized, with decreased metabolite excretion and nutritional and developmental catch-up. There is complete protection against recurrent crises [22, 23]. The risks of early liver transplantation in PA are high yet cumulative disease morbidity increases the risk of delayed transplantation. As a result, deciding on appropriate timing of transplantation is difficult and in general it should be reserved for those with metabolic instability despite maximal treatment [23].

The situation is more positive with MSUD where transplantation gives excellent long-term control, much improved quality of life, and facilitates developmental catch up [22].

The situation is not as clear in severe MMA. Although, liver transplantation in infancy has a poor outcome, it results in a useful functional correction of the metabolic defect but does not completely protect from metabolic stroke [24]. As some children with MMA develop renal failure, it is reasonable to consider combined liver–kidney transplantation at this juncture, even though the risk is higher than in other candidates for combined transplantation. For some children with MMA and renal failure, isolated renal transplantation may be a reasonable option [25].

Antenatal diagnosis

Prenatal diagnosis is possible for by enzymatic measurement in cultured amniocytes or amniotic fluid measurement of methylcitrate or methylmalonate. DNA analysis from chorionic villi is feasible in families with known mutations. In adenosylcobalamin synthetic defects, prenatal B_{12} treatment may be given.

Other organic acidemias

Isovaleric acidemia and multiple carboxylase deficiency are rarer organic acidemias due to deficiencies in isovaleryl-CoA dehydrogenase and holocarboxylase synthetase, respectively.

Clinical presentation and diagnosis

Both conditions present with severe neonatal illness, with similar presentation to other organic acidemias. Distinguishing features include a "sweaty feet" odor in isovaleric acidemia. Diagnostic biochemical features include elevated plasma isovaleric acid and urinary isovalerylglycine in isovaleric acidemia, and urinary 3-methylcrotonylglycine and 3-hydroxyvaleric acid in multiple carboxylase deficiency. Enzymatic diagnosis is confirmed in cultured fibroblasts in both disorders.

Management and prognosis

Emergency management is similar to the other organic acidemias. Most patients with multiple carboxylase deficiency are responsive to pharmacological doses of biotin (10 mg/day) and in the majority no other treatment is required other than an "emergency regimen" for use during intercurrent illness. In isovaleric acidemia a low-protein high-calorie diet should be used in combination with glycine supplementation.

Transaldolase deficiency

Transaldolase deficiency is a disorder of the pentose phosphate pathway due to mutations in *TALDO1*.

Clinical presentation and diagnosis

This is a heterogeneous condition which usually presents in infancy with hepatosplenomegaly, pancytopenia, and bleeding tendency which can progress to liver failure.

Growth retardation, dysmorphic features, cutis laxa, and congenital heart disease are also common, Presentation with cirrhosis in later childhood or hydrops fetalis are also reported, Development progress is usually normal [26].

Urine anaylsis for polyols is usually diagnostic but can be confirmed by detection of mutations in *TALDO1*. There is no specific treatment.

Hepatocyte transplantation

Hepatocytes can be efficiently isolated from donor livers which are not suitable for whole organ transplantation. These can subsequently be used immediately as fresh cells or cryopreserved for future use as needed. Transplantation is minimally invasive, requiring only infusion into the portal vein using either a percutaneous or surgically placed catheter and can be repeated. Similar levels of immunosuppression compared to whole organ transplantation are necessary.

More than 50 subjects have received hepatocyte transplants for metabolic disease. In general the procedure has been well tolerated, but the metabolic effect has been modest and usually short lived. The major role for the first generation of this procedure appears to be in newborn infants with urea cycle disorders where it appears to provide some stability and acts as a bridge to subsequent liver transplantation [15].

Methods to improve the efficacy of cell transplantation are needed and should be multifaceted. Options include increasing the number of cells transplanted, improving the hepatocyte repopulation rate or using different cell types.

Increasing cell numbers is problematic as few organs are now unsuitable for whole organ transplantation. Efforts to increase hepatocyte repopulation may require a noxious stimulus to the native liver to provide a survival advantage to transplanted cells, hence changing the risk benefit balance. Mature hepatocytes are terminally differentiated cells with limited proliferation potential, hence the use of stem cells, which may retain proliferative potential to repopulate the liver, are very attractive [27]. The ability to develop patient-specific induced pleuripotent stem cells, which in theory can be genetically corrected and then replaced without the need for immunosuppression, provides an exciting future potential [28].

Screening for inborn errors of metabolism

The advent of tandem mass spectrometry has changed the paradigm for neonatal screening. This technique has the potential to detect more than 30 inborn metabolic disorders simultaneously from a single blood spot with a high degree of sensitivity. This has been used for disorders of amino acids, organic acids and fatty acids to date. However it has the potential to be applied to disorders such as lysosomal disorders [20]. However, the potential technical, logistical and ethical difficulties posed by application of this technique to mass screening programs should not be underestimated. The technique has to date been validated in only a small number of individual disorders and the challenge in the coming years will be to learn how to harness the potential of this technique for mass neonatal screening. The applications chosen will likely differ from country to country and may change depending on clinical developments. For example the excellent outcome for presymptomatic use of nitisinone makes the case for screening for tyrosinemia much stronger [10]. The role for screening in the diagnosis of liver disease has yet to be established.

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CHAPTER 10

Neonatal Hemochromatosis

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Key points

- Neonatal hemochromatosis is a clinical-anatomic phenotype consisting of severe liver disease in the newborn accompanied by siderosis of extrahepatic tissues.
- Gestational alloimmune liver disease (GALD), a condition where maternal immunoglobulin G alloantibodies bind fetal liver antigen and activate the terminal complement cascade, is responsible for almost all cases of neonatal hemochromatosis (GALD-NH).
- GALD should be considered in any neonate presenting with severe liver disease or in cases of unexplained stillbirth or fetal demise.
- Infants with GALD-NH should be treated with double-volume exchange transfusion to remove existing reactive antibody and high-dose intravenous immunoglobulin to block antibody activation of complement. Recurrence of GALD-NH can be prevented during future pregnancies via administration of intravenous immunoglobulin during gestation.

Neonatal hemochromatosis (NH) is defined as severe neonatal liver disease that is accompanied by siderosis of extrahepatic tissues in the distribution seen with hereditary hemochromatosis [1, 2]. First described in the mid-20th century, NH was initially believed to be an inborn error of iron metabolism with liver injury occurring as the result of iron overload. NH was thus classified as part of the family of hereditary hemochromatosis disorders (OMIM231100). Over the last decade the understanding of NH has grown exponentially [3]. It is now clear that NH does not result from an inherited disorder of iron homeostasis with primary iron overload; but rather, is a phenotypic expression of severe fetal liver disease with secondary iron overload [4]. NH is now considered to be an endpoint of fetal liver injury due to any cause [5]. It is a symptom rather than a disease.

Etiology of neonatal hemochromatosis

Initially, NH was believed to be a hereditary disorder of iron regulation and metabolism. However, infants with NH were often born with cirrhosis suggesting that an intrauterine process had affected their liver [6], although it was unclear what caused the intrauterine injury. As multiple siblings were also affected, it was hypothesized that perhaps there was an underlying genetic defect. However, no gene locus was identified and the pattern of NH recurrence in maternal sibships was not typical of autosomal inheritance. A mother might have had a number of healthy infants prior to giving birth to an affected infant, but the subsequent recurrence rate in subsequent pregnancies was approximately 90% [7]. Half-siblings sharing a father were unaffected, but half-siblings with the same mother were affected. Female survivors of NH could have healthy unaffected infants. Therefore, NH appeared to be congenital and familial, but not hereditary. These findings led to the hypothesis that NH is caused by a maternofetal alloimmune disorder [8].

The evidence needed to support the alloimmune causation theory of NH etiology came in 2010 when it was shown that the liver from cases of NH displays complement-mediated hepatocyte injury [9]. Using immunohistochemistry for the C5b-9 complement complex (a neoantigen formed with activation of the terminal complement cascade and synonymous with membrane attack complex, MAC) the

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investigators showed pathologic deposition of MAC in over 80% of residual hepatocytes in liver specimens from proven NH cases as compared to small amounts of MAC in less than 20% of hepatocytes in disease controls and 5% in infants with asphyxia. MAC is the product of complement activation, which in the setting of fetal liver injury is highly likely to result from immunoglobulin G (IgG) antibody binding to fetal hepatocytes. Since the fetus has no innate IgG production, the offending antibody must have come from the mother; thus, complement-mediated injury to fetal hepatocytes must be the result of gestational alloimmunity. The disease state was named gestational alloimmune liver disease (GALD) [10]. GALD results in liver injury and acute liver failure of the fetus with fetal death with or without extrahepatic siderosis [11]. GALD and NH are not synonymous. GALD is a disease process causing severe fetal liver injury, whereas NH is the phenotypic expression in a neonate of severe liver injury initiated in utero [4, 5]. The discovery of an immune basis of GALD has changed the way infants with NH are treated [12] and has allowed for a preventative therapeutic strategy to prevent disease in future pregnancies [7, 13].

While nearly all NH is due to GALD, there are other causes, which should be excluded. Trisomy 21 (Down syndrome) is the most frequent non-GALD cause of NH, but because of the obvious clinical features, the differential diagnosis of NH is straightforward and the diagnosis of trisomy 21 eliminates the need for further evaluation of the cause of newborn liver failure. Other syndromes that have been associated with NH are Martinez-Frias syndrome, tricho-hepato-enteric syndrome, GRACILE syndrome (fetal growth restriction, aminoaciduria, cholestasis, iron overload, lactoacidosis, and early death), fetal growth restriction, aminoaciduria, and infantile generalized myofibromatosis.

Two inherited disorders that may present diagnostic confusion with GALD-NH are the mitochondrial DNA depletion syndrome due to deoxyguanosine kinase deficiency (*DGUOK* mutations) and the bile acid synthesis defect δ-4-3-oxosteroid 5-β-reductase deficiency (*SRD5B1* mutations). The latter may be difficult to distinguish from GALD-NH because neonatal liver failure may produce secondary 5-β-reductase enzyme deficiency. Although the mechanisms of liver injury differ among these disorders, they produce neonatal iron overload by impairing the fetal liver's ability to regulate maternofetal iron homeostasis [5].

Finally, NH has been reported in association with perinatal infection. The mechanism may be that as the fetal liver is the major iron storage site, in severe acute perinatal hepatic necrosis, large amounts of non-transferrin bound iron (NTBI) are released into the circulation, which are taken up by extrahepatic tissues as in fetal iron overload. In addition, hepcidin signaling may be impaired in acute hepatic necrosis, which would result in operative ferroportin and lack of iron in reticuloendothelial cells, as in GALD-NH.

Pathogenesis of gestational alloimmune liver disease

Maternofetal alloimmunity is mediated by IgG, the only subclass of antibodies that crosses the placenta. IgG antibodies are actively transported across the placenta to the fetus beginning in the 12th week of gestation when the neonatal crystallizable fragment receptor (FcRn) that chaperones IgG is first expressed. Gestational alloimmunity occurs when a mother is exposed to a fetal antigen that she does not recognize as "self." This exposure leads to sensitization and the subsequent production of IgG antibodies that cross the placenta to incite immune injury against fetal tissues. In GALD, unlike other gestational alloimmune diseases such as hydrops fetalis, ABO incompatibility hemolysis, and alloimmune thrombocytopenia, the maternal IgG antibodies are directed against a solid organ, specifically against fetal hepatocytes [9]. Non-hepatocyte liver cells and extrahepatic tissues are not attacked in GALD. Complement-mediated hepatocyte injury was identified when immunohistochemical staining demonstrated deposition of C5b-9 complex (the neoantigen created during terminal complement cascade activation and culmination) in the majority of hepatocytes from a large cohort of NH cases [9]. IgG1 and IgG3 are both capable of crossing the placenta and activating the terminal complement cascade via the classical pathway. Since activation of the terminal complement cascade in a fetus must involve binding of maternal IgG to a fetal hepatocyte antigen, this confirmed the theory that NH results from maternofetal alloimmunity.

It is not clear which specific fetal alloantigen is responsible for GALD and why the mother does not have tolerance for this protein. Fetal liver injury appears to begin around mid-gestation [14] when fetal liver is rapidly producing hepatocytes. Recent work demonstrates that the liver in GALD has a full complement of epithelial progenitors but is depleted of hepatocytes [15]. The only hepatocyte forms found in these livers were multinucleate giant cells with no necrotic hepatocytes or hepatocytes in cords. These findings suggest that the target of GALD may be expressed in developing hepatocytes and that the antigen is uniquely expressed during fetal development and not in mature liver. This might explain why the mother sees the antigen as non-self (she has lost immune memory (i.e., has no peripheral tolerance) of her fetal self-antigen) and why the mother's own liver is not injured by high-titer antibody, as mature liver does not under normal, non-regenerative circumstances express this antigen. It is not known how this antigen gains access to the maternal circulation. That about 10% of GALD cases occur in the first pregnancy suggests that, unlike the antigens involved in alloimmune blood diseases, sensitization may occur during pregnancy and not just at parturition.

It is possible that the fetal liver antigen might gain access by becoming trapped in/on exocytotic vesicles, which cross the

placenta and thus expose the mother, or that spillage of apoptotic soluble protein into the fetal circulation during rapid liver development leaks across the placenta. Exposure to the GALD antigen may be a common event in pregnancy but central tolerance (expression on thymic endothelial cells) prevents most women becoming sensitized to it upon exposure.

Some cases of NH have been associated with lupus antibodies and anti-nuclear antibodies in the mother, but there is no evidence to suggest that GALD is the result of passive autoimmune disease.

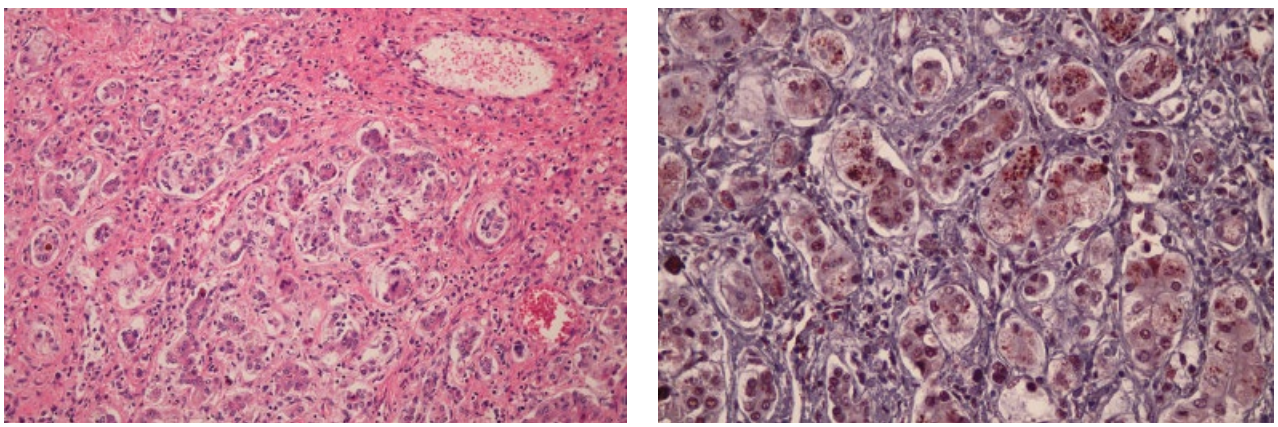
Liver pathology

Most descriptions of the liver pathology of NH come from the study of autopsy specimens [1, 2] and before the discovery of GALD as the principal cause of NH. It is assumed that these pathologic descriptions are mainly the description of GALD-NH liver pathology as the liver pathology of non-GALD causes of NH has been poorly studied. The liver pathology in these disorders is assumed to be characteristic of the individual disorders and dissimilar to that seen in GALD, though further study is needed to confirm that assumption.

The liver pathology of GALD-NH is not pathognomonic for the disease but is highly characteristic (Figure 10.1). Liver tissue displays severe loss of hepatocyte mass (10–25% of that in normal newborn liver tissue) [14, 15], and the remaining hepatocytes consist almost entirely of multinucleate giant cells, which may contain coarsely granular siderosis and occasional bile. In some infants, there may be no remaining hepatocytes.

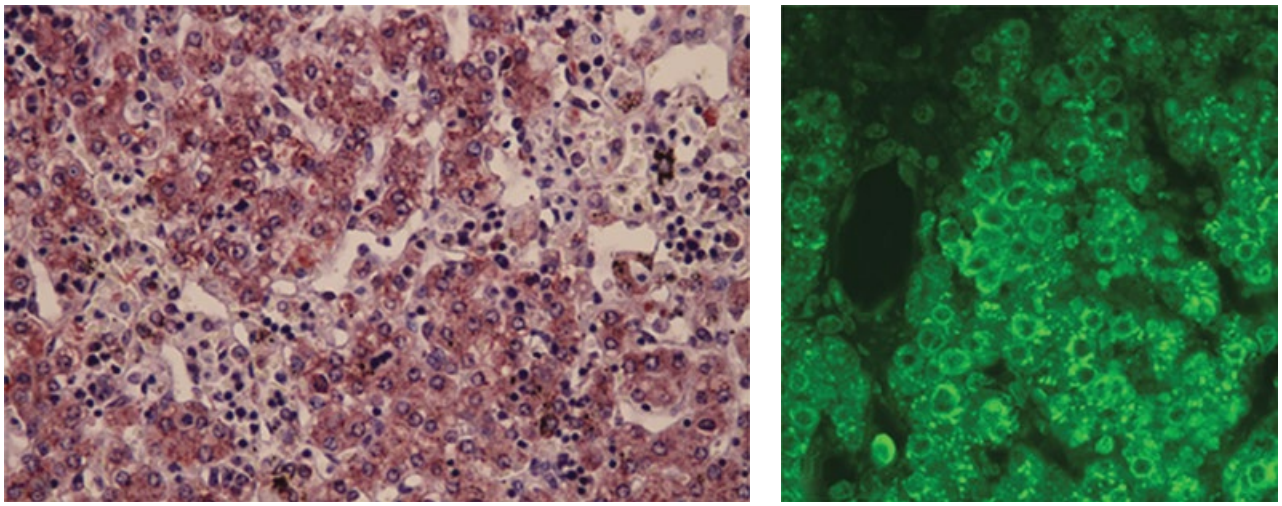
In the parenchyma there is an abundance of narrow, elongated, tubular forms consisting of epithelial cells surrounding a narrow central lumen that is usually devoid of bile [15]. These parenchymal tubules resemble reactive ductules and may be formed when foregut derived stem cells are activated in an attempt to replace lost/damaged hepatocytes. The tubules express KRT7/19, EPCAM, and SOX9 and exhibit active hyperactive hedgehog (Hh) signaling which results in excess production of SPP1 (osteopontin) [15]. Hh-pathway activation results in fibrogenic signals that lead to extensive pan-lobular fibrosis. Regenerative nodules are common, and the majority of patients have cirrhosis at the time of birth. In contrast to the extensive parenchymal injury and fibrosis, the portal tracts are spared from injury. There is a notable paucity of inflammation and of extramedullary hematopoiesis. The minimal inflammation present in the parenchyma consists mainly of macrophages and neutrophils, innate immune cells that are recruited by C3a and C5a during activation of the terminal complement cascade.

The pathognomonic immunohistochemical finding in hepatocytes of infants affected by GALD-NH is accumulation of C5b-9 complex in giant cells and residual hepatocytes (see Figure 10.1) [9]. This complement complex is the neoantigen created with activation terminal complement cascade to form MAC. Its presence on or in cells indicates the formation of MAC on the surface of the cell. In GALD-NH it is observed almost exclusively as coarse granular deposits in the cytoplasm of giant cells. Multinucleate giant cells are thought to be the product of fusion of injured mononuclear hepatocytes. Finding C5b-9 complex inside giant cells suggests that hepatocytes are injured by complement activation on their plasma membrane and then fuse into giant cells, carrying MAC along with their plasma membranes into the newly formed giant cell. Much work is needed to fully understand the implications of this finding, but it suggests that NH is immunomediated [9]; and that GALD is the cause of most cases of NH [10].



(A) (B)

Figure 10.1 Liver histopathology of a term infant with gestational alloimmune liver disease–neonatal hemochromatosis (GALD-NH). This infant was noted to have liver failure at birth and died on the 3rd day of life. Extrahepatic siderosis was identified at postmortem examination. (A) Routine histology (H&E, × 100) shows typical pathology of GALD. Hepatic parenchyma consists of numerous tubules and occasional giant cells and pseudorosettes surrounded by extensive fibrous stroma. (B) Immunohistochemistry for C5b-9 complex (red reporter assay, × 200) shows deposition in nearly all residual hepatocyte forms, consistent with complement mediated alloimmune injury.



(A) (B)

Figure 10.2 Liver histopathology from a 27-week gestational age infant with gestational alloimmune liver disease (GALD). This infant was born by emergency C-section for fetal distress and died on the first day of life. No extrahepatic siderosis was identified at postmortem examination. Routine histology showed loss of hepatocyte mass and immature hepatocytes with evidence of injury including hydropic swelling and vacuolization. There was no fibrosis. (A) Immunohistochemistry for C5b-9 complex (red reporter assay, × 200) shows deposition in nearly all residual hepatocytes, consistent with complement-mediated alloimmune injury. (B) Immunofluorescence staining for membranous MAC (membrane attach complex, green fluorescent reporter assay, × 200) shows punctate and confluent MAC deposition on the plasma membrane of nearly all parenchymal cells, which have a progenitor or oval cell appearance.

GALD can cause an acute liver injury to the fetal liver that results in fetal demise and stillbirth typically at 18–24 weeks' gestation [11]. Liver tissue from these infants demonstrates only acute complement-mediated hepatocyte injury, with no evidence of chronicity such as fibrosis and formation of regenerative nodules. Study of these cases by immunofluorescence shows MAC on the surface of small, round parenchymal epithelial cells with anatomy consistent with hepatoblasts or early hepatocyte forms (Figure 10.2). This finding suggests that GALD actually targets some early hepatocyte form that is abundant in the fetal liver in mid-gestation, which may explain why GALD-related fetal liver injury has its onset at this time in development [14]. The majority of these cases show no siderosis of hepatic or extrahepatic tissues, presumably because of the precipitous nature of the disease leaving inadequate time to accrue excess iron. It remains unclear why certain fetuses experience hyperacute liver injury while others develop congenital cirrhosis. Cases of fetal death and cases of congenital cirrhosis are seen within the same maternal sibships [10]. It is possible that the antibody and the antigen target are the same, but genetic differences in hepatocyte development, defense or susceptibility result in different outcomes. A good animal model of GALD is required to understand this intriguing phenomenon.

Extrahepatic manifestations

Extrahepatic siderosis. Siderosis of extrahepatic tissues is the defining feature of NH, and demonstration of extrahepatic siderosis is required to establish the diagnosis of NH in the clinical setting of newborn liver failure or at autopsy [2]. The mechanism by which GALD results in iron overload involves failure of control of placental iron flux [4]. The fetus must closely regulate placental iron transport allowing the influx of enough iron for growth and oxygen-carrying capacity while preventing toxic overload from the mother's large iron pool. Fetal control over placental iron flux is similar to postnatal control of intestinal iron. In states of iron sufficiency, the fetal liver produces hepcidin, which acts to down regulate cell surface expression of ferroportin, a transmembrane protein that permits movement of iron out of cells. Hepcidin produced by the fetal liver binds placental ferroportin causing its internalization and proteosomal degradation, which leads to decreased functional ferroportin and reduced iron influx. However, in fetuses affected with GALD, liver injury results in significantly decreased expression of hepcidin [4]. This results in less negative feedback on ferroportin and allows for excess iron to be transported across the placenta, resulting in fetal iron overload. In addition, transferrin gene expression is decreased, which results in reduced iron binding capacity and excess circulating NTBI.

The specific distribution of iron in extrahepatic tissues is determined by the ability of each tissue to manage excess circulating NTBI. Affected tissues express ZIP14, a zinc transporter also capable of iron transport, making them capable of NTBI uptake and accrual. Other tissues similarly express ZIP14, but what distinguishes tissues with siderosis is lack of ferroportin expression. They can accrue iron, but they cannot rid themselves of it [4]. Reticuloendothelial cells are spared siderosis because they express ferroportin, which is not limited in function because of the dearth of hepcidin.

Hepatocytes express ferroportin in addition to transporters for transferrin bound iron and NTBI, but they accrue hemosiderin because of direct tissue injury and inability to make hepcidin. The tissues most frequently affected by pathologic siderosis in NH autopsy cases are acinar epithelium of the exocrine pancreas, myocardium, epithelia of thyroid follicles, the adrenal glands, and renal tubular epithelium, all of which are ZIP14-expressing and ferroportin-non-expressing. Minor salivary glands of the oral mucosa may be sampled in the clinical setting to establish the presence of extrahepatic siderosis. The reticuloendothelial system is relatively spared so the spleen, lymph nodes, and bone marrow do not contain significant amounts of stainable iron.

Renal dysgenesis. Renal hypoplasia and dysgenesis of proximal tubules with paucity of peripheral glomeruli have been reported in association with NH and may be linked as a renal developmental abnormality secondary to fetal liver injury. Expansion of the proximal tubule and associated glomeruli occurs starting in the 24th week of gestation and is dependent upon angiotensinogen, which is made exclusively in the liver. A study of NH cases showed that liver angiotensinogen expression is markedly reduced and that expression correlates closely with the mass of hepatocytes present in the liver [14]. In infants with GALD-NH with reduced hepatocyte mass, there may be reduced angiotensinogen gene expression and defective renal development [14] as all cases of GALD-NH, in this study, had some degree of impairment of renal development. Renal failure commonly accompanies severe GALD-NH and may be refractory to treatment. It should not be considered part of hepatorenal syndrome, as it does not recover with liver transplantation. A recently reported case of GALD-NH with glyceroluria suggests that proximal tubular dysfunction may occur in the absence of renal failure [16]. Careful study of renal function in many case of GALD-NH will be required to fully understand the clinical spectrum of this condition.

Clinical presentation

Liver failure in neonates is classified as "acute" because of timing, with birth being the point of onset of evidence of liver disease. However, in most cases of GALD-NH, the onset of liver injury is many weeks before parturition, and they have evidence of chronic liver disease or cirrhosis at birth.

The typical presentation of GALD-NH consists of:

- Liver failure detected in the first few days of life, most often within hours of birth.
- Intrauterine growth restriction, oligohydramnios, and premature birth [6]. In many cases, prenatal ultrasound has shown ascites to be present before birth, and neonatal ultrasound may show persistent patency of the ductus venosus. These are both manifestations of cirrhosis with onset before birth.
- Perinatal acute liver failure. In these cases, the liver shows acute injury only, without evidence of chronicity such as fibrosis or cirrhosis. Such cases are rare among cases of GALD-NH [8], but may be a cause of acute liver failure in neonates with gestational ages beyond 34 weeks.

Atypical presentations include:

- Fetal loss or stillbirth. The gestational histories of women who have had a baby affected by GALD show that 1 in 7 of their pregnancies ended with fetal loss [7]. Examination of the liver from eight cases of fetal death (six stillbirths and two cases of extreme premature birth) drawn from sibships comprising one or more GALD-NH cases showed diffuse complement-mediated hepatocyte injury [11]. These fetal losses occurred at 20–34 weeks of gestation and present evidence that GALD may cause acute fetal liver failure and fetal death.
- Postpartum death. A recent study of autopsies performed on infants <90 days of age showed that GALD may be an unappreciated cause of newborn death [17]. Among 209 infant autopsies performed over 16 years in a single institution, seven were identified as having an obscure or indeterminate cause of death, with final diagnoses such as "bleeding diathesis" and "non-immune hydrops." Examination of the livers from these cases showed acute complement-mediated hepatocyte damage in all. These infants all died before 3 days of age without clinical suspicion for having liver disease.
- Not all cases of GALD-NH present with liver failure at birth. For example, in rare cases liver disease can manifest days to weeks after birth. A spectrum of disease severity is possible with some infants presenting with liver failure while other "affected" babies having no clinical disease. For example, twin sets may have one twin severely affected and the other minimally so [18]. Spontaneous recovery from apparently mild liver disease has been recorded in members of sibships comprising lethal NH cases. The variability in severity may explain the apparent 10% "skip rate" for recurrence of severe disease within maternal sibships comprising a case of lethal NH [7].

Clinical and laboratory findings

Clinical features include:

- Hypoglycemia, jaundice, and coagulopathy with an average INR of >4.
- Hypoalbuminemia, edema, non-immune hydrops.
- Renal dysfunction with oliguria and anuria, which has a poor prognosis.
- Low serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations relative to the degree of liver failure. Recovery is associated with an increase in aminotransferase concentrations, presumably as hepatocytes are generated. The low aminotransferase

Table 10.1 Contrasting clinical and laboratory findings in the newborn with gestational alloimmune liver disease–neonatal hemochromatosis (GALD-NH) versus perinatal viral infection. (Adapted from: Whitington 2014 [21]. Reproduced with permission of Springer.)

Finding	GALD-NH	Perinatal viral infection
Premature birth	Most (70–90%)	Usual population incidence
Oligohydramnios	Most (70–90%)	Exceedingly rare
Intrauterine growth restriction	Most (70–90%)	Rare
Ascites	Common (40–60%)	Rare–never
Patent ductus venosus	Most (70–90%)	Rare–never
Hepatomegaly	Uncommon (10–20%)	Common
Hard liver	Always when palpable	Rare–never
Splenomegaly	Uncommon (10–20%)	Common but can be mild
Maternal history of stillbirth or infant with liver failure	Common (up to 30%)	Rare
Hypoglycemia	Common	Common
Coagulopathy	Always	Common
ALT	Almost always <100 IU/L	Almost always >100 IU/L and often >1000 IU/L
Bilirubin	Elevated total and direct after DOL 3	Usually normal or minimally elevated
Ferritin	Almost always >800 ng/mL	Usually <800 ng/mL
α-Fetoprotein	Almost always >80,000 and often >300,000–600,000	Almost always <80,000
Iron saturation	Almost always >90%	Almost always <80%
Plasma amino acids	Tyrosine and methionine often elevated	Usually normal

levels in an infant with liver failure distinguish GALD-NH from acute hepatic necrosis from viral or other causes.

- α-Fetoprotein (AFP) levels are typically high, ranging between 100,000 and 600,000 ng/mL (normal term newborn values are <80,000 ng/mL) [1]. Elevated AFP is sensitive for detection of GALD-NH with well over 95% of cases having elevated levels, but has minimal positive predictive value because many other newborn conditions show similar elevations.
- Serum ferritin levels >800 ng/mL. As ferritin levels are elevated in most neonatal liver diseases, it has little positive predictive value for the diagnosis of GALD-NH. High ferritin levels reflect release from hepatic stores and a very high level (i.e., >7000 ng/mL) suggests an alternative diagnosis such as hemophagocytic lymphohistiocytosis (HLH) [19].
- Iron saturation typically reveals low transferrin with hypersaturation of iron-binding capacity (95–105%) [4]. These findings reflect hepatic dysfunction and iron overload, but have little diagnostic value.
- Cholestasis depends upon the time course of disease. In the first few days of life, the ability to clear and conjugate bilirubin is evident, with total bilirubin levels higher than in normal newborns, often >30 mg/dL (>500 μmol/L). The direct or conjugated fraction of serum bilirubin remains less than 20% of the total. Subsequently, there will be a rise in conjugated bilirubin, which may persist for months in infants who recover with medical therapy. Bile acid synthesis is severely impaired in GALD-NH, and delayed time to recovery of this important liver function is likely the cause of protracted cholestasis.

Clinically, infants with GALD-NH are unique in that they have evidence of fetal insult and neonatal liver failure, which results in unique clinical and laboratory findings in infants with GALD-NH compared to infants with acute liver failure from perinatal viral infections (Table 10.1).

Diagnosis and differential diagnosis

GALD is possibly underdiagnosed and should be considered *first* in the differential diagnosis of newborns with liver failure, death from obscure cause in the first month of life, stillbirths occurring in second and third trimesters of gestation, and early death of prematurely born infants.

Establishing a diagnosis of GALD-NH depends upon identifying extrahepatic siderosis [19].

- In autopsy specimens, extrahepatic siderosis can be demonstrated by iron staining (Prussian blue, Perl's stain) of tissues affected by siderosis, in particular the pancreas, thyroid, and myocardium where it is most prominent.
- In the clinical setting, it can be demonstrated by magnetic resonance imaging (MRI) as a T2-weighted MRI will show iron excess in extrahepatic tissues, in particular pancreas, heart, and adrenal gland. Tissues with excess iron appear dark using the spleen as a reference (Figure 10.3).
- Iron staining of submucosal glands obtained from a biopsy of the oral mucosa. Biopsy of the oral mucosa is minimally invasive and can be performed at the bedside without need for fresh frozen plasma or recombinant factor VII beforehand. One must be sure to obtain a sample that obtains submucosal glands.

- Where feasible, liver histology usually demonstrates cirrhosis, pericellular fibrosis, giant cell transformation, ductular proliferation, and regenerative nodules. Less commonly, acute changes with hepatocyte necrosis are seen. The diagnosis of GALD can be confirmed by staining liver tissue for C5b-9 complex (MAC) by immunohistochemistry or immunofluorescence [19].

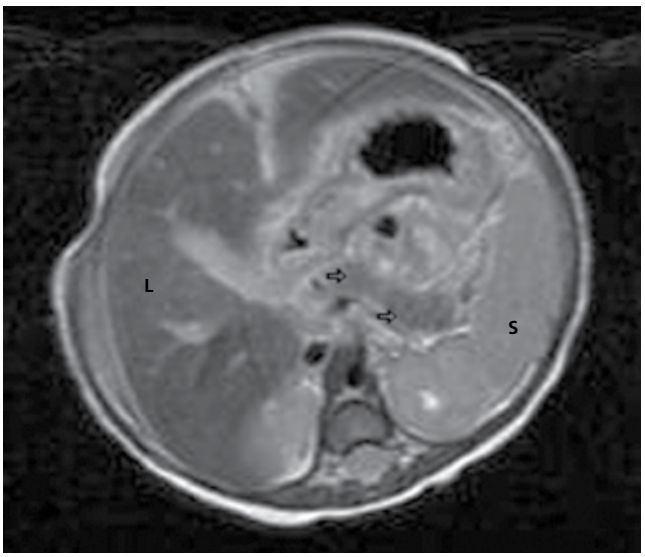


Figure 10.3 MRI showing extrahepatic siderosis in a term infant with liver failure. The T2-weighted series image shows liver (L) and pancreas (arrows) to be dark relative to spleen (S), indicating excess iron (siderosis) of these tissues. This along with liver failure established the diagnosis of neonatal hemochromatosis.

Oral mucosal biopsy and T2-weighted MRI each have about 60% sensitivity for identifying extrahepatic siderosis in cases with autopsy proven NH. It is not necessary to do both tests unless one test is negative. Siderosis in the liver alone is not diagnostic as stainable iron is present in the normal newborn and in other neonatal liver disease. The amount of iron content in the liver does not accurately discriminate between NH and other causes of iron overload. In addition, some infants with NH with extensive hepatocyte destruction do not have liver siderosis.

Properly performed autopsies including iron stains will identify all cases of GALD-NH; however, not all GALD cases have NH. Thus, the C5b-9 stain may be used to identify GALD if there is strong suspicion, even when no extrahepatic siderosis can be found [19]. This approach may be particularly useful in examination of stillbirths [11]. The availability of C5b-9 staining to diagnosticians remains an issue, and this approach has not been rigorously tested for diagnostic efficacy. Caution in using it is advised.

A diagnostic pathway has been developed that should minimize missed diagnosis of GALD (Figure 10.4) [19].

Autopsy should be performed in all cases of unexplained fetal death, stillbirth, and infant death suspicious for liver

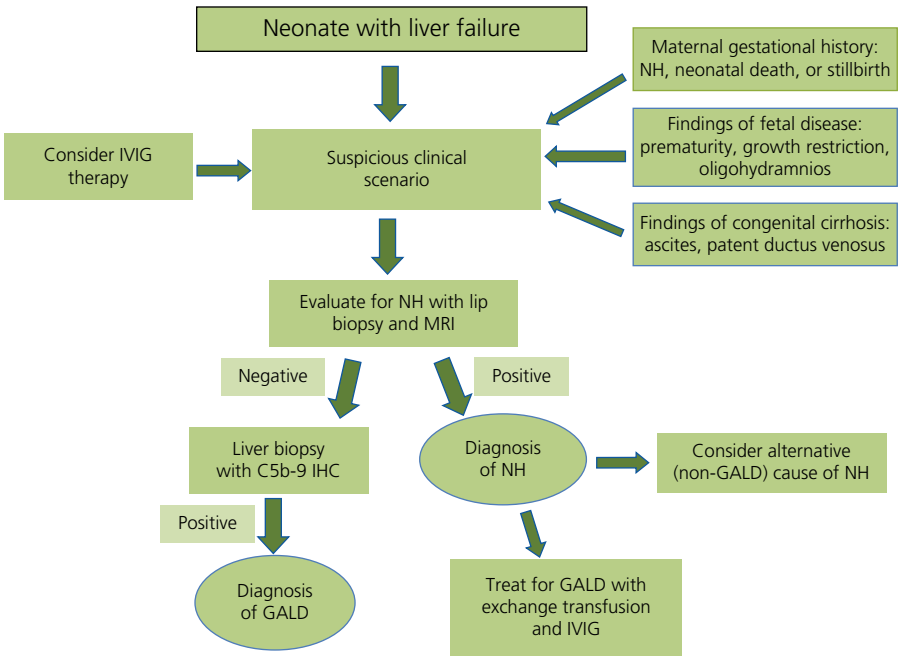


Figure 10.4 Diagnostic algorithm for neonatal hemochromatosis (NH). The diagnosis of NH should be considered in any neonate born with liver failure or clinical signs of congenital cirrhosis. Intravenous immunoglobulin (IVIG) should be given while diagnostic evaluation (including a lip biopsy or MRI for extrahepatic siderosis) is performed. If either lip biopsy or MRI is positive, the diagnosis of NH can be made and additional treatment (IVIG and double-volume exchange transfusion) can be started. If lip biopsy and MRI are negative and suspicion for gestational alloimmune liver disease–neonatal hemochromatosis (GALD-NH) is high, liver biopsy with immunohistochemistry for C5b-9 complex can be performed.

failure, which should include careful examination for siderosis of extrahepatic tissues. When possible, serum, dried blood spot, urine, DNA, fibroblasts, and frozen liver tissue should be collected as quickly as possible in order to diagnose non-GALD causes. C5b-9 staining is reserved for cases in which NH cannot be demonstrated and there is no alternate diagnosis for severe liver disease. Stillborn liver is often macerated making C5b-9 testing less useful in these cases.

Treatment

When NH was believed to be the result of iron overload resulting in secondary oxidative injury, a treatment combination of antioxidants and an iron chelator was utilized with little or no success.

Current therapy is directed towards removing existing reactive antibody using the combination of double-volume exchange transfusion followed by administration of high-dose intravenous immunoglobulin (IVIG) (to block antibody-induced complement activation) [12]. Experience in treating over 60 infants with this regimen has shown survival rates exceeding 80% [10].

The success of this relatively simple and well-tolerated therapy has changed the management of newborns with liver failure or suspected liver failure while diagnosis is being established. As soon as NH is considered in the differential, the infant should be given one dose of IVIG (1 g/kg body weight). This treatment can do no harm if the infant has a non-GALD cause of liver disease, and if GALD is the cause, it may substantially improve the patient's condition, making diagnosis easier. If NH is proven, a double-volume exchange transfusion may be performed followed by a second dose of IVIG. Successful outcome has been achieved with IVIG only and with exchange transfusion only, but combining these elements is recommended if possible. Normalization of the INR may take several weeks as the therapy can reduce ongoing immune injury but cannot reverse injury that has already occurred [12].

The ability for regeneration of the neonatal liver allows for a good long-term outcome following medical therapy. It may take 2–4 years for the liver to fully recover; however, there is biopsy documentation that surviving patients have undergone complete reversal of cirrhosis after receiving treatment [20].

Liver transplantation should be considered in those who do not respond to medical therapy, but it is important to allow supportive treatment sufficient time to be effective. The operation is difficult as the patient is both critically ill and small but can be successful despite considerable technical difficulties, with survival after transplant in about 50% in these cases. Successful transplantation seems to be curative.

Prevention in future pregnancies

Once a woman has delivered an infant with NH, she has a 90% likelihood that the next pregnancy will be affected unless she has adequate prevention [7]. Administration of IVIG during gestation effectively prevents recurrence of severe NH [7, 13]. The current guideline (available at neonatalhemochromatosis.org, last accessed June 2016) is that subsequent pregnancies be treated with 1 g/kg body weight (maximum 60 g) of IVIG at 14 weeks, 16 weeks, and then weekly from the 18th week of pregnancy until the end of gestation.

Data from over 160 pregnancies treated under these guidelines demonstrate a good outcome in >95% of cases. One pregnancy was lost at 22 weeks due to severe acute GALD (this woman did not start treatment until 18 weeks), and four infants born at near-term had severe liver disease resulting in death or need for liver transplantation. Four other infants had clinical liver disease as newborns and recovered. All other infants showed no signs of fetal distress or liver disease.

Administration of maternal IVIG is an onerous and expensive therapy, but likely to succeed. It is not known whether the dosage of IVIG can be reduced without reducing its effectiveness. Side effects of IVIG are rare, but include maternal hemolysis, aseptic meningitis, and anaphylaxis in IgA-deficient women.

The dramatic change in outcome of subsequent pregnancies with this therapy highlights the importance of making a diagnosis of NH in any fetal demise, stillbirth, or infant with signs and symptoms of liver failure. Irrespective of antenatal treatment all subsequent siblings should be assessed for evidence of NH and as a minimum should have iron status (iron, transferrin, and ferritin) and coagulation checked soon after birth.

Conclusion

Major changes have occurred in our understanding of NH over the last decade. It is now clear that the disease is not an inborn error of metabolism, but rather a phenotype of severe liver injury that results in inability of the fetal liver to properly regulate iron homeostasis. The most common cause of NH is GALD, an alloimmune process that can be both prevented and treated. Hence it is of upmost importance to consider NH in all cases of fetal demise, stillbirth, and infantile death due to liver disease. The prevalence of NH may be underestimated and future studies are needed to evaluate the role of GALD in fetal loss, miscarriage, and stillbirth. Obstetricians, pediatricians, and neonatologists should be aware of GALD in their clinical practices.

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SECTION 6

Liver Disease in Older Children

CHAPTER 11

Autoimmune Liver Disease

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Key points

- There are three types of pediatric liver disease with an autoimmune component to their pathogenesis: autoimmune hepatitis, autoimmune sclerosing cholangitis, and de novo autoimmune hepatitis after liver transplantation.
- Autoimmune hepatitis is divided into two subtypes: type 1, positive for antinuclear (ANA) and/or antismooth muscle (SMA) autoantibodies; and type 2, positive for anti-liver kidney microsomal type 1 (anti-LKM1) and/or anti-liver cytosol type 1 (anti-LC-1) autoantibodies.
- The histological feature typical common to autoimmune hepatitis, autoimmune sclerosing cholangitis and de novo autoimmune hepatitis is interface hepatitis.
- Autoimmune sclerosing cholangitis is serologically (ANA/SMA) and histologically similar to autoimmune hepatitis type 1, but in addition has bile duct damage demonstrable by cholangiography.
- Both in autoimmune hepatitis and autoimmune sclerosing cholangitis the parenchymal inflammation responds satisfactorily to standard immunosuppressive treatment with steroids ± azathioprine, but in autoimmune sclerosing cholangitis the bile duct disease progresses in about 50% of cases, leading to liver transplantation.
- Autoimmune sclerosing cholangitis is frequently associated to inflammatory bowel disease and deterioration of liver disease, as well as the risk of autoimmune sclerosing cholangitis recurrence after transplant, is correlated to the activity of the gut disease.
- The minority of patients, who do not respond to standard treatment, or who relapse frequently should be offered alternative immunosuppression (including in order of priority mycophenolate mofetil and calcineurin inhibitors).
- Relapse is frequently due to non-adherence to treatment, particularly in adolescents.
- Both autoimmune hepatitis and autoimmune sclerosing cholangitis can recur after liver transplantation, recurrence being more common in autoimmune sclerosing cholangitis than autoimmune hepatitis.
- Regulatory T cells defective both in function and number are likely to play a major role in the loss of tolerance that leads to autoimmune liver disease.
- De novo autoimmune hepatitis after liver transplantation for non-autoimmune conditions responds to the classical treatment of autoimmune hepatitis, but not to standard antirejection treatment.

Definition

Autoimmune liver diseases are characterized histologically by a dense mononuclear cell infiltrate in the portal tract (interface hepatitis; Figure 11.1) and serologically by high levels of transaminases and immunoglobulin G (IgG) and positive autoantibodies, in the absence of a known etiology. These disorders typically respond to immunosuppressive treatment, which should be instituted as soon as a diagnosis is made [1].

In pediatrics, there are three liver disorders in which liver damage is likely to arise from an autoimmune attack: autoimmune hepatitis (AIH); autoimmune sclerosing cholangitis (ASC); and de novo AIH after liver transplantation.

Diseases of the Liver and Biliary System in Children, Fourth Edition. Edited by Deirdre A. Kelly.

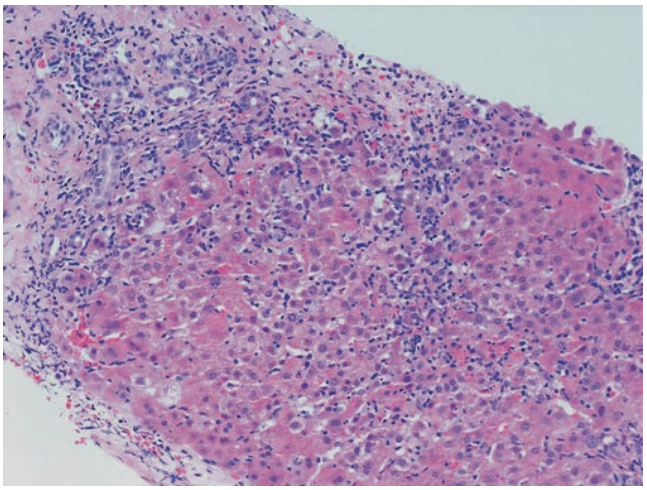

Figure 11.1 Portal and periportal lymphocyte and plasma cell infiltrate, extending to and disrupting the parenchymal limiting plate (interface hepatitis). Swollen hepatocytes, pyknotic necroses, and acinar inflammation are present (H&E, original magnification ×40). (Image kindly provided by Dr Alberto Quaglia.)

Autoimmune hepatitis

History and epidemiology

AIH was described for the first time in 1950 by Waldenström [2]. Seropositivity for antinuclear antibody (ANA), the hallmark of systemic lupus erythematosus, led to the name of "lupoid hepatitis," a term that is no longer used. As the disease frequently presents acutely, the term "chronic active hepatitis," which implied that the disease should be chronic, i.e., of at least 6 months' duration, before institution of treatment, is similarly obsolete. Before the efficacy of immunosuppression was established, untreated severe AIH had a mortality of 50% at 5 years and 90% at 10 years. The prevalence of AIH is unknown [1]. Reported prevalences vary from 1 in 200,000 in the general population in the US to 20 in 100,000 in females over 14 years of age in Spain, although probably both figures are underestimates. Data collected at a tertiary center suggest that there is an increasing annual incidence of both AIH and ASC in childhood. In the 1990s, these conditions represented 2.3% of children older than 4 months referred to the King's Paediatric Liver Service, UK, during 1 year, whereas in the 2000s, their incidence has increased to 12%.

Etiology and pathogenesis

The etiology of AIH is unknown, although both genetic and environmental factors are involved in its expression [1–3].

Genetics. AIH is a "complex-trait" disease, i.e., a condition not inherited in a mendelian autosomal dominant, autosomal recessive, or sex-linked fashion. The mode of inheritance of a complex-trait disorder is unknown and involves one or more genes operating alone or in concert to increase or reduce the risk of the trait, and interacting with environmental factors.

Susceptibility to AIH is imparted by genes in the human leukocyte antigen (HLA) region on the short arm of chromosome 6, especially those encoding DRB1 alleles. These class II major histocompatibility complex (MHC) molecules are involved in peptide antigen presentation to CD4 T cells, suggesting the involvement of MHC class II antigen presentation and T-cell activation in the pathogenesis of AIH.

In Europe and North America, susceptibility to AIH type 1 in adults is conferred by the possession of HLA DR3 (*DRB1*0301*) and DR4 (*DRB1*0401*), both heterodimers containing a lysine residue at position 71 of the DRB1 polypeptide and the hexameric amino acid sequence LLEQKR at positions 67–72. In Japan, Argentina, and Mexico, susceptibility is linked to *DRB1*0405* and *DRB1*0404*, alleles encoding arginine rather than lysine at position 71, but sharing the motif LLEQ-R with *DRB1*0401* and *DRB1*0301*. Thus, K or R at position 71 in the context of LLEQ-R may be critical for susceptibility to AIH, favoring the binding of autoantigenic peptides, complementary to this hexameric sequence.

The lysine-71 and other models for AIH type 1 cannot explain the disease completely, since in European and North American patients, e.g., the presence of lysine-71 is associated with a severe and mainly juvenile disease in those who are positive for *DRB1*0301*, but to a mild and adult-onset disease in those who are positive for *DRB1*0401*. Other genes inside and/or outside the MHC are therefore likely to be involved in determining the phenotype. Possible candidates are the MHC-encoded complement and tumor necrosis factor α genes, mapping to the class III MHC region, and the MHC class I chain-related A and B genes. Patients with AIH, whether positive for anti-liver–kidney microsomal antibody type 1 (anti-LKM-1) or ANA/SMA, have isolated partial deficiency of the HLA class III complement component C4, which is genetically determined.

In northern Europe, pediatric AIH-1, similar to adult AIH, is associated with the possession of HLA *DRB1*03*. In contrast to adult patients, possession of *DRB1*04* does not predispose to AIH in childhood, and can even exert a protective role. Susceptibility to AIH type 2 is conferred by the possession of HLA DR7 (*DRB1*0701*) and, in DR7-negative patients, with possession of DR3 (*DRB1*0301*), those patients positive for *DRB1*0701* having a more aggressive disease and a more severe prognosis. In Egypt AIH-2 appears to be associated also with possession of *HLA-DRB1*15*. In Brazil and in Egypt, the primary susceptibility allele for AIH-1 is *DRB1*1301*, but a secondary association with *DRB1*0301* has also been identified. Interestingly, in South America, possession of the HLA *DRB1*1301* allele not only predisposes to pediatric AIH-1, but is also associated with persistent infection with the endemic hepatitis A virus. Homozygosity for DR3 plays a major role in the predisposition to juvenile autoimmune liver disease [4].

A form of AIH resembling AIH type 2 affects some 20% of patients with autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED), a condition also known as autoimmune polyendocrine syndrome 1 [3, 5]. APECED is a monogenic autosomal recessive disorder caused by homozygous mutations in the *AIRE1* gene and characterized by a variety of organ-specific autoimmune diseases, the most common of which are hypoparathyroidism and primary adrenocortical failure, accompanied by chronic mucocutaneous candidiasis. The *AIRE1* gene sequence consists of 14 exons containing 45 different mutations, with a 13-base pair deletion at nucleotide 964 in exon 8 accounting for more than 70% of APECED alleles in the UK. The protein predicted to be encoded by *AIRE1* is a transcription factor. *AIRE1* is highly expressed in medullary epithelial cells and other stromal cells in the thymus that are involved in clonal deletion of self-reactive T cells. Studies in a murine model indicate that the gene inhibits organ-specific autoimmunity by inducing thymic expression of peripheral antigens in the medulla, leading to central deletion of autoreactive T cells. Interestingly, APECED has a high level of variability in symptoms, especially between populations. Since various gene mutations have the same effect on thymic transcription of ectopic genes in animal models, it is likely that the clinical variability across human populations is related to environmental or genetic modifiers. Of the various genetic modifiers, perhaps the most likely to synergize with *AIRE* mutations are polymorphisms in the HLA region. HLA molecules are not only highly variable and strongly associated with multiple autoimmune diseases, but are also able to affect thymic repertoire selection of autoreactive T-cell clones. Carriers of a single *AIRE* mutation do not develop APECED. However, although the inheritance pattern of APECED indicates a strictly recessive disorder, there are anecdotal reports of mutations in a single copy of *AIRE* being associated with human autoimmunity of a less severe form than classically defined APECED. The role of the *AIRE1* heterozygote state in the development of AIH remains to be established.

AIRE1 mutations have been reported in three children with severe AIH type 2 and extrahepatic autoimmune manifestations [3].

Immune mechanisms [6]. Immunohistochemical studies have identified the phenotype of the cells infiltrating the portal tract and invading the parenchyma in the typical AIH histological picture of interface hepatitis. T lymphocytes mounting the α/β T-cell receptor predominate. Among the T cells, the majority are positive for the CD4 helper/inducer phenotype, and a sizable minority are positive for the CD8 cytotoxic phenotype. Lymphocytes of non-T-cell lineage are fewer and include (in decreasing order of frequency) natural killer cells (CD16/CD56-positive), macrophages, and B lymphocytes. Natural killer T cells, which simultaneously express markers of both natural killer (CD56) and T cells (CD3), were found to be involved in liver damage in an animal model of AIH.

Powerful recruiting stimuli must be promoting the formation of the massive inflammatory cell infiltrate that is present at diagnosis. Whatever the initial trigger, it is most probable that such a high number of activated inflammatory cells cause liver damage.

There are different possible pathways that an autoimmune attack can follow to inflict damage on hepatocytes (Figure 11.2). It is believed that liver damage is orchestrated by $CD4^+$ T lymphocytes recognizing a self-antigenic peptide on hepatocytes. To trigger an autoimmune response, the peptide has to be embraced by an HLA class II molecule and presented to uncommitted (naive) $CD4^+$ T helper (Th0) cells by professional antigen-presenting cells (APC), with the co-stimulation of ligand–ligand ($CD28^+$ on Th0, $CD80^+$ on APC) fostering interaction between the two cells. Th0 cells become activated, differentiate into functional phenotypes according to the cytokines prevailing in the microenvironment and the nature of the antigen, and initiate a cascade of immune reactions determined by the cytokines these activated T cells produce. Th1 cells, arising in the presence of the macrophage-produced interleukin 12 (IL-12), secrete mainly IL-2 and interferon gamma (IFN-γ), which activate macrophages, enhance expression of HLA class I (increasing liver cell vulnerability to a $CD8^+$ T-cell cytotoxic attack) and induce expression of HLA class II molecules on hepatocytes. Th2 cells, which differentiate from Th0 if the microenvironment is rich in IL-4, mainly produce IL-4, IL-10, and IL-13, which favor autoantibody production by B lymphocytes. Physiologically, Th1 and Th2 antagonize each other. Th17 cells arise in the presence of transforming growth factor β (TGF-β) and IL-6 and appear to have an important effector role in inflammation and autoimmunity. The process of autoantigen recognition is strictly controlled by regulatory mechanisms, such as those exerted by $CD4^+CD25^+$ regulatory T cells, which are derived from Th0 in the presence of TGF-β, but in the absence of IL-6. If regulatory mechanisms fail, the autoimmune attack develops and persists.

Various aspects of the above pathogenic scenario have been investigated during the last 35 years [6]:

- *Regulatory T cells* [7]. Autoimmunity arises against a background of defective immunoregulation, and this has been repeatedly reported in AIH. Early studies showed that patients with AIH have low levels of circulating T cells expressing the $CD8^+$ marker, and impaired suppressor cell function, which segregates with the possession of the disease-predisposing HLA haplotype *B*08/DRB1*03* (formerly B8/DR3) and is correctable by therapeutic doses of corticosteroids. It is possible, although not formally tested, that these early characterized $CD8^+$ T cells with a suppressor function represent the recently defined $CD8^+CD28^-$ suppressor T cells. Furthermore, patients

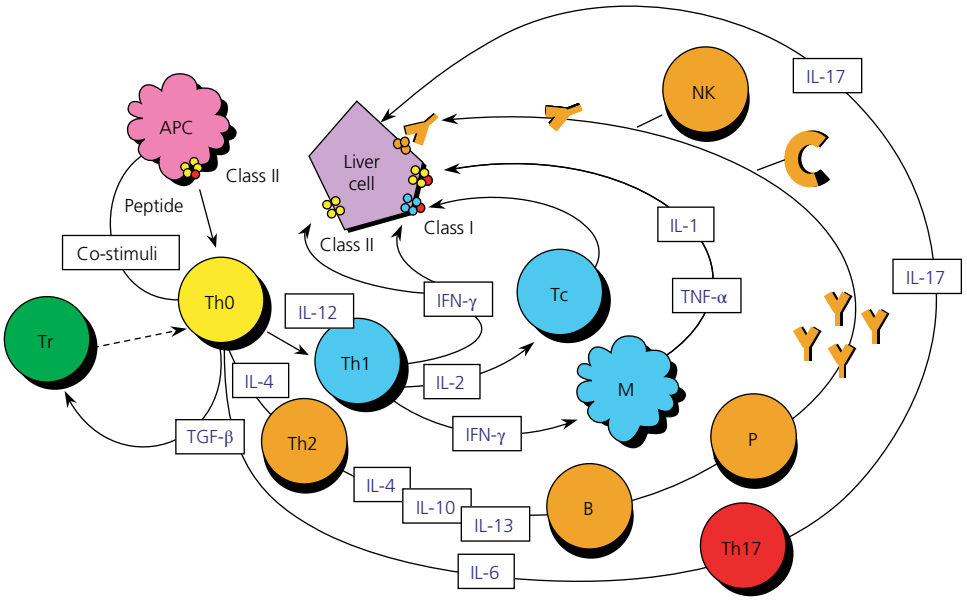

Figure 11.2 Autoimmune attack on the liver cell. A specific autoantigenic peptide is presented to an uncommitted T-helper (Th0) lymphocyte within the HLA class II molecule of an antigen-presenting cell (APC). Th0 cells become activated and, depending on the presence in the microenvironment of interleukin-12 (IL-12) or IL-4 and the nature of the antigen, differentiate into Th1 or Th2 and initiate a series of immune reactions determined by the cytokines they produce. Th2 cells mainly secrete IL-4, IL-10, and IL-13, and direct autoantibody production by B lymphocytes. Th1 cells secrete IL-2 and interferon gamma (IFN-γ), which stimulate T cytotoxic (Tc) lymphocytes, enhance expression of class I, and induce expression of class II HLA molecules on hepatocytes and activate macrophages. Activated macrophages release IL-1 and tumor necrosis factor alpha (TNF-α). If regulatory T cells (Tr) do not oppose, a variety of effector mechanisms are triggered: liver cell destruction could result from the action of Tc lymphocytes; cytokines released by Th1 and recruited macrophages; complement activation or engagement of Fc receptor-bearing cells such as natural killer (NK) lymphocytes by the autoantibody bound to the hepatocyte surface. The role of the recently described Th17 cells, which arise in the presence of tissue growth factor beta (TGF-β) and IL-6, is under investigation.

with AIH have been shown to have a defect in a subpopulation of T cells controlling the immune response to liver-specific membrane antigens. Novel experimental evidence confirms an impairment of the immunoregulatory function in AIH. Amongst T-cell subsets with potential immunosuppressive function, $CD4^+$ T cells constitutively expressing the IL-2 receptor α-chain (CD25; T-regulatory cells, T-regs) have emerged as the dominant immunoregulatory subset of lymphocytes. These cells, which represent some 5–10% of the total population of peripheral $CD4^+$ T cells in health, control innate and adaptive immune responses by preventing proliferation and effector function of autoreactive T cells. Their mechanism of action involves mainly a direct contact with the target cells, and to a lesser extent the release of immunoregulatory cytokines, such as IL-10 and TGF-β. In children with AIH, T-regs are defective in number and function in comparison with normal controls, and this impairment relates to the stage of disease, being more evident at diagnosis than during drug-induced remission. The percentage of T-regs inversely correlates with markers of disease severity, such as antisoluble liver antigen (anti-SLA) and anti-LKM1 autoantibody titers, suggesting that a reduction in regulatory T cells favors the serological expression of autoimmune liver disease. Importantly, several studies show that T-regs from AIH patients at diagnosis are impaired in their ability to control the proliferation of CD4 and CD8 effector cells compared to T-regs isolated from AIH patients at remission or from healthy subjects. Effector CD4 T cells isolated from patients with AIH are less susceptible to the regulatory control exerted by T-regs. This defect is linked to reduced expression of the inhibitory receptor T-cell immunoglobulin and mucin-domain containing molecule 3 (Tim-3), which upon ligation of galectin-9 expressed by T-regs, induces effector cell death. If loss of immunoregulation is central to the pathogenesis of AIH, treatment should concentrate on restoring the T-regs' ability to expand, with a consequent increase in their number and function. This is at least partially achieved by standard immunosuppression, since numbers of T-regs increase during remission.

- *Autoreactive T cells* [6]. As mentioned above, to trigger an autoimmune response, a peptide embraced by an HLA class II molecule has to be presented to uncommitted T-helper (Th0) cells by professional APCs (see Figure 11.2). Given the impaired regulatory function described above, it is suspected that in AIH, an autoantigenic peptide is indeed presented to the helper/inducer T cells, leading to their sustained activation. There is direct, albeit limited, evidence that an autoantigenic peptide is presented and

recognized in AIH type 2. Activation of T-helper cells has been documented in earlier studies on AIH, both in the liver and in the peripheral blood. These activated cells are mainly of the CD4 phenotype, and their numbers are highest in the most active stages of the disease.

Major advances in the study of T cells have occurred in AIH type 2, since the knowledge that CYP2D6 is the main autoantigen has made it possible to characterize both CD4 and CD8 T cells targeting this cytochrome. One study has shown that CD4 T cells from patients with type 2 AIH who are positive for the predisposing HLA allele *DRB1*0701* recognize seven regions of CYP2D6, five of which have later been shown to be also recognized by CD8 T cells. High numbers of antigen-specific IFN-γ-producing CD4 and CD8 T cells are associated with biochemical evidence of liver damage, suggesting a combined cellular immune attack.

What triggers the immune system to react to an autoantigen is unknown. A lesson may be learned by the study of humoral autoimmune responses during viral infections. Thus, studies aimed at determining the specificity of the LKM-1 antibody – present in both the juvenile form of AIH and in some patients with chronic hepatitis C virus (HCV) infection – have shown a high amino acid sequence homology between the HCV polyprotein and CYP2D6, the molecular target of LKM-1, implicating a mechanism of molecular mimicry as a trigger for the production of anti-LKM-1 in HCV infection [6]. It is therefore conceivable that an as yet unknown virus infection may be at the origin of the autoimmune attack in AIH.

Titers of antibodies to liver-specific lipoprotein, a macromolecular complex present on the hepatocyte membrane, and to its well-characterized component asialoglycoprotein receptor, correlate with the biochemical and histological severity of AIH. Immunofluorescence studies of monodispersed suspensions of liver cells obtained from patients with AIH have shown that these cells are coated with antibodies in vivo. A pathogenic role for these autoantibodies has been indicated by cytotoxicity assays showing that autoantibody-coated hepatocytes from patients with AIH are killed when incubated with autologous lymphocytes. The effector cell was identified as an Fc receptor positive mononuclear cell. T-cell clones obtained from liver biopsies of patients with AIH and expressing the γ/δ T-cell receptor have been shown to be cytotoxic to a variety of targets, but to preferentially kill liver-derived cells as opposed to cell lines derived from other organs.

The establishment of cell lines and clones enabled to show that the majority of T-cell clones obtained from the peripheral blood and a proportion of those from the liver of patients with AIH are CD4 positive and use the conventional α/β T-cell receptor. Some of these CD4-positive clones were further characterized and were found to react with partially purified antigens, such as crude preparations of liver cell membrane or liver-specific lipoprotein, and with purified asialoglycoprotein receptor or recombinant CYP2D6, and to be restricted by HLA class II molecules in their response. Co-culture of these clones with autologous B lymphocytes resulted in a dramatic increase in autoantibody production, confirming their role of Th cells. All of the above experimental evidence suggests that cellular immune responses are involved in the liver damage that occurs in AIH, although the evidence that the trigger is an autoantigen is still incomplete.

The possible role of Th17 cells in the pathogenesis of AIH is under investigation. Th17 cells contribute to autoimmunity by producing the proinflammatory cytokines IL-17, IL-22, and TNF-α, and inducing hepatocytes to secrete IL-6, which further enhances Th17 activation. Th17 cells have been shown to be elevated in the circulation and liver of patients with AIH.

Clinical features

AIH affects mainly females and is divided into two main types according to the autoantibody profile: type 1 is positive for ANA and/or antismooth muscle (SMA) antibody, type 2 is positive for anti-LKM-1. Pediatric series, including the King's College Hospital one, have reported a similarly severe disease in ANA/SMA-positive and anti-LKM-1-positive patients [3, 8]. We reviewed the clinical, biochemical, and histological features and outcomes of type 1 and 2 AIH in 52 children referred between 1973 and 1993 (Table 11.1) [9]. Thirty-two patients were positive for ANA and/or SMA, and 20 were positive for anti-LKM-1.

The clinical features included:

- 75% female preponderance.
- Variable age at onset (median of 10 years in type 1 and 7.4 years in type 2 AIH), with occasional presentation in infancy.
- Other autoimmune disorders affecting patients in 20% and a first-degree relative in 40% of cases.
- Similar severity and outcome.

The mode of presentation was variable, with the following predominant types emerging:

- Acute presentation resembling that of viral hepatitis (in 50% of patients with type 1 and 65% of patients with type 2 AIH) with non-specific symptoms of malaise, nausea/vomiting, anorexia, and abdominal pain, followed by jaundice, dark urine, and pale stools.
- Fulminant hepatic failure (in 11%, five out of six of these patients having type 2); with grade II to IV hepatic encephalopathy developing 2 weeks to 2 months (median 1 month) after the onset of symptoms.
- Insidious onset, characterized by progressive fatigue, relapsing jaundice, headache, anorexia, and weight loss, lasting from 6 months to 2 years (median 9 months) before diagnosis (25% of patients with type 2 and 38% of patients with type 1 AIH).

Table 11.1 Autoimmune hepatitis (AIH) type 1, autoimmune hepatitis type 2, and autoimmune sclerosing cholangitis (ASC): clinical, laboratory, and histological features at presentation [9, 17].

	Type 1 AIH	Type 2 AIH	ASC
Median age in years	11	7	12
Females (%)	75	75	55
Mode of presentation (%)			
• acute hepatitis	47	40	37
• acute liver failure	3	25	0
• insidious onset	38	25	37
• complication of portal hypertension	12	10	26
Associated autoimmune diseases (%)	22	20	48
• inflammatory bowel disease (%)	20	12	44
Family history of autoimmune disease (%)	43	40	37
Abnormal cholangiogram (%)	0	0	100
Interface hepatitis (%)	92	94	60
Biliary features (%)	28	6	31
Cirrhosis (%)	69	38	15
Remission after immunosuppressive treatment (%)	97	87	89
Increased frequency of HLA *DR*0301*	Yes	No*	No
Increased frequency of HLA *DR*0701*	No	Yes	No
Increased frequency of HLA *DR*1301*	No	No	Yes
ANA/SMA (%)	100	25	96
Anti-LKM-1 (%)	0	100	4
pANCA (%)	45	11	74
Anti-SLA (%)†	58	58	41
Increased IgG level (%)	84	75	89
Partial IgA deficiency (%)	9	45	5
Low C4 level (%)	89	83	70

ANA, antinuclear antibodies; C4, C4 component of complement; HLA, human leukocyte antigen; IgA, immunoglobulin A; IgG, immunoglobulin G; LKM-1, liver–kidney microsomal type 1 antibody; pANCA, perinuclear antineutrophil cytoplasmic antibody; SLA, soluble liver antigen; SMA, antismooth muscle antibody.
* Increased in patients who are negative for HLA *DR*0701*.
† Measured by radioligand assay.

- Complications of cirrhosis and portal hypertension. In six patients (two of whom were positive for anti-LKM-1), there was no history of jaundice, and the diagnosis followed presentation with complications of portal hypertension, such as hematemesis from esophageal varices, bleeding diathesis, chronic diarrhea, weight loss, and vomiting.
- Incidental finding of raised hepatic aminotransferases.

Associated disorders

There was also no significant difference in the frequency of associated autoimmune disorders and a family history of autoimmune disease. Associated autoimmune disorders included:

- Behçet disease.
- Insulin-dependent diabetes.
- Graves disease.
- Celiac disease.
- Inflammatory bowel disease.
- Sjögren syndrome.
- Hemolytic anemia.
- Glomerulonephritis.
- Idiopathic thrombocytopenia.
- Urticaria pigmentosa in type 1 AIH.
- Thyroiditis, vitiligo, hypoparathyroidism, and Addison disease in type 2 AIH.

AIH may also occur in 10–20% of patients with autoimmune polyglandular syndrome type 1 (APECED).

The mode of presentation of AIH in childhood is therefore variable, and the disease should be suspected and excluded in all children presenting with symptoms and signs of prolonged or severe liver disease.

Differential diagnosis

The differential diagnosis, depending on presentation, includes:

- AIH.
- Sclerosing cholangitis.
- Chronic viral hepatitis.
- Acute infective hepatitis.
- Drug-induced liver disease.
- Metabolic liver disease.
- Cystic fibrosis.
- α_1-Antitrypsin deficiency.
- Wilson disease.

A careful history is invaluable in considering this wide range of disorders.

Diagnosis and laboratory findings

These should include the following: elevated serum transaminase and IgG/γ-globulin levels, and presence of ANA, SMA, or anti-LKM, anti-LC-1 autoimmune markers [2, 8].

Biochemistry

Biochemical abnormalities in AIH hepatitis are non-specific:

- Serum aminotransferases – alanine aminotransferase (ALT) and aspartate aminotransferase (AST) – are usually raised.
- Serum alkaline phosphatase and γ-glutamyltransferase (GGT) are usually normal or mildly elevated.
- Serum bilirubin is variable.
- Albumin may be low.
- Coagulation may be abnormal, particularly in chronic disease or fulminant hepatitis.

Overall, anti-LKM-1-positive patients had higher median levels of bilirubin and AST than those who were ANA/SMA-positive, but if the six patients presenting with acute hepatic failure are excluded, the differences for these two parameters are not significant. Severely impaired hepatic synthetic function, as assessed by prolonged prothrombin time and hypoalbuminemia, tended to be more common in patients with type 1 AIH (53%) than in those with type 2 AIH (30%).

Immunoglobulins

The majority (80%) of the patients had increased levels of IgG, but 10 (five of whom were positive for anti-LKM-1) had a normal serum IgG level for age, including three patients who presented with acute hepatic failure – indicating that normal IgG values do not exclude the diagnosis of AIH. As previously reported, we found that partial IgA deficiency is significantly more common in type 2 than in type 1 AIH (45% versus 9%).

Histology

Liver biopsy is necessary to establish the diagnosis. The typical histological picture includes:

- A dense mononuclear and plasma cell infiltration of the portal areas, which expands into the liver lobule.
- Destruction of the hepatocytes at the periphery of the lobule, with erosion of the limiting plate ("interface hepatitis"; see Figure 11.1).
- Connective tissue collapse resulting from hepatocyte death and expanding from the portal area into the lobule ("bridging collapse").
- Hepatic regeneration with "rosette" formation.
- Cirrhosis.

The severity of interface hepatitis is similar in both type 1 and 2 at diagnosis. Cirrhosis on initial biopsy was more frequent in ANA/SMA-positive patients (69%) than in anti-LKM-1-positive patients (38%). Fifty-seven percent of patients who were already cirrhotic at diagnosis presented with a clinical picture reminiscent of that of prolonged acute virus-like hepatitis. Multi-acinar or pan-acinar collapse, which suggests an acute liver injury, was present in eight patients (15%, five of whom were anti-LKM-1-positive), six of whom had acute liver failure. In these patients, it was not possible to ascertain the degree of fibrosis or the presence or absence of cirrhosis. The question of whether the acute presentation in these patients represented a sudden deterioration of an underlying unrecognized chronic process or a genuinely acute liver damage remains open.

The diagnosis of AIH has been advanced by the criteria developed by the International Autoimmune Hepatitis Group (IAIHG) [1–3], in which negative criteria such as evidence of infection with hepatitis B or C virus or Wilson disease are taken into account in addition to the positive criteria mentioned above. The IAIHG has provided a scoring system for the diagnosis of AIH, mainly used for research purposes to allow ready comparison between series from different centers, but has also been used clinically, including in pediatric series. The IAIHG have later published a simplified scoring system based on autoantibodies, IgG, histology, and exclusion of viral hepatitis that is better suited to clinical application. However, neither scoring system is suitable to the juvenile form of the disease, where diagnostically relevant autoantibodies often have titers lower than the cut-off value considered positive in adults. In addition, neither system can distinguish between AIH and ASC, which can only be differentiated if a cholangiogram is performed at presentation.

Autoantibodies [8]. Autoantibody detection by immunofluorescence (Figure 11.3) not only assists in the diagnosis, but also allows differentiation of AIH types. ANA and SMA, which characterize type 1 AIH, and anti-LKM-1, which defines type 2 AIH, are usually mutually exclusive. In the instances in which they are present simultaneously, the disease is classified as type 2 AIH. Recognition and interpretation of the immunofluorescence patterns is not always simple. The operator dependency of the technique and the relative rarity of AIH explain the not infrequent occurrence of errors in reporting, particularly of less frequent findings such as anti-LKM-1. There are problems between laboratory reporting and the clinical interpretation of the results, which are partly dependent on insufficient standardization of the tests, but also partly dependent on a degree of unfamiliarity of some clinicians with the disease spectrum of AIH. With regard to standardization, guidelines have been provided by the IAIHG serology committee. The basic technique for the routine testing of autoantibodies relevant to AIH is indirect immunofluorescence on a freshly prepared rodent substrate, which should include kidney, liver, and stomach, to allow the detection of ANA, SMA, anti-LKM-1 as well as anti-LC-1, but also of antimitochondrial antibody (AMA), the serological hallmark of primary biliary cirrhosis. Commercially available sections are of variable quality because, in order to lengthen their shelf-life, they are treated with fixatives (acetone, ethanol, or methanol), which readily result in enhanced background staining that may hinder the recognition of diagnostic autoantibodies, especially when these are present at low titer. In healthy children, autoantibody reactivity is infrequent, so that titers of 1/20 for ANA and SMA and 1/10 for anti-LKM-1 are clinically relevant. Positive sera should be titrated to extinction. The laboratory should report any level of positivity from 1/10 in children and 1/40 in adults, and the attending physician should interpret the result within the clinical context.

ANA is readily detectable as a nuclear stain in kidney, stomach, and liver. On the latter in particular, the ANA pattern may be detected as homogeneous, or coarsely or finely speckled. In most cases of AIH, but not in all, the pattern is homogeneous. To obtain a much clearer and easier definition of the nuclear pattern, HEp2 cells that have prominent nuclei should be used. However, HEp2 cells, derived from a laryngeal carcinoma, should not be used for screening purposes, because nuclear reactivity to these cells is frequent at a low serum dilution (1/40) in the normal population. For ANA, likely molecular targets include nuclear chromatin and histones, akin to lupus, but there are probably several others.

SMA is detected on kidney, stomach, and liver, where it stains the walls of the arteries. In the stomach, it also stains the muscularis mucosa and the lamina propria. On the renal

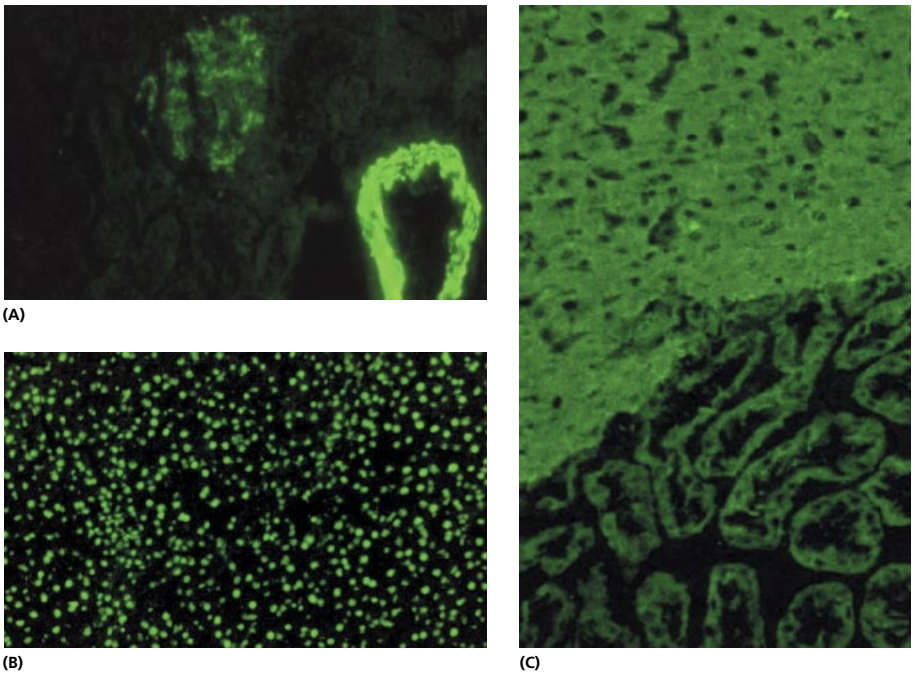

Figure 11.3 Immunofluorescence appearance of smooth muscle (SMA), antinuclear (ANA), and liver–kidney microsomal type 1 (LKM-1) autoantibodies on renal and liver rodent sections. SMA stains the small artery and the glomerulus in a renal section (A), ANA the nuclei in a liver section (B), and LKM-1 the cytoplasm of hepatocytes and proximal renal tubules (C). SMA and/or ANA are the markers of autoimmune hepatitis type 1; their molecular targets are still unknown. LKM-1 characterizes autoimmune hepatitis type 2, and its target is cytochrome P4502D6.

substrate, it is possible to visualize the V, G, and T patterns; V refers to vessels, G to glomeruli, and T to tubules. The V pattern is present also in non-autoimmune inflammatory liver disease, in autoimmune diseases not affecting the liver, and in viral infections, but the VG and VGT patterns are more specific for AIH. The VGT pattern corresponds to the so-called "F actin" or microfilament (MF) pattern observed using cultured fibroblasts as substrate. However, neither the VGT nor the anti-MF patterns are entirely specific for the diagnosis of AIH type 1 Although it has been suggested that the VGT-MF pattern is due to a specific antibody uniquely found in AIH type 1, it probably just reflects high-titer SMA. The molecular target of the microfilament reactivity that is observed in AIH type 1 has yet to be identified. Although "anti-actin" reactivity is strongly associated with AIH type 1, some 20% of SMA-positive AIH type 1 patients do not have the F actin/VGT pattern. The absence of anti-actin SMA therefore does not exclude the diagnosis of AIH.

Anti-LKM-1 brightly stains the liver cell cytoplasm and the P3 portion of the renal tubules, but does not stain gastric parietal cells. Anti-LKM-1 is often confused with AMA, since both autoantibodies stain liver and kidney. In comparison with anti-LKM-1, AMA stains the liver more faintly and the renal tubules more diffusely, with an accentuation of the small distal ones. In contrast to anti-LKM-1, AMA also stains the gastric parietal cells. AMA positivity in childhood AIH is exceedingly rare. The identification of the molecular targets of anti-LKM-1, i.e., cytochrome P4502D6 (CYP2D6), and of AMA, i.e., enzymes of the 2-oxo-acid dehydrogenase complexes, has led to the establishment of immunoassays based on the use of the recombinant or purified antigens. Commercially available enzyme-linked immunosorbent assays (ELISAs) are accurate for detecting anti-LKM-1, at least in the context of AIH type 2, and reasonably accurate for the detection of AMA. Therefore, if any doubt remains after immunofluorescence examination, it can be resolved by using molecular-based immunoassays.

Other autoantibodies less commonly tested, but of diagnostic importance, include those to LC-1, antineutrophil cytoplasmic antibody (ANCA), and soluble liver antigen (SLA). Anti-LC-1, which may be present on its own but frequently occurs in association with anti-LKM-1, is an additional marker for AIH type 2 and targets formiminotransferase cyclodeaminase (FTCD). ANCA can also be positive in AIH. There are three types of ANCA – namely, cytoplasmic (cANCA), perinuclear (pANCA), and atypical perinuclear, the target of which is a peripheral nuclear and not cytoplasmic perinuclear antigen (hence the suggested term "pANNA," i.e., peripheral antinuclear neutrophil antibody). The type found in AIH type 1 is pANNA, which is also found in inflammatory bowel disease and sclerosing cholangitis, while it is virtually absent in type 2 AIH. Anti-SLA, which was originally described as the hallmark of a third type of AIH, is also found in some 50% of patients with type 1 and 2 AIH, in whom it defines a more severe course. Screening of cDNA expression libraries using high-titer anti-SLA serum has made it possible to identify the molecular target antigen as UGA tRNA suppressor-associated antigenic protein (tRNP(Ser)Sec).

After assessment of all the specific findings described above, there is still a small proportion of patients with AIH who do not have detectable autoantibodies. This condition, which responds to immunosuppression in the same way as the seropositive form, represents seronegative AIH, and its prevalence and clinical characteristics have yet to be defined.

Management and prognosis

AIH is exquisitely responsive to immunosuppression [1–3, 10]. The rapidity and degree of the response depends on the disease severity at presentation. All types of presentation, with the exception of fulminant hepatic failure with encephalopathy, respond to standard treatment with prednisolone with or without azathioprine.

Standard treatment for AIH consists of prednisolone 2 mg/kg/day (maximum 40–60 mg/day), which is gradually decreased over a period of 4–8 weeks in parallel to the decline of transaminase levels. Once normal liver function tests are obtained, which may take several weeks or even a few months, the patient is maintained on the minimal dosage that is capable of sustaining normal transaminase levels – usually 5 mg/day. During the first 6–8 weeks of treatment, liver function tests are checked weekly to allow frequent fine-tuning, avoiding severe steroid side effects. The initial goal is to obtain at least 80% reduction of the transaminase levels by 8 weeks of treatment. If progressive normalization of the liver function tests is not obtained over this period of time, or if too high a dose of prednisolone is required to maintain normal transaminases, azathioprine is added at a starting dose of 0.5 mg/kg/day, which – in the absence of signs of toxicity – is increased up to a maximum of 2.0–2.5 mg/kg/day until biochemical control is achieved. Azathioprine is not recommended as first-line treatment because of its hepatotoxicity in severely jaundiced patients, but 85% of the patients will eventually require the addition of azathioprine.

Children who present with acute hepatic failure pose a particularly difficult therapeutic problem. If not encephalopathic, they usually benefit from conventional immunosuppressive therapy, but only one of the six children with acute liver failure and encephalopathy in the King's College Hospital series responded to immunosuppression and survived without transplantation. Steroid therapy may predispose to sepsis and make encephalopathy worse.

Interestingly, it has been shown that neither thiopurine methyltransferase genotype nor activity predicts azathioprine hepatotoxicity in AIH, which appears instead to be related to the degree of liver fibrosis [11]. A preliminary report in a cohort of 30 children with AIH suggests that the measurements of the azathioprine metabolites 6-thioguanine and 6-methylmercaptopurine are useful in identifying drug toxicity and nonadherence and in achieving a level of 6-thioguanine considered therapeutic for inflammatory bowel disease [12] – although an ideal therapeutic level for AIH has not been determined. However, it has been reported that patients with AIH can achieve remission with azathioprine metabolites levels lower than those needed for inflammatory bowel disease [13]. Although an 80% decrease in the initial transaminase levels is obtained within 6 weeks of the start of treatment in most patients, complete normalization of liver function may take several months. In the King's College Hospital series, normalization of transaminase levels occurred at median of 6 months in children who were positive for ANA/SMA and 9 months in children who were positive for LKM-1. In pediatric care, apart from the transaminase levels, an important role in monitoring the response to treatment is the measurement of autoantibody titers and IgG levels, the fluctuation of which correlates with disease activity.

Withdrawal of treatment

Treatment should be continued for at least 3 years before considering its cessation, after which period stopping treatment can be attempted but only if liver function tests and IgG levels have been persistently normal, and autoantibodies are either undetectable or detectable at very low titer (ANA/SMA <1 : 10; anti-LKM-1 should be negative) over at least 12 months, and a liver biopsy shows no inflammatory changes. However, it is advisable not to attempt treatment withdrawal during or immediately before puberty, when relapses are more common, possibly due to non-adherence. In the King's College Hospital experience, successful long-term withdrawal of treatment was achieved in 20% of patients with AIH type 1, but in none with AIH type 2 [9].

Outcome

Progression to cirrhosis was more common in type 1 than in type 2 AIH. Overall, 74% of ANA/SMA-positive and 44% of anti-LKM-1-positive patients showed evidence of cirrhosis on initial or follow-up histological assessment, indicating that, apart from the higher tendency to present with acute liver failure, the severity of type 1 and 2 AIH is similar. A more severe disease and a higher tendency to relapse are associated with the possession of antibodies to SLA, which are present in about half of patients with AIH type 1 or 2 at diagnosis [3, 8].

Side effects of steroid treatment were mild, the only serious complication being psychosis during induction of remission in 4%, which resolved after withdrawal of prednisolone. All patients developed a transient increase in appetite and mild cushingoid features during the first few weeks of treatment. After 5 years of treatment, 56% of the patients maintained the baseline centile for height or went up across a centile line, 38% dropped across one centile line, and only 6% dropped across two centile lines. In addition, it has been shown that long-term daily treatment with prednisolone in children with autoimmune liver disease does not affect their expected final adult height relative to parental stature [3].

Sustained remission, achieved with prednisolone and azathioprine, has been maintained with azathioprine alone

in some patients with AIH type 1, akin to the experience in adults, but not in AIH type 2 [10]. Whether this is effective long term and whether it offers any benefit on possible side effects compared to low-dose prednisolone/azathioprine maintenance is unclear.

Relapse during treatment is common, occurring in about 40% of patients and requiring a temporary increase in the steroid dose. An important role in relapse is played by nonadherence, which is common, particularly in adolescents and young adults. The risk of relapse may also be higher if steroids are administered on an alternate-day schedule, which is often instituted because it may have a less negative effect on the child's growth. Small daily doses are more effective in maintaining disease control and minimize the need for high-dose steroid pulses during relapses (with the consequent more severe side effects).

Treatment can be safely continued during pregnancy. Although the experience is limited, there do not appear to be any adverse events for mother and baby [14]. In particular, no teratogenic effects have been described with azathioprine in humans, although for women who are concerned about its use, treatment with steroids alone can be considered.

Despite the efficacy of standard immunosuppressive treatment, severe hepatic decompensation may develop even after many years of apparently good biochemical control, leading to transplantation 10–15 years after diagnosis in 10% of the patients. Overall, in the King's College Hospital series, over 97% of patients treated with standard immunosuppression were alive between 0.3 and 19 years (median 5 years) after diagnosis, including 8% after liver transplantation.

Other immunosuppressive agents

Mycophenolate mofetil. Mycophenolate mofetil (MMF) is the prodrug of mycophenolic acid. Its effect on purine synthesis leads to decreased T- and B-lymphocyte proliferation. In patients (up to 10%) in whom standard immunosuppression is unable to induce stable remission, or who are intolerant to azathioprine, MMF at a dose of 20 mg/kg twice daily, together with prednisolone, is successfully used [10]. If there is a persistent absence of response or if there is intolerance for MMF (headache, diarrhea, nausea, dizziness, hair loss, and neutropenia), the use of calcineurin inhibitors (ciclosporin A or tacrolimus) should be considered.

Ciclosporin. Induction of remission has been obtained in 71% of treatment naïve children with AIH using ciclosporin A alone for 6 months, followed by maintenance with low-dose prednisone and azathioprine. A 5-year follow-up of this study shows that 94% of the patients eventually achieved remission, with minor side effects [10]. Whether this mode of induction has any advantage over the standard treatment has yet to be evaluated in controlled studies in specialized centers. However, as the side effects of ciclosporin – including renal impairment, gingival hyperplasia, and hirsutism – may cause more significant morbidity than those of prednisolone, it has not become established as a first-line treatment.

Ciclosporin is effective in patients with AIH type 1 and 2 who are resistant to prednisolone/azathioprine, and it is well tolerated [10].

Tacrolimus. Tacrolimus is a more potent immunosuppressive agent than ciclosporin, but it also has significant toxicity. There is limited evidence supporting its role in the treatment of AIH apart from anecdotal evidence, but it may be useful in combination with prednisolone as second-line therapy.

Budesonide. Budesonide has a hepatic first-pass clearance of >90% of oral dose and fewer side effects than prednisone/prednisolone, but cannot be used in the presence of cirrhosis, which affects at least two-thirds of AIH patients. The results among the children recruited into a large European study, where the efficacy of azathioprine plus budesonide at a dose of 3 mg three times daily, decreased upon response, was compared to that of azathioprine plus prednisone 40 mg once daily, reduced per protocol irrespective of response, were disappointing, with a similarly low remission rate of 16% for budesonide and 15% for prednisone after 6 months of treatment and of 50% and 42% respectively after 12 months of treatment, with similar steroid side effects in both groups, apart from higher frequency of weight gain in children on prednisone [15]. Large studies in a pediatric population are needed to establish whether budesonide has a role in the treatment of juvenile AIH [16]. Nevertheless, budesonide could be a valid alternative in selected non-cirrhotic patients who are at risk of adverse effects from steroids.

Indications for liver transplantation

Despite an apparent initial response to immunosuppression, gradual histological progression may occur over a period of years. Failure of medical treatment is more likely when established cirrhosis is present at diagnosis, or if there is a long history before the start of treatment.

Approximately 10–20% of children with AIH require liver transplantation for the following indications:

- Fulminant hepatic failure.
- Complications of cirrhosis.
- Failure of medical therapy.

AIH recurs in approximately 25%, and this needs to be included in pre-transplantation counseling, as does the need for lifelong steroid therapy.

Autoimmune hepatitis/sclerosing cholangitis overlap syndrome

Sclerosing cholangitis

Sclerosing cholangitis is a chronic inflammatory disorder that may affect both the intrahepatic and extrahepatic bile ducts and may lead to fibrosis. The diagnosis is based on typical

bile duct lesions being visualized on cholangiography. The increasing recognition of this disease in children – rising from five cases before 1987 to several hundred reported since – probably reflects the introduction of endoscopic and magnetic resonance cholangiography into pediatric practice, rather than an actual increase in the prevalence of the condition.

In childhood, sclerosing cholangitis may occur as an individual disease or may develop in association with a wide variety of disorders, including Langerhans cell histiocytosis, immunodeficiency, psoriasis, cystic fibrosis, and chronic inflammatory bowel disease. An overlap syndrome between AIH and sclerosing cholangitis has been reported both in adults and children, most of the reported cases of overlap having been originally diagnosed as AIH.

AIH/sclerosing cholangitis overlap syndrome (ASC) has the same prevalence as AIH type 1 in childhood. This has been shown in a prospective study conducted over a period of 16 years [17], in which all children with serological features (i.e., autoantibodies, high IgG levels) and histological features (i.e., interface hepatitis) of autoimmune liver disease underwent cholangiography at the time of presentation. Approximately 50% of these patients had alterations in the bile ducts characteristic of sclerosing cholangitis, although the changes were generally less advanced than those observed in adult primary sclerosing cholangitis (Figure 11.4). A quarter of the children with ASC, despite abnormal cholangiograms, had no histological features suggesting bile duct involvement, and the diagnosis of sclerosing cholangitis was only possible because of the cholangiographic studies. Currently in our center imaging of the biliary system by magnetic resonance cholangiopancreatography (MRCP) followed by endoscopic retrograde cholangiopancreatography (ERCP) if MRCP is not informative, as well as colonoscopy to investigate for possible inflammatory bowel disease, are part of the evaluation of all children with liver disease associated with autoimmune features.

The IAIHG scoring systems for the diagnosis of AIH, as currently formulated, do not allow distinguishing AIH from ASC, as they not include cholangiographic investigations at presentation.

Susceptibility to ASC in children is conferred by the possession of HLA *DRB1*1301*. The clinical, laboratory, and histological features of type 1 and 2 AIH and ASC are compared in Table 11.1.

Clinical features of ASC include:

- 50% of the patients are male.
- Abdominal pain, weight loss, and intermittent jaundice, resembling AIH type 1.
- Inflammatory bowel disease is present in about 45% of children with ASC, in comparison with about 20% of those with classical AIH.
- Virtually all patients are seropositive for ANA and/or SMA.
- 90% of children with ASC have greatly increased serum IgG levels.

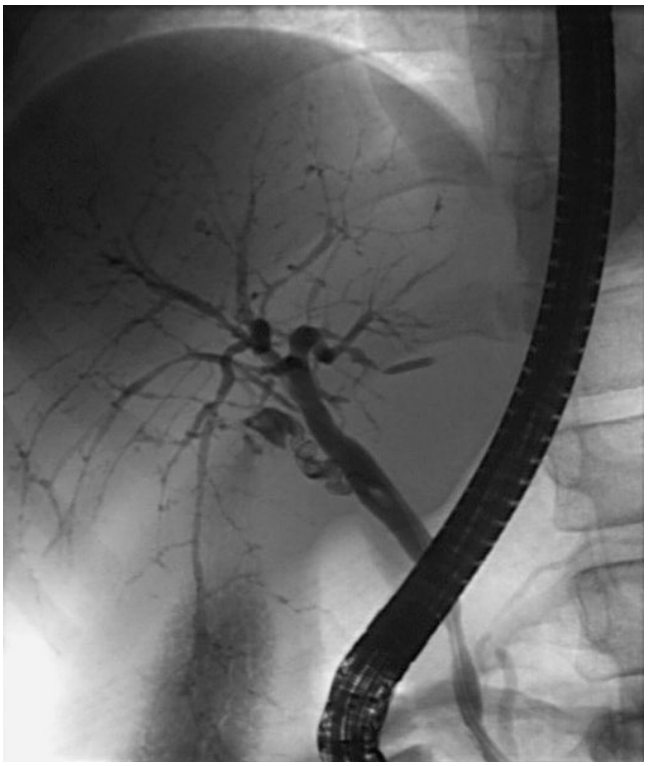

Figure 11.4 Endoscopic retrograde cholangiopancreatography (ERCP) of a child with autoimmune sclerosing cholangitis, demonstrating cholangiopathy with strictures and dilations affecting the intrahepatic and extrahepatic bile ducts.

Table 11.2 Autoimmune hepatitis (AIH) and autoimmune sclerosing cholangitis (ASC): liver function tests at presentation [9, 17].

	AIH (n = 28)	ASC (n = 27)
Bilirubin (normal <20 µmol/L)	35 (4–306)	20 (4–179)
AST (normal <50 IU/L)	333 (24–4830)	102 (18–1215)
GGT (normal <50 IU/L)	76 (29–383)	129 (13–948)
AP (normal <350 IU/L)	356 (131–878)	303 (104–1710)
AP/AST ratio	1.14 (0.05–14.75)	3.96 (0.20–14.20)
Albumin (normal >35 g/L)	35 (25–47)	39 (27–54)
INR (<1.2)	1.2 (0.96–2.5)	1.1 (0.9–1.6)

Results are presented as medians (range).
AP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; INR, international normalized ratio (prothrombin).

Standard liver function tests do not help in discriminating between AIH and ASC (Table 11.2).

- pANCA is present in 75% of patients with ASC, in comparison with 45% of patients with AIH type 1 and 10% of those with AIH type 2.

Laboratory investigation

Elevated alkaline phosphatase and GGT may be the most consistent biochemical abnormalities, except in early disease.

- *Alkaline phosphatase.* Although occasionally normal at presentation, it subsequently becomes elevated during the course of the disease.

- *GGT*. This is elevated usually when the diagnosis is made late.
- *Bilirubin*. The bilirubin level in serum may be normal at presentation in at least 50% of cases, contributing to the diagnostic delay. It may be intermittently elevated during the course of disease, with persistent elevation being associated with a poorer prognosis.
- *Hepatic transaminases*. These are moderately elevated in the majority of cases, and may be raised to 50 times the upper limit of normal.
- *Prothrombin time and albumin*. Hepatic synthetic function is usually preserved unless decompensation has occurred following progression to cirrhosis. An elevated prothrombin time may occur due to fat-soluble vitamin deficiency and may therefore be responsive to vitamin K.

Diagnostic imaging

Ultrasound. Ultrasonography may reveal intrahepatic and extrahepatic bile duct dilation, a heterogeneous or nodular echotexture characteristic of cirrhosis, or the manifestations of portal hypertension including splenomegaly, ascites, and varices. However, the appearance may be normal in up to 50% of cases, particularly in early cases.

Cholangiography. The diagnosis of sclerosing cholangitis is confirmed by cholangiography (see Figure 11.4), which reveals lesions typical of:

- Irregular intrahepatic ducts.
- Focal saccular dilation.
- Intervening short annular strictures (producing a beaded appearance).
- An abnormally large gallbladder.
- Increased diameter of the common bile duct.
- Extrahepatic ductal irregularity.

Cholangiography can be carried out either with ERCP, a technique associated with a risk of pancreatitis, or preferably, in the absence of adequate ERCP expertise, with MRCP, a noninvasive method of diagnosis. Experience with MRCP in children is increasing (Figure 11.5), although ERCP remains more accurate than MRI, particularly in early disease.

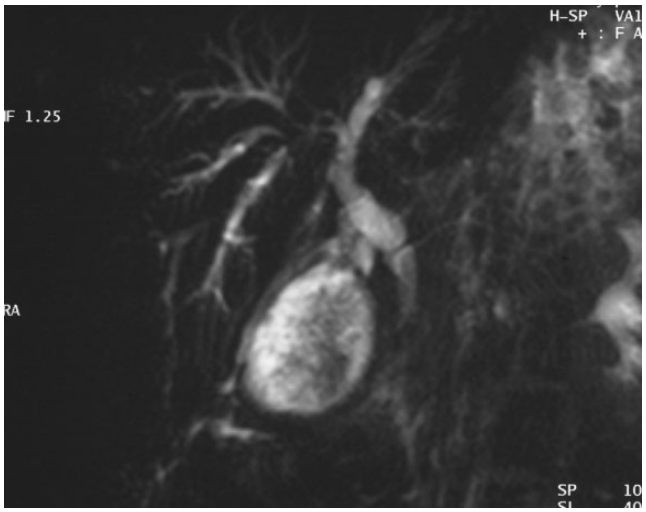

Figure 11.5 Magnetic resonance imaging is less invasive than endoscopic retrograde cholangiopancreatography and demonstrates the characteristic features of an enlarged gallbladder, with irregularity of the intrahepatic ducts due to septal dilation and short strictures.

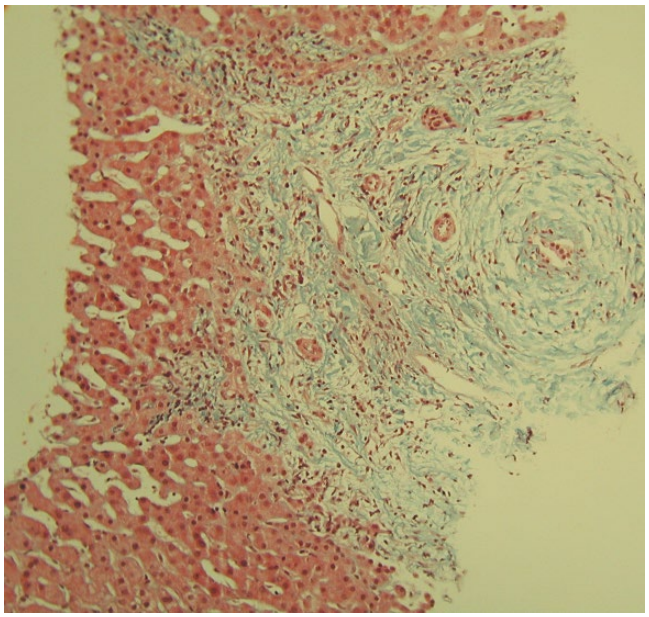

Figure 11.6 Liver histology in autoimmune sclerosing cholangitis may demonstrate the characteristic "onion-skin" appearance of the bile ducts secondary to fibrosis, in association with an inflammatory infiltrate, usually at an advanced stage of the disease.

Histology

The pathognomonic feature of sclerosing cholangitis – i.e., fibrous obliterative cholangitis with periductular fibrosis – is rarely seen in early cases, in which the patients mostly show inflammatory changes similar to those in AIH (Figure 11.6).

Treatment and prognosis

Children with ASC respond to the same immunosuppressive treatment described above for AIH. Liver test abnormalities resolve in most patients within a few months after treatment has been started. However, although steroids and azathioprine are beneficial in abating the parenchymal inflammatory lesion, they appear to be less effective in controlling the bile duct disease. Following favorable reports in adult primary sclerosing cholangitis, ursodeoxycholic acid is usually added to the treatment of ASC, but whether it is helpful in arresting the progression of bile duct disease remains to be established. In adults with primary sclerosing cholangitis high-dose UDCA was reported as more beneficial than standard doses, but a randomized double-blind controlled study from the Mayo Clinic shows that high-dose UDCA has a negative effect [18]. It is prudent, therefore, to use doses not higher than 15–20 mg/kg/day. Fat-soluble vitamin supplements are required if cholestasis develops. As in AIH, measurement of autoantibody

titers and IgG levels is useful in monitoring disease activity and the response to treatment [3]. The medium- to long-term prognosis of ASC is worse than that of AIH because of progression of bile duct disease despite treatment in some 50% of patients, with 20% of them eventually requiring liver transplantation. Reactivation of the liver disease often follows flares of the intestinal disease in sclerosing cholangitis patients with inflammatory bowel disease. It is therefore essential to control the bowel pathology to avoid progression of liver disease. A beneficial effect of oral vancomycin (500 mg t.d.s.) has been reported in 14 patients with sclerosing cholangitis and inflammatory bowel disease [19]. All patients showed improvement of liver function tests and erythrocyte sedimentation rate, which was more marked in those without cirrhosis. These results await confirmation in a larger number of patients. Whether vancomycin acts through its antibiotic or immunomodulatory properties remains to be elucidated.

Evolution from AIH to ASC has been documented, suggesting that AIH and ASC may be part of the same pathogenic process [17].

Recurrence of autoimmune liver disease after transplantation

Recurrence of AIH after liver transplantation has been reported in several studies [20]. The diagnosis is based on the reappearance of clinical symptoms and signs, histological features of periportal hepatitis, elevation of transaminases, circulating autoantibodies, and elevated IgG, associated with a response to steroids and azathioprine. Possession of the HLA DR3 allele appears to confer a predisposition to disease recurrence, as it does to the original AIH, although this has not been universally confirmed. Recurrence has been noted in both adult and pediatric series, and although the rate of this complication increases with the post-transplantation interval, it may appear as early as 1 month after surgery. Most transplant recipients with recurrent AIH respond to an increase in the dosage of corticosteroids and azathioprine, but in a few cases, recurrence can lead to graft failure and to a need for retransplantation. Caution should be exercised in weaning immunosuppression in patients who undergo transplantation for AIH, since discontinuation of corticosteroid therapy may increase the risk for recurrent disease.

ASC recurs after liver transplantation more frequently and with more severe consequences than AIH [20]. While recurrence of AIH does not usually affect post-transplant outcome, recurrence of ASC leads to re-transplantation in a high proportion of patients. Recurrence of sclerosing cholangitis after transplantation is often associated to uncontrolled inflammatory bowel disease. In this context it is of interest that primary sclerosing cholangitis recurrence in adults with inflammatory bowel disease can be prevented by pre-liver transplant colectomy.

De novo autoimmune hepatitis after transplantation

Tissue autoantibodies after liver transplantation – particularly ANA and SMA – are also common in patients who undergo transplantation for non-autoimmune liver disease [20]. Anti-LKM-1 is the third most frequently reported antibody, but its fluorescence pattern is at times atypical, staining the renal tubules preferentially and sparing the liver. The reported prevalence of post-liver transplantation autoantibodies is variable – probably reflecting the different techniques used for detecting them, the cut-off point above which the autoantibodies are considered positive, the time after transplantation at which they are tested, the nature of the clinical condition leading to transplantation, and the presence or absence of post-transplantation complications. In the late 1990s, it was observed that AIH can arise de novo after liver transplantation in children who had not undergone transplantation for autoimmune liver disease [20]. After the original report, de novo AIH after liver transplantation has been confirmed by several studies, both in adult and pediatric patients. Importantly, treatment with prednisolone and azathioprine, using the same schedule for classical AIH, is also effective in de novo AIH, leading to excellent graft and patient survival. It is of interest that these patients do not respond satisfactorily to standard antirejection treatment, making it essential to reach an early diagnosis in order to avoid graft loss. Sirolimus has been reported to be effective in patients unresponsive to standard AIH treatment. In patients who undergo transplantation during childhood, progressive liver damage over a 10-year follow-up period is often associated with serological features of autoimmunity and a histological picture of chronic hepatitis (see Chapter 31).

The recurrence of AIH after transplantation can be readily explained. The recipient's immune system is sensitized to species-specific antigens and has a pool of memory cells, which are re-stimulated and re-expanded when the target antigens, "autoantigens," are presented to the recipient's immune system either by the recipient's APC re-populating the grafted liver, or by the donor's APC sharing histocompatibility antigens with the recipient. In contrast, akin to autoimmune liver disease outside the context of transplantation, the pathogenesis of post-transplantation de novo AIH remains to be defined. There are several possible explanations, which are not mutually exclusive. In addition to the release of autoantigens from damaged tissue, one possible mechanism is molecular mimicry, in which exposure to viruses that share amino acid sequences with autoantigens leads to cross-reactive immunity. Viral infections, which are frequent after transplantation, may also lead to autoimmunity through other mechanisms, including polyclonal stimulation, enhancement and induction of membrane expression of MHC class I and II antigens, and/or interference with immunoregulatory cells. Another possible mechanism has been suggested by animal experiments showing that the use

of calcineurin inhibitors predisposes to autoimmunity and autoimmune disease, possibly by interfering with the maturation of T lymphocytes or with the function of regulatory T cells, with the consequent emergence and activation of autoaggressive T-cell clones. Another proposed mechanism stems from observation that patients with de novo AIH often have an antibody directed to glutathione-*S*-transferase T1 (GSTT1) [20]. Since the gene encoding this protein is defective in a fifth of white individuals and the encoded enzyme was absent in patients experiencing de novo AIH, the authors speculated that graft dysfunction resulted from the recognition as foreign of GSTT1 acquired with the graft. However, we have been unable to confirm this observation, having investigated reactivity against GSTT1 sequentially on 60 occasions in 20 patients with post-transplantation de novo AIH.

It has been demonstrated in murine models of heart allograft that allogeneic transplantation of a solid organ can lead to the development of autoimmunity; heart transplantation from an allogeneic donor resulted not only in signs of rejection, but also in the production of antibodies and $CD4^+$ T cells directed against cardiac myosin in the recipient. The relative importance of autoantigenic and allogeneic stimuli in the development of de novo AIH after liver transplantation remains to be elucidated [20].

Conclusion

Autoimmunity is an important cause of liver disease in childhood. The prognosis with immunosuppression treatment is excellent, with symptom-free long-term survival in the majority of patients. However, a failure to diagnose and promptly treat these conditions can have severe consequences, including cirrhosis, end-stage liver disease, transplantation, or death. During the past 35 years, several pathogenic aspects of liver autoimmunity have been elucidated, including predisposing genetic factors and disease-specific humoral and cellular immune responses. Research tasks for the future include further elucidation of the pathogenesis and the establishment of novel treatments aimed at specifically arresting liver autoaggression or, ideally, at reinstating tolerance to liver antigens.

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CHAPTER 12

Drug-Induced Liver Disease

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Key points

- Drug-induced liver injury (DILI) is the most common cause of identifiable acute liver failure in children between the ages of 3 and 18 years old.
- DILI may be associated with a wide spectrum of severity of liver injury and can resemble all other forms of liver disease.
- The diagnosis of DILI requires a high degree of suspicion on the part of the clinician as in most cases the primary treatment is immediate withdrawal of the offending agent.
- Children with DILI with hypersensitivity features have improved survival compared to those who do not present with fever, eosinophilia, or rash.
- Survival without liver transplantation is better in children with paracetamol-induced acute liver failure compared to children with acute liver failure caused by other medications.

In modern society, exposure to synthesized pharmacological agents or herbal medications in the treatment of disease is widespread. In addition, children and young people use recreational drugs for pleasure or as self-prescribed remedies. For most medications, the benefits outweigh the risks of toxicity. Nevertheless, many of these "safe" compounds are potentially hepatotoxic, and awareness of this allows early recognition and prevention of severe toxicity. Some drugs have a dose-dependent toxicity, whereas others cause hepatotoxicity with an idiosyncratic pattern (Table 12.1). With medications that have dose-dependent toxicity, such as paracetamol and minocycline, the toxicity becomes apparent after an overdose or prolonged exposure. With paracetamol, both acute overdose and accumulated toxicity with chronic use result in hepatotoxicity. Minocycline can cause both an acute reaction and an autoimmune reaction, which becomes apparent months to years after the exposure. Medications that have an idiosyncratic pattern exhibit a less predictable pattern of toxicity. In either case, the diagnosis of drug-induced liver injury (DILI) may be complicated by the disease state for which the medication is being used, and consequently a high index of suspicion is required for accurate diagnosis.

Role of the liver in drug metabolism

The liver plays a crucial role in the metabolism of virtually all drugs (see Chapter 1). Most drugs are lipophilic and thus to be detoxified and excreted in bile or filtered by the renal glomerulus, they must be rendered hydrophilic. In a healthy child, drug metabolism includes an initial "phase I" reaction where oxidation, reduction, and hydrolysis alter the structure of the drug molecule; oxidation is frequently reliant on cytochrome P450 isoenzymes located in the smooth endoplasmic reticulum of the liver. In "phase II" reactions, the initially metabolized drug molecules are conjugated, often to more water-soluble compounds for excretion. In addition, other enzymes such as alcohol dehydrogenase, are required for the metabolism of certain drugs. Electrophilic intermediate metabolites generated during these reactions are potentially harmful and binding of glutathione, catalyzed by glutathione-*S*-transferase, provides a mechanism for detoxification of these reactive species.

Diseases of the Liver and Biliary System in Children, Fourth Edition. Edited by Deirdre A. Kelly.

Table 12.1 Exposure pattern of hepatotoxicity.

Dose dependent	Idiosyncratic
Paracetamol	Antiepileptic medications
Isoniazid	Antibiotics other than those listed on the left
Tetracyclines	
Oxypenicillins	
Ureidopenicillins	

Mechanisms of drug-induced liver injury

DILI is caused by two distinct mechanisms: direct hepatotoxicity and adverse drug reaction. The distinction lies in the predictability of the toxic effect:

- Direct hepatotoxicity arises from the administration of a drug with intrinsic toxicity to the liver and is usually dose dependent (e.g., paracetamol, aspirin).
- Adverse drug reactions comprise the majority of cases of DILI. An adverse drug reaction is unpredictable and idiosyncratic and may occur despite recommended treatment regimens being prescribed. This type of reaction occurs with a low frequency in the population exposed to the drug, is variable in presentation, is dose independent, and may result in diverse pathology. Host factors may be of particular importance (see later).

Pathogenesis

DILI most commonly arises in the hepatocyte, due to its central role in drug metabolism – although biliary cells, Ito cells, and sinusoidal endothelium may also be targets. A drug itself or an intermediate metabolite may:

- Impair cell structure.
- Inhibit enzyme activity.
- Overwhelm glutathione cytoprotection.
- Form dysfunctional protein adducts (drug binding to protein), or adducts that promote injurious immunological responses [1].
- Impair bile transport leading to biliary epithelium, canalicular, and hepatocyte injury [1].
- Evoke a type IV systemic hypersensitivity reaction.

These mechanisms may occur in combination and for many drugs, the precise mechanism remains unknown. The outcome for a given agent may be largely determined by host factors.

Epidemiology

DILI may be regarded as the end result of an interaction between a pharmacologically active compound after host metabolism, in the presence of environmental influences. Consideration of factors that can affect drug metabolism is crucial if inappropriate prescribing and the risk of DILI are to be minimized.

In a prospective study of children, 50% of cases of DILI were attributed to the use of antimicrobials of which minocycline was the most common drug (prescribed for acne in 13% of patients) [1]. In this same study of DILI, additional implicated medications included: 20% antiepileptic drugs, 13% medications for attention deficit hyperactivity disorder (ADHD), 7% antidepressants, and 3% herbal medications [1].

Host factors affecting enzyme activity

Age

When comparing neonates, infants, young children, and adolescents, differences exist in the proportion of drug bound to plasma proteins (albumin, α1-acid glycoprotein and lipoproteins), total body water affecting the distribution of water-soluble drugs and cytochrome P450 isoenzyme activity, which does not reach adult levels until around 10 years of age. Additionally, differences may be explained by variation in drug receptor number and affinity, drug concentration, and the proportion of drug metabolites to the parent compound. There is an increased risk of adverse drug reactions with age, which may reflect polypharmacy or an increased likelihood of concurrent disease. However, younger age is a risk factor for toxicity from some drugs such as valproic acid, which is particularly prone to hepatotoxicity in children less than 2 years old.

Both the Pediatric Exclusivity Provision of the Best Pharmaceuticals for Children Act as well as the Pediatric Research Equity Act continue to encourage drug research in children, further recognizing the unique differences in drug metabolism between adult and pediatric populations.

Gender

For unknown reasons, many forms of DILI – especially acute and chronic hepatitis – are more common in women.

Concomitant drug therapy

Enzyme activity, especially of cytochrome P450, may be enhanced or inhibited by other drugs. Induction (e.g., by ethanol, phenobarbital, phenytoin, carbamazepine, rifampicin, isoniazid (INH), omeprazole) may increase the rate of generation of toxic metabolites, increasing the susceptibility to DILI. Individuals with a history of an adverse drug reaction are also more likely to experience a reaction to another agent.

Genetic factors

The cytochrome P450 system is composed of a family of almost 300 genes that code for P450 enzymes. These genes are distributed among several chromosomes and the encoded enzymes are thus susceptible to considerable polymorphism. Genetic variation in these enzymes influences drug metabolism and may contribute to DILI, either by excess toxic metabolite production or deficient precursor metabolism.

Table 12.2 Clinical category and timing after exposure of drug toxicity.

Drug	Hepatitis	Jaundice	Hypersensitivity
Paracetamol	1–2 days	3–5 days	
Halothane	7–13 days	10–28 days	7–13 days
Isoniazid	<2 months (50%) 2–14 months (50%)	2–15 months	
Rifampicin		Weeks	
Amoxicillin–clavulanic acid		Days–2 months	
Flucloxacillin		2–5 weeks	
Tetracycline	4–6 days		
Minocycline	Months		Days–weeks
Erythromycin	1–3 weeks		
Trimethoprim–sulfamethoxazole	2–3 weeks	2–3 weeks	2–3 weeks
Ketoconazole	2–3 weeks		
Carbamazepine	2–3 weeks	2–3 weeks	Weeks
Phenytoin	1–3 weeks	1–3 weeks	Weeks

Table 12.3 General histological categories of drug-induced hepatotoxicity.

Hepatocellular inflammation	Cholestasis	Vanishing bile duct syndrome
Paracetamol	Amoxicillin–clavulanic acid	Flucloxacillin
Halothane	Erythromycin	Tetracycline
Isoniazid	Sulfonamides	Ampicillin/amoxicillin
Cephalosporins	Ketoconazole	Amoxicillin–clavulanic acid
Tetracyclines (steatosis)	Carbamazepine	
Erythromycin		
Ketoconazole		
Carbamazepine		
Phenytoin		
Cannabis		
Amphetamines		

Other hepatic enzymes are also polymorphic. For example, genetic variation in *N*-acetyltransferase-2 activity is expressed phenotypically as a slow or fast acetylator and is relevant to INH toxicity.

Host factors affecting glutathione cytoprotection

Alcohol and starvation may lead to glutathione depletion and thus diminished cytoprotection, especially relevant in paracetamol toxicity. Drugs that induce enzyme activity may also lead to glutathione depletion, because of the increased generation of metabolites requiring conjugation.

Clinicopathological spectrum of drug-induced liver injury

DILI may be associated with a wide spectrum of severity of liver injury and can resemble all other forms of liver disease. There is considerable overlap in the type of disease produced and many drugs may produce more than one syndrome. A particular feature of DILI is significant biochemical and histological abnormality occurring with few clinical symptoms (Table 12.2). As such, early symptoms are non-specific, although fever, rash, and eosinophilia may be present in hypersensitivity reactions.

The histological features of drug-induced hepatotoxicity are frequently non-specific, but the patients can be categorized into those with: primarily acute inflammatory hepatocellular injury, prominent cholestasis, or vanishing bile duct syndrome (Table 12.3). The predominant clinicopathological manifestations of DILI are outlined below.

Enzyme induction without disease

Induction of hepatic enzymes may be of no clinical significance and not associated with hepatic disease or dysfunction, for example, γ-glutamyltransferase (GGT) induction by phenytoin/phenobarbital.

Acute hepatitis/hepatocellular necrosis/acute liver failure

This is the most common manifestation of DILI. The clinical presentation may be similar to those in:

- Acute viral hepatitis with fever, anorexia, nausea, or vomiting, followed by right upper quadrant tenderness with variable jaundice.

- Allergic hepatitis, presenting with fever, rash, eosinophilia, and lymphadenopathy, which may resemble infectious mononucleosis. Drug rash with eosinophilia and systemic symptoms (DRESS) is a term applied when the clinical presentation is that of a cutaneous eruption, fever, multiple peripheral ganglions, and potentially life-threatening multiorgan failure.
- Acute liver failure (ALF; Figures 12.1 and 12.2).

DILI is the most common cause of identifiable acute liver failure (ALF) in children between the ages of 3 and 18 years old and represents approximately 20% of pediatric cases of ALF [2]. Of the initial 348 children enrolled in the Pediatric Acute Liver Failure (PALF) study group, a clinical research network established by the National Institutes of Health in the US (including 21 sites in the US, one in Canada, and two in the UK), 5% of cases of ALF were due to an identifiable drug other than paracetamol, 14% to paracetamol, and 48% were indeterminate; some of the latter may have been drug related [3]. In a study of children in India, antituberculous medications were the most common cause of drug-induced ALF, leading to death in 83.3% of these patients [4]. Of children with ALF requiring liver transplantation in the US, 29% had DILI secondary to paracetamol, 23% from antiepileptic medications (of which 73% were taking valproic acid), 9% from antituberculosis drugs, 6% from propylthiouracil, 3% from antineoplastics, 2% from antibiotics, and 9% from other drugs including disulfiram, halothane, iron, pemoline, isoflurane, and vitamin A [2]. The prognosis in the drug-induced ALF population depends on the cause. Survival without liver transplantation following paracetamol poisoning has been reported as 94%, but only 41% in children who developed ALF due to other drugs [3].

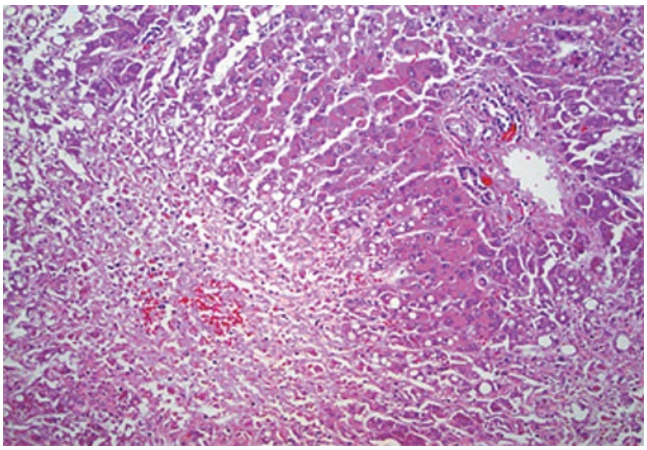


Figure 12.1 Paracetamol-induced liver disease, showing pericentrivenular necrosis. (H&E, original magnification × 200.)

Histological changes in acute hepatitis reflect hepatocellular damage with degeneration and necrosis, accompanied by an inflammatory cell infiltrate, with a variable degree of acute hepatic necrosis (Figures 12.1–12.3). Extensive necrosis may be seen with drugs that cause fulminant liver failure, but are not useful in differentiating between drug-induced or other causes of liver injury.

Cholestasis

Acute cholestasis manifests with a rapid onset of jaundice and pruritus. Without hepatitis, recovery is usually rapid and complete, but with hepatitis there may be a more prolonged course. Histological features include inspissated bile within

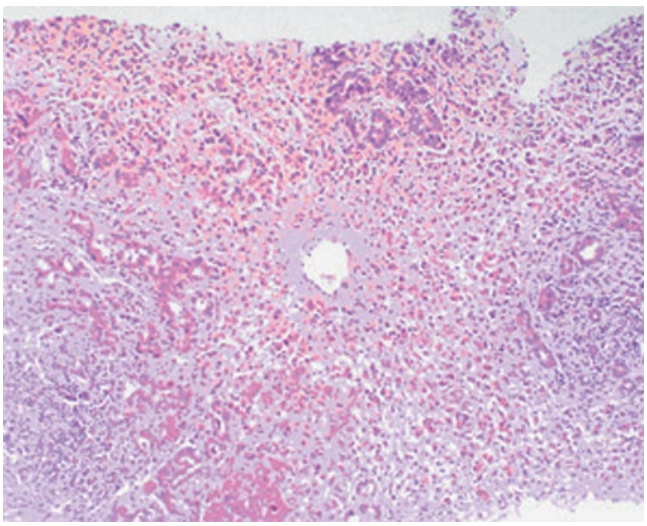


Figure 12.2 Acute liver failure secondary to halothane exposure. Postnecrotic confluent cell loss with inflammation, including pigmented macrophages, periportal ductular reaction, and only a few islands of surviving hepatocytes. (Courtesy of Professor Bernard Portmann, Institute of Liver Studies, King's College Hospital, London, UK.)

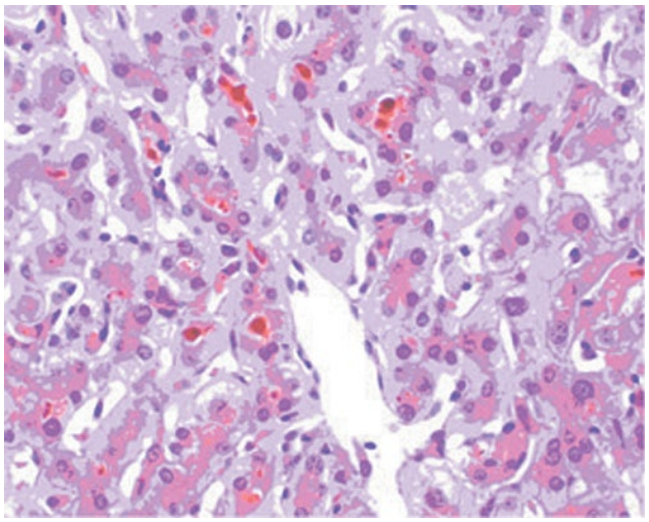


Figure 12.3 Minocycline-induced hepatitis. Portal inflammation with mononucleated cells, plasma cells, and eosinophils spilling over the parenchymal limiting plates, resembling an autoimmune hepatitis. (Courtesy of Professor Bernard Portmann, Institute of Liver Studies, King's College Hospital, London, UK.)

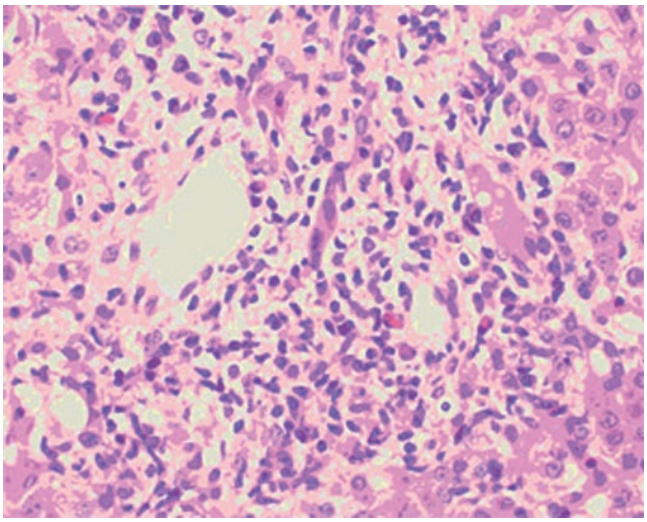


Figure 12.4 Clavulanic acid related cholestasis. Liver biopsy specimen, showing severe canalicular bile plugging with only minimal necroinflammatory activity. (Courtesy of Professor Bernard Portmann, Institute of Liver Studies, King's College Hospital, London, UK.)

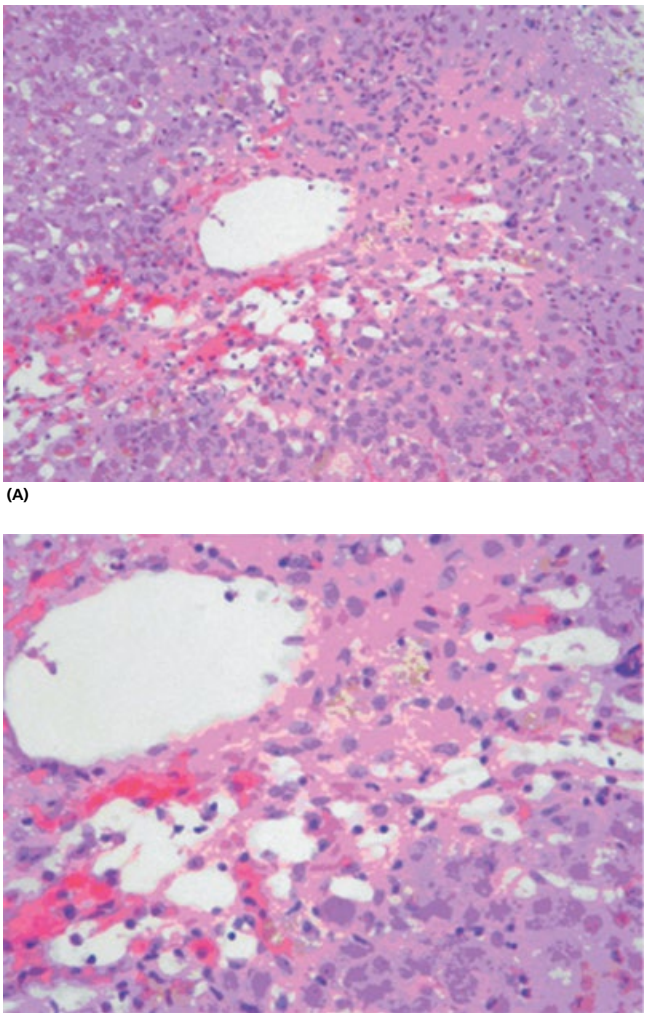
the canaliculi and bile pigment in hepatocytes and Kupffer cells. Significant bile duct injury may occur and lead to more severe and chronic symptoms and resemble sclerosing cholangitis or vanishing bile duct syndrome. Cholestasis may be accompanied by mild or moderate hepatocellular necrosis (Figure 12.4).

Granulomatous hepatitis

Granulomatous infiltrates are typically non-necrotizing either within the parenchyma or in periportal regions. On histology, the presence of eosinophils and granulomas are associated with immunoallergic reactions. A wide variety of drugs may be responsible, although the most frequent cause is carbamazepine (Figures 12.5 and 12.6). Clinical features include fever and malaise. The associated degree of hepatitis and cholestasis is variable.

Drug-induced chronic hepatitis

Chronic hepatitis is one of the least common forms of DILI, but is important as prevention is possible. Examples of responsible agents include methyldopa, nitrofurantoin, minocycline, and chronic paracetamol ingestion. Drug-induced chronic hepatitis, e.g., from lamotrigine, may resemble autoimmune hepatitis, with either an acute hepatitic onset or a more insidious onset of fatigue, lethargy, anorexia, weight loss, and hepatic discomfort. Jaundice and pruritus are unusual at presentation. A prolonged course is usually due to continued ingestion, as there is rarely perpetuation of the hepatic injury after withdrawal of the drug. Signs of liver disease are more likely than in acute hepatitis.



(A)

(B)

Figure 12.5 Carbamazepine-induced cholestatic liver disease, with perivenular necroinflammation and cholestasis shown. (H&E, original magnification: A, × 200; B, × 400.)

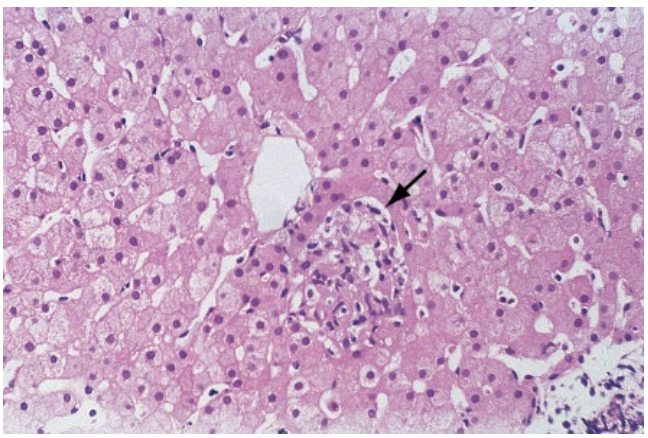


Figure 12.6 Carbamazepine may also cause granulomatous hepatitis or acute liver failure.

Vascular disorders

Vascular injury, specifically sinusoidal obstruction syndrome, is most often associated with chemotherapy and immunomodulatory drugs (see Chapter 22). These range from sinusoidal dilation (with estrogens) to sinusoidal obstruction syndrome (with cytotoxic chemotherapy, e.g., thioguanine). Anabolic steroids and cytotoxic chemotherapy have been implicated in peliosis hepatitis, in which hepatic sinusoids are destroyed forming blood-filled lakes or lacunae within the liver, which may rupture spontaneously.

Hepatic tumors

These are the least common form of DILI, accounting for <1%, with oral contraceptive steroids being the most commonly implicated agent.

Diagnosis of suspected drug-induced liver injury

Clinical history

DILI is a diagnosis of exclusion and should be suspected when introduction of a drug agent correlates with clinical or laboratory features of hepatotoxicity for which there is no better explanation. It is important to note that signs or symptoms of hepatotoxicity may not present until after a medication has already been discontinued, for example after short courses of antibiotics; therefore a detailed medication history is required.

An accurate history should be obtained, including:

- All drugs ingested at minimum in the 3 months before symptoms, including those bought over the counter and accidentally ingested.
- Total dosage given and previous courses:
  - timing of symptoms in relation to drug administration
  - symptomatology: presence of fever, rash, etc.
- Risk factors: previous drug reaction, family history, alcohol intake, concomitant drug therapy, underlying liver disease.
- Exclusion of other causes of the symptoms and biochemical/histological pattern: risk factors for other diseases should be considered (e.g., hepatitis A/B, autoimmune disease, etc.).

Clinical symptoms and findings may include abdominal pain, nausea, jaundice, pruritus and dark urine, but these are not required for the diagnosis of DILI [1, 5]. In a prospective study of DILI that excluded paracetamol toxicity, clinical signs and symptoms of hepatotoxicity occurred on average 140 days (median 32 days) after drug initiation, while laboratory findings consistent with hepatotoxicity were found on average 196 days (median 45 days) after drug initiation [1]. Minocycline and atomoxetine were notable outliers in this study with a median of 569 and 510 days, respectively [1].

Significant improvement after withdrawal of the suspected inciting drug suggests causality. Intentional re-exposure with the proposed offending drug is not typically recommended in children and is contraindicated in those with hypersensitivity features. A validated scoring system to assess the probability and causality of DILI is not currently available for children [6].

Laboratory investigation

The diagnosis of DILI is typically based on circumstantial evidence and exclusion of other causes. Supportive laboratory findings of drug-induced hepatotoxicity have been defined as:

- Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >5 times the upper limit of normal (or >5 times the pre-drug patient baseline); or alkaline phosphatase >2 times the upper limit of normal (or >2 times the pre-drug patient baseline) on two consecutive occasions.
- Serum bilirubin >2.5 mg/dL plus any elevation of AST, ALT, or alkaline phosphatase.
- International normalized ratio (INR) >1.5 with any elevation of AST, ALT, or alkaline phosphatase [1].
- Peripheral eosinophilia on blood film.

In a prospective study, 64% of children with DILI had positive autoantibodies: 46% were for positive for antinuclear antibody and 42% positive for antismooth muscle antibody (23% were positive for both) [1]. Medications associated with positive autoantibodies in decreasing order included: minocycline, atomoxetine, lamotrigine, azithromycin, and amoxicillin.

Histology

Liver biopsy is of value unless rapid resolution of liver function occurs following drug withdrawal. Liver biopsy may also be of value when there are multiple drugs being considered as the cause of liver injury. Histological assessment makes it possible to establish the severity of the condition, as well as supporting causation and excluding differential diagnoses such as autoimmune causes or viral infections (Figures 12.1–12.9).

Histological features are often non-specific and resemble those of autoimmune hepatitis. There may be eosinophilic portal and lobular inflammatory infiltrates, with centrizonal cholestasis and mild hepatocellular necrosis. Overall, no histology finding is pathognomonic for DILI and must be interpreted within the clinical context. The severity of the histological changes is the most important factor predicting the outcome (Table 12.3).

Cholestasis

A cholestatic hepatitis is typical of carbamazepine or oxcarbazepine, while vanishing bile duct syndrome has been associated with tetracycline and doxycycline.

Granulomatous hepatitis

A granulomatous hepatitis is found with a number of medications such as sulfonamides, aspirin, and carbamazepine and suggests a drug reaction depending on the clinical setting.

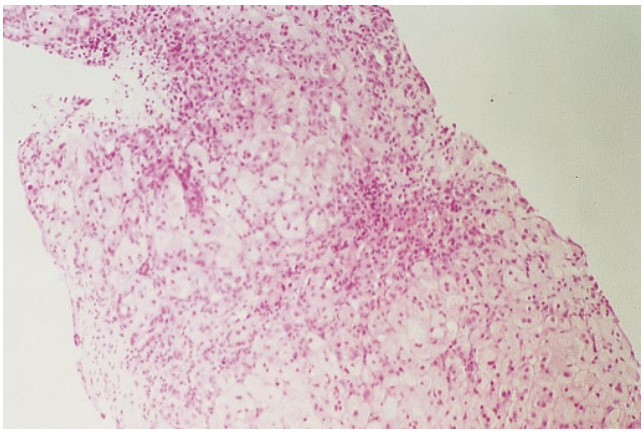


Figure 12.7 Sodium valproate and tetracycline both cause fatty liver with microvesicular fat in the hepatocytes, which is associated with hepatocellular dysfunction and may progress to acute liver failure.

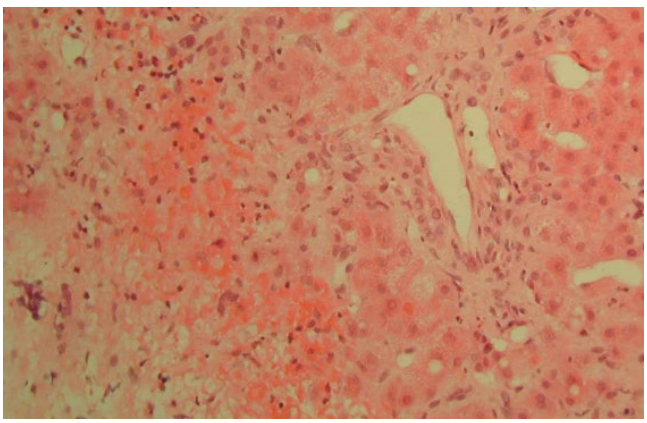


Figure 12.8 Actinomycin and other forms of chemotherapy for malignancy may cause hepatocellular necrosis and acute liver failure.

Fatty liver

Microvesicular fat within hepatocytes is associated with cellular dysfunction and is typical of Reye syndrome, valproic acid and tetracycline hepatotoxicity. Macrovesicular fat may be seen with amiodarone and may lead to rapid progression and cirrhosis (see Figure 12.7).

Fibrosis

Progressive fibrosis without clinical manifestations may occur, for example, due to methotrexate, vitamin A, and actinomycin D (see Figure 12.8).

Necrosis

Extensive necrosis may be seen with drugs that cause fulminant liver failure, but are not useful in differentiating between drug-induced or other causes of liver injury.

Treatment and outcomes

The primary treatment for DILI is withdrawal of the offending agent. The expectation is that liver injury should improve days to weeks after the cessation of the drug, however patients who develop chronic liver disease or ALF might show minimal or no improvement [5]. In a prospective study of children with DILI, ALT normalized on average 119 days after initial injury (median 79 days) and bilirubin normalized on average after 44 days (median 34 days) [1]. If no biochemical or clinical improvement is seen after withdrawal, the diagnosis of DILI should be reconsidered.

Children with DILI with hypersensitivity features have excellent survival with no deaths in one study compared to a 31% mortality rate in patients without hypersensitivity features in an area where liver transplantation was not available [4]. Better survival in children with hypersensitivity features may be secondary to earlier identification and withdrawal of the offending medication as laboratory findings of hepatotoxicity lag behind skin findings, lymphadenopathy, fever, and eosinophilia in these patients [4].

Steroids may be considered in children with severe hepatitis with hypersensitivity features, particularly if no improvement is noted 4 days after withdrawal of the inciting agent [5, 6]. Consensus guidelines in adults currently recommend 1 mg/kg/day of oral or intravenous steroids for DRESS for life-threatening cases with significant systemic involvement, though similar pediatric guidelines are not available. Steroids may also be indicated for drug-induced autoimmune hepatitis, most classically secondary to minocycline. Ursodeoxycholic acid may also be considered in cases with prolonged drug-induced cholestasis, and may improve hepatic laboratory abnormalities, jaundice, and pruritus in up to two-thirds of patients [5, 6].

Identifying the inciting drug agent in DILI is critical in determining the prognosis. Children with paracetamol-induced ALF have a better prognosis with a spontaneous recovery rate of 96% compared to 41% of patients with non-paracetamol-induced ALF [3]. In the first 348 patients in the pediatric ALF study group, children with paracetamol-induced ALF had a pre-liver transplantation mortality rate of 2% compared to 29% of children with non-paracetamol-induced ALF [3]. In this same study group, 4% of patients with paracetamol-induced ALF required liver transplantation compared to 29% of patients with non-paracetamol drug exposures. Furthermore, liver transplant for antiepileptic-induced ALF increased the post-transplant risk of mortality by a factor of 4.13, when compared to paracetamol-induced liver injury, with a 1-year survival of only 27% [2]. For all pediatric patients requiring liver transplant secondary to drug-induced ALF, 1- and 2-year survival is 68% and 67%, respectively [2].

Rarely, children may also develop chronic liver disease defined as laboratory, radiological, histological, or clinical findings of portal hypertension 6 months after the initial DILI [1].

Prevention

Careful prescribing of medications with known hepatotoxic effects can minimize the risk of DILI and specific risk factors in an individual patient should be considered. Prescribing of

drugs with a dose-dependent effect or toxic threshold (e.g., methotrexate) requires careful monitoring of levels and of liver function.

Analgesic medications

The most common analgesic medications associated with hepatotoxicity are paracetamol and non-steroidal anti-inflammatory drugs.

Paracetamol

Epidemiology and pathogenesis

Paracetamol is safe when given in appropriate dosages: 10–15 mg/kg/dose with no more than five doses per day for a total maximum daily dose no greater than 75 mg/kg/day. Although paracetamol overdose occurs most commonly following suicide (in adolescents) or accidental acute poisoning (in children aged 1–4 years), deliberate poisoning by care providers is a possibility. Paracetamol accounts for 14% of cases of ALF and can occur after a single overdose or therapeutic misadventure with paracetamol [3].

Paracetamol hepatotoxicity results when the normal toxin scavenger mechanisms are overwhelmed and has a bimodal presentation. In healthy patients, 90% of ingested paracetamol is converted to glucuronide and sulfate conjugates (non-toxic compounds), 5% is metabolized by cytochrome P450 enzymes (primarily CYP 2E1) to *N*-acetyl-*p*-benzoquinone imine (NAPQI) and 5% is renally excreted without modification [7]. With therapeutic dosing of paracetamol, NAPQI, which is a reactive oxygen radical, combines with glutathione to form a non-toxic compound. However in paracetamol overdose, endogenous glutathione stores are depleted and reactive NAPQI causes hepatic injury (see Figure 12.9) [7]. Toxin accumulation may also occur with chronic paracetamol use, due to the inadvertent depletion of glutathione, especially if other hepatotoxins such as alcohol or recreational drugs are present.

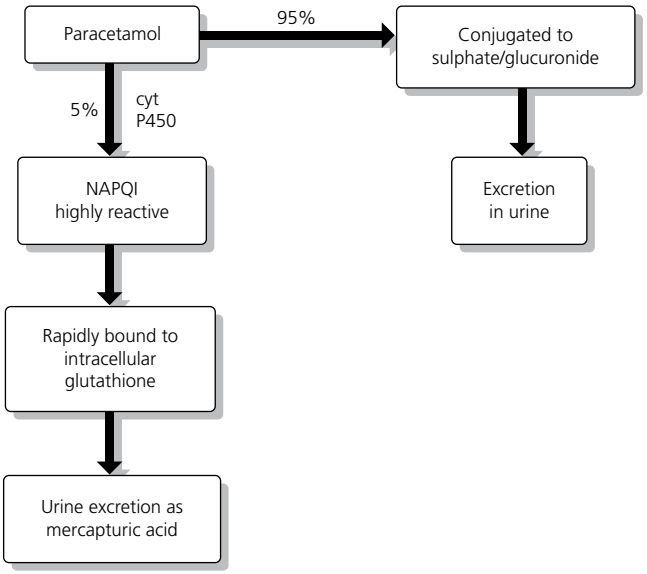


Figure 12.9 Metabolism of paracetamol. cyt P450, cytochrome P450; NAPQI, *N*-acetyl-*p*-benzoquinone imine.

Diagnosis and clinical presentation

In patients presenting with potential paracetamol overdose, clinical history should elicit the milligrams of paracetamol ingested, time of the ingestion, if there were multiple overdoses, the presence of clinical symptoms, and any coingestions (drugs, alcohol, toxins) that may also carry a risk of hepatotoxicity or impact hepatic metabolism.

Initial symptoms of paracetamol-induced toxicity include:

- First 24 h: anorexia, nausea, and vomiting. In very severe ingestions, this early phase may be complicated by hypoglycemia and lactic acidosis.
- 24–48 h: minimal symptoms; hepatic enlargement and right upper quadrant tenderness.
- Days 2–4: overt hepatic injury; marked elevation of hepatic transaminases, jaundice, coagulopathy out of proportion to the rise in bilirubin.
- Days 3–5: jaundice and encephalopathy, associated with renal failure due to acute tubular necrosis, with oliguria or anuria and metabolic acidosis in 25–30%.
- Days 4–5: recovery may occur.

Initial laboratory studies should include: a paracetamol level obtained ≥4 h after ingestion, ALT, AST, conjugated and unconjugated serum bilirubin, GGT, albumin, INR and prothrombin time, blood urea nitrogen (BUN), creatinine, electrolytes, glucose, and urine toxicology screen. BUN and creatinine should be evaluated in patients with paracetamol overdose as impairment of renal function can occur in up to 25% of patients due to direct nephrotoxic effects of NAPQI [7].

The Rumack–Matthew nomogram is a clinical predictive tool to predict the risk of hepatotoxicity based on serum paracetamol concentration between 4 and 24 h after paracetamol ingestion; potentially toxic paracetamol levels start at 150 µg/mL (993 µmol/L) at 4 h. This 4-h time point was chosen for the Rumack–Matthew nomogram as peak paracetamol plasma values occur between 20 and 90 min after therapeutic dosing of oral paracetamol in healthy children and plasma values are not expected to increase beyond 4 h. Current studies do not support the use of paracetamol levels obtained prior to 4 h to exclude the need for therapy with *N*-acetylcysteine (NAC). Acute paracetamol toxicity is diagnosed when either toxic serum paracetamol levels are present based on the Rumack–Matthew nomogram or a paracetamol dose of 140 mg/kg is ingested in a 24-h period [7]. As metabolism of paracetamol leads to a progressive reduction in measured levels following ingestion, the diagnosis may not be confirmed if a prolonged interval has lapsed prior to assay.

Within the first 24 h following ingestion, ALT, AST, bilirubin, and liver synthetic function are typically normal with elevation occurring more frequently between 24 and 48 h following ingestion [7]. Maximal hepatotoxicity typically occurs between 72 and 96 h after ingestion and in patients that do not progress to ALF, improvement in laboratory studies and ultimate normalization of liver function occurs between 4 days to 2 weeks [7].

Serum paracetamol adduct concentrations are currently being explored and were detected in 86% of children with ALF in the setting of known paracetamol overdose and 11% of children with an indeterminate cause of ALF [8]. Serum paracetamol adduct testing is not recommended nor available for clinical evaluation in suspected paracetamol-related DILI.

Liver biopsy is not necessary for the diagnosis of paracetamol-induced liver injury, but if performed typically shows centrilobular necrosis (see Figure 12.1).

Treatment and outcome

Activated charcoal should be considered in patients presenting within 3 h of paracetamol ingestion. Activated charcoal and NAC doses should be separated by 1 h if possible, though this should not delay the first dose of NAC [7]. Charcoal can be administered orally or by nasogastric tube in a dose of: 1 g/kg for children <1 year old; 25 g for children 1–12 years old; and 50 g for children >12 years old.

The primary treatment for paracetamol overdose is NAC, which acts by increasing the availability of glutathione and also can substitute for glutathione, directly binding NAPQI [7]. NAC should be initiated ideally within 8 h of paracetamol overdose as hepatotoxicity increases if treatment is started >8–10 h after ingestion [7]. Therefore, treatment should not be delayed while awaiting paracetamol levels. Even patients presenting >24 h after paracetamol overdose may still show some benefit from therapy with NAC and treatment should be made available to these patients.

NAC is available as an oral and intravenous preparation. Oral formulations may exacerbate vomiting and has been shown to delay treatment by 4.5 h in children. Nasogastric tube administration and antiemetics may improve tolerability of oral preparations. A successfully retained dose is defined as no emesis for 1 h following the oral administration of NAC. Oral dosing includes: an initial loading dose of 140 mg/kg/dose followed by 70 mg/kg/dose every 4 h for 72 h total. Intravenous dosing of NAC is an initial loading dose of 150 mg/kg/dose over 15 min followed by 50 mg/kg/dose to run over 4 h then finally 100 mg/kg/dose to run over 16 h [7]. While the dosage remains the same, the volume of fluid that the intravenous formulation is delivered in is dependent on patient age and weight due to risk of hyponatremia and subsequent seizures secondary to increased administration of free water when adult diluent volumes are used. The current convention is for 20% NAC to be diluted to a final concentration of 40 mg/mL with final volumes based on patient weight [7]. The intravenous formulation of NAC is recommended only for patients that cannot tolerate oral therapy and in patients with ALF from paracetamol toxicity. Further pediatric prospective studies are needed to determine if oral versus intravenous preparations influence outcomes based on patient-specific factors including time from overdose to initial presentation, >4 h paracetamol level or other laboratory values.

Adverse reactions to oral NAC are seen in 0.4% of patients and include vomiting and diarrhea, whereas adverse reactions to intravenous formulations can be seen in 3–14% of patients and include rash, pruritus, bronchospasm, and rarely anaphylaxis typically responsive to antihistamines, corticosteroids, inhaled β-agonists, and then decreased infusion rates and typically does not require withdrawal of the medication [7].

Duration of therapy with NAC is guided by the idea that treatment should continue as long as the liver is able to produce the toxic metabolite of NAPQI. A secondary rise in hepatic enzymes has been found in patients with early termination of NAC. Studies however have not borne out the ability to predict length of treatment based on the ≥4 h paracetamol level. Following an overdose, paracetamol half-life is initially normal (around 2 h), but may become prolonged if hepatic function is affected (up to 12 h). Therefore, current guidelines regarding duration of therapy are to continue NAC (beyond 20 h for intravenous preparations and beyond 72 h for oral preparations) if paracetamol levels continue to be measurable and/or AST/ALT is not improving. Thereafter, NAC may be discontinued when paracetamol levels are undetectable (<10 μg/mL) and AST/ALT is improving with normal coagulation parameters.

Unlike in adults, most children with paracetamol overdose-induced ALF survive. The liver injury is increased if the medication is taken with alcohol, recreational or other drugs. The development of lactic acidosis or renal or other organ failure, as well as cerebral edema, are poor prognostic signs that should prompt early evaluation for liver transplantation. In one study, 96% of children had complete recovery while 4% required liver transplantation; of those requiring liver transplantation 2% died while awaiting liver transplantation [1]. In patients who proceed to liver transplant, 1-year post-transplant patient survival in a combined adult and pediatric study was 75% [9].

Liver injury secondary to chronic paracetamol use is a more recently investigated phenomenon. In an observational cohort study utilizing the PALF study group, 12% of patients with ALF had chronic paracetamol exposure defined as multiple dose exposures over ≥2 days (51% of patients had 3–7 days of exposure). Of children with chronic paracetamol exposure, only 68% of children were alive without liver transplantation at 21 days after study enrollment compared to 92% of children with single dose paracetamol-induced ALF.

NAC is not currently recommended for the treatment of DILI from chronic paracetamol exposure. Additionally, NAC is not recommended for use in non-paracetamol ALF as it has been associated with poorer 1-year liver transplantation-free survival when compared to placebo [10].

Prevention

In the US, the ability to purchase paracetamol in bulk allows easy access for both accidental and non-accidental overdose. In the UK, the issue of preventing accidental and deliberate overdose from paracetamol has been addressed by limiting the pack size available for purchase to 16 tablets from supermarkets and 32 tablets from pharmacies. This change in packaging has led to a 22% reduction in suicide deaths and a 30% reduction in admissions to liver units for ALF in the UK.

Non-steroidal anti-inflammatory drugs

Although a structurally heterogeneous group, most non-steroidal anti-inflammatory drugs (NSAIDs) not only share similar therapeutic anti-inflammatory and antipyretic effects, but also adverse effects. Appreciation of their toxicity in children, particularly with over-the-counter medications and in children with chronic rheumatic conditions is increasing. In patients with elevated baseline aminotransferase activity, the more frequently hepatotoxic NSAIDs should be avoided. Serum aminotransferase activity should be monitored monthly for the first 6 months, and more frequently if they become elevated.

Aspirin

In the majority of cases, aspirin hepatotoxicity is mild, asymptomatic, and reversible.

Pathogenesis

Aspirin is a non-competitive irreversible inhibitor of tissue cyclo-oxygenase 1 (Cox-1). Following absorption, aspirin is metabolized to salicylate then to hydroxyhippurate and gentisate by hepatocyte mitochondria. Salicylates have been hypothesized to cause a dose-dependent hepatotoxicity by the uncoupling of oxidative phosphorylation or by inhibiting mitochondrial oxidation of long-chain fatty acids. Aspirin-induced liver injury has more commonly been described in patients with connective tissue diseases or those on chronic aspirin therapy who have typically have been taking the medication for at least 6 days prior to the onset of hepatoxicity.

Diagnosis and histopathology

As clinical symptoms are uncommon other than rare allergic features (rash, fever), the diagnosis of aspirin-induced liver injury is based on laboratory findings of hepatoxicity (hepatocellular pattern) supported by elevated salicylate levels; as hepatic injury is more likely to occur in patients receiving doses in excess of 100 mg/kg/day or with salicylate levels >25 mg/dL. In the majority of cases, AST is <500 IU/L and bilirubin is normal. Periportal and lobular necroinflammatory changes, autoimmune hepatitis, and granulomatous hepatitis have all been described in aspirin-induced liver injury with normal size and number of mitochondria [11].

Treatment

Withdrawal of aspirin therapy typically results in rapid resolution of hepatotoxicity even in the setting of severe hepatocellular injury. Re-exposure to aspirin after complete resolution of resultant hepatotoxicity is usually tolerated with lower dosing in patients on chronic aspirin therapy.

Aspirin and Reye syndrome

Aspirin use is associated with Reye syndrome based on epidemiological data, though a clear pathophysiological mechanism to explain the end result of mitochondrial failure has not yet been delineated. Reye syndrome in children is associated with ingestion of aspirin in 90% of cases, especially in those with chickenpox or influenza. Its pathogenesis is multifactorial, and may reflect a genetic predisposition and mitochondrial enzyme abnormality in which the viral infection or the aspirin ingestion may promote the hepatocellular insult. There is, however, no relationship between the salicylate level and the severity of hepatic dysfunction, and Reye syndrome may also occur in the absence of aspirin ingestion [11]. Due to broad public health education, aspirin is no longer used as an antipyretic in children and the incidence of Reye syndrome has subsequently declined from a peak of 555 cases per year in 1980 to 2 cases per year between 1994 and 1997.

Reye syndrome is a diagnosis of exclusion presenting as an acute non-inflammatory encephalopathy with either sterile cerebrospinal fluid (≤8 white blood cells/mm^3) or lack of evidence of meningeal or perivascular inflammation on histopathology. Diagnostically, there must also be evidence of hepatic dysfunction with ALT, AST, or ammonia greater than three times the upper limit of normal or a liver biopsy that demonstrates characteristic findings including: diffuse microvesicular fatty change without an inflammatory reaction or significant hepatocellular necrosis (see Figure 12.7) Electron microscopy reveals abnormal, enlarged pleomorphic mitochondria with disrupted cristae. Treatment is supportive.

Other non-steroidal anti-inflammatory drugs

These include indometacin, sulindac, ibuprofen, naproxen, piroxicam, and diclofenac.

Toxicity is rare, especially in childhood. Unlike aspirin, which causes dose-dependent liver injury, hepatoxicity from other NSAIDs is typically idiosyncratic as enzymes that metabolize NSAIDs are influenced by genetic factors.

Table 12.4 Liver dysfunction associated with non-steroidal anti-inflammatory drugs (NSAIDs).

NSAID	Type	Manifestations	Onset	Additional
Indometacin	Indole acetic acid derivative	Hepatocellular dysfunction Variable cholestasis Occasional microvesicular fat	1–7 months	Severe toxicity reported in patients with JCA also receiving diclofenac
Sulindac	Indole acetic acid derivative	Mixed hepatocellular damage/ cholestasis. Hypersensitivity with fever, rash, facial edema.	Within 8 weeks	Mortality 5% with hypersensitivity
Ibuprofen	Propionic acid derivative	Mixed hepatocellular damage/cholestasis Fever, Stevens–Johnson syndrome Vanishing bile duct syndrome reported	Within 1–12 weeks	Rare cause, sporadic reports of DILI. No toxic metabolites formed. Recovery may take 1–5 months
Naproxen	Propionic acid derivative	Cholestasis	Within 12 weeks	
Piroxicam	Oxicam	Massive hepatocellular necrosis Prolonged cholestatic hepatitis	Within 3 months	Rare, but high mortality Recovery typically weeks to several months
Diclofenac	Phenylacetic acid derivative	Mixed hepatocellular damage/ cholestasis. Hypersensitivity with fever, rash, eosinophilia	Within 1–3 months	ALT >3–10× ULN in 3% of cases. Polymorphisms of UGT2B7 and CYP 2C8 may play a role

AST, aspartate aminotransferase; DILI, drug-induced liver injury; JCA, juvenile chronic arthritis; ULN, upper limit of normal.

The incidence of NSAID-induced hepatoxicity has been primarily studied in adults and occurs in descending order of frequency: diclofenac (34%), ibuprofen (15%), suldinac (12%), aspirin (12%), naproxen (11%), and piroxicam (9%). Table 12.4 lists the typical manifestations of commonly used NSAIDs.

Antimycobacterial medications

Worldwide, children comprise up to 6% of all reported cases of tuberculosis. Current guidelines by the World Health Organization for the treatment of tuberculosis in children includes 2 months of daily INH (10 mg/kg/day, maximum dose 300 mg/day), rifampicin (15 mg/kg/day, maximum dose 600 mg/day), pyrazinamide (35 mg/kg/day, maximum dose 2 g/day), and ethambutol (20 mg/kg/day, maximum dose 2.5 g/day) followed by 4 months of daily INH and rifampicin.

Children may have elevated transaminases prior to the initiation of therapy with antituberculous medications due to bacillary dissemination to the liver [12]. As a consequence, baseline laboratory monitoring with AST, ALT, conjugated and unconjugated bilirubin, and alkaline phosphatase are recommended prior to the start of antituberculosis treatment and prothrombin time/INR should be considered in patients with pre-existing liver disease. Routine laboratory monitoring is recommended in patients with a history of liver disease, prior history of DILI or taking potentially hepatotoxic medications; monthly testing is recommended in high-risk individuals [13].

Children develop hepatotoxicity in less than 0.3% of cases. In one study of children under 16 years of age receiving combination antituberculosis therapy (INH, rifampicin ± pyrazinamide) found that 8% of 99 children evaluated developed elevation of their transaminases to five times the upper limit of normal, which was more likely with age under 5 years and treatment with pyrazinamide. Treatment should be interrupted if there is an asymptomatic rise in ALT above five times the upper limit of normal, or greater than three times the upper level of normal with symptoms of hepatitis or jaundice [12].

Isoniazid

INH is acetylated in the liver by *N*-acetyltransferase 2 (NAT-2) and then further metabolized primarily to monoacetyl hydrazine (MAH) that may generate free radicals in susceptible individuals causing hepatocyte injury [12]. There is an increased risk of INH-induced hepatotoxicity with age and with concomitant medication that increases the activity of the P450 system, such as with pyrazinamide therapy. A genetic predisposition has been suggested due to specific polymorphisms of *N*-acetyltransferase leading to differing rates of activity. "Rapid acetylators" may have an increased risk of toxicity due to an increased rate of production of MAH. "Slow acetylators" may also be at risk, however, due to longer exposure to MAH before its subsequent detoxification, or due to the generation of an alternative hepatotoxic metabolite.

INH causes serum transaminase elevations in 6.8–13.6% of children and overt hepatitis in approximately 0.1% of children. In a pediatric study utilizing the DILI Network, INH was one of the most implicated drugs causing hepatotoxicity (based on laboratory evidence) accounting for 10% of all cases of DILI in children [4]. However, symptomatic INH-induced liver injury, which includes clinical symptoms (jaundice, nausea, vomiting, anorexia) in addition to laboratory evidence of hepatotoxicity occurred in only 1–2% of patients. In 50% of patients, clinical symptoms typically occurred within the first 2 months of therapy while the other half had a later presentation, up to 14 months after the

start of therapy [6]. Histopathology findings may include an acute hepatitis, granulomatous hepatitis, or massive hepatic necrosis (in 10% of severe cases) [12].

Once INH has been discontinued, the hepatitis resolves over 1–4 weeks in most individuals. In general, patients who develop delayed-onset hepatitis (>2 months) have a worse prognosis, with a case fatality rate of approximately 10%. Conversely, if the INH is continued despite symptomatic hepatitis, patients may develop fulminant hepatic failure resulting in death or requiring liver transplantation. In a study of 20 children with INH-induced hepatic failure, 20% recovered spontaneously (over an average of 42 days) with withdrawal of the medication, 30% died prior to liver transplant and 50% underwent liver transplantation; of which 20% died on average 21 days following transplantation. In this study, the incidence of hepatic failure was estimated to be 3.2 per 100,000 children treated for latent tuberculosis.

Rifampicin

Rifampicin is relatively safe when used alone. Although asymptomatic elevation of transaminases is common, symptomatic hepatitis has been noted in only 0.6–2.7% (meta-analysis mean of 1.1%). Rifampicin is rapidly eliminated in bile, undergoes enterohepatic circulation, and progressively undergoes deacetylation in the liver. It impairs bilirubin uptake and may lead to a conjugated hyperbilirubinemia. Some 1–3% of the reactions are thought to be allergic, whereas the majority are thought to result from rifampicin competing with bilirubin uptake at the plasma membrane of the hepatocyte. Hepatocellular injury is rarer and is typically associated with hypersensitivity type reactions occurring in the first month of treatment [12].

In the context of multidrug antituberculosis treatment, rifampicin also induces cytochromes as well as uridine diphosphate glucoronosyl transferases [12]. By this mechanism, coadministration of rifampicin may potentiate the hepatotoxicity of INH. In a meta-analysis, laboratory plus clinical findings of hepatoxicity were found in 2.6% of patients taking INH plus rifampicin; a higher incidence than that found in patients taking either drug alone [12].

The vast majority of patients who develop asymptomatic elevation of transaminases will have complete recovery upon discontinuation of the medication. For those who develop symptomatic hepatitis, the symptoms are similar to viral hepatitis with jaundice. The histopathology is patchy and generally shows less periportal inflammation than is seen with INH hepatitis.

Pyrazinamide

Pyrazinamide is metabolized in the liver to 5-hydroxy pyrazinoic acid. Hepatotoxicity is both dose dependent and idiosyncratic and may potentially be related to the generation of free radicals. DILI with hypersensitivity features may also occur with pyrazinamide [12]. In children treated with antituberculosis medications, the administration of pyrazinamide (in addition to INH and rifampicin) was a significant risk factor for the development of hepatotoxicity. Therefore, more frequent laboratory monitoring is recommended in children receiving pyrazinamide as part of combination therapy for the treatment of tuberculosis, particularly in those <5 years old.

Antibiotics

Antibiotics are commonly prescribed in pediatric practice, but hepatotoxicity is rare. Drug reactions are idiosyncratic and dose unrelated, often with features of hypersensitivity such as fever, rash, and eosinophilia. It may be difficult to differentiate manifestations of drug toxicity from the underlying illness, as it may be complicated by multiple antibiotic exposures and other medications. Erythromycin, flucloxacillin, and tetracycline have most clearly been implicated in DILI (see later). Table 12.5 lists those antimicrobials in widespread use in pediatric practice in which DILI has been described.

There is no specific therapy other than immediate discontinuation of the medication, supportive care of the patient and monitoring of the laboratory values.

Beta-lactam antibiotics

Amoxicillin

The synthetic penicillins cause a subclinical cytolytic hepatocellular injury, whereas the natural penicillins do so less commonly. In a pediatric study utilizing the Drug-Induced Liver Injury Network (DILIN), amoxicillin accounted for 7% of cases of drug-induced hepatoxicity and was associated with autoantibodies [1]. Presentation is with hepatocellular injury [6]. Amoxicillin-induced ALF has not been described in children.

Amoxicillin–clavulanic acid

Drug injury secondary to amoxicillin–clavulanic acid (ACA) is due to the clavulanic acid component as the incidence of hepatotoxicity is 1.7 cases per 10,000 prescriptions compared to 0.3 cases for amoxicillin alone in adults. The incidence in children has not been well described, though ACA accounted for 3% of cases of DILI in one pediatric study [4]. Additionally, patients often are able to tolerate re-challenge with amoxicillin alone, but develop recurrent hepatotoxicity with repetitive dosing of ACA [6]. The proportion of amoxicillin dosage to clavulanic acid dosage has not been identified as a risk factor for hepatotoxicity.

The proposed mechanism of DILI secondary to ACA is immunoallergic as 30–60% of adults present with hypersensitivity features (fever, rash, serum eosinophilia) and may be affected by histocompatibility leukocyte antigen (HLA) haplotypes; the incidence is not known in children [6].

Table 12.5 Antimicrobial-associated drug-induced liver injury (DILI).

Drug	DILI reported
Antibacterial	
Amoxicillin/ampicillin	Increased transaminases rarely clinically significant, case reports of more severe injury Anecdotal granulomatous hepatitis
Augmentin (amoxicillin/clavulanic acid)	Cholestatic hepatitis within 1–6 weeks, rare in children, usually mild but may be protracted Accelerated onset with subsequent exposure
Cephalosporins	Rare and usually not significant; mild abnormality of aminotransferases or mild, reversible cholestasis
Co-trimoxazole	Acute and cholestatic hepatitis, vanishing bile duct syndrome, increased risk with human immunodeficiency virus infection
Erythromycin	Cholestatic hepatitis
Flucloxacillin	Cholestasis
Imipenem	Minor liver injury and cholestasis
Quinolones	Cholestasis, mild hepatitis, reports of severe liver injury
Sulfonamides	Hepatitic, cholestatic or mixed hepatic injury, occurs within 4 weeks with features of hypersensitivity
Tetracycline	Microvesicular steatosis
Antifungals	
Ketoconazole	Rare, range from mild hepatitis to fulminant hepatic failure
Itraconazole	Rare, occasional hepatitis
Fluconazole	No hepatic injury
Antiviral agents	Liver toxicity not a major problem, but may be unrecognized due to underlying viral dysfunction

In a prospective pediatric study, 64% of children presented with clinical and laboratory findings of hepatocellular injury, 9% with cholestatic findings and 18% with mixed (hepatocellular/cholestatic) patterns of injury [13]. Patients may present with jaundice as late as 45 days after the initiation of ACA. As treatment courses with ACA typically ranging from 5–14 days, patients often present after the medication has already been discontinued, requiring a high degree of suspicion by the clinician.

Liver histology demonstrates predominant bile duct damage and biliary proliferation, with less specific features of centrilobular canalicular cholestasis (see Figure 12.4), eosinophil, neutrophil, and lymphocyte-rich inflammatory infiltrates, and portal edema. In those who develop chronic liver disease, focal destruction of the bile ducts with extensive inflammatory infiltrates and associated bile ductular wall necrosis is found, similar to primary sclerosing cholangitis. In one case report, a 3-year-old child developed vanishing bile duct syndrome requiring liver transplantation after receiving a 10-day course of ACA for otitis media.

Treatment is discontinuation of the medication and normalization of laboratory values may take up to 8 weeks. ACA-induced ALF has not been described in children.

Regarding prevention, in a study of children with ACA-induced liver injury, this medication was prescribed without a clear indication in 82% of cases when amoxicillin was the preferred alternative. Avoidance of this more hepatotoxic medication may help prevent future cases of DILI.

Other penicillins

The risk of hepatotoxicity with flucloxacillin, a semi-synthetic penicillin, ranges from 1 in 10,000 to 1 in 30,000. The risk factors are: older age, treatment longer than 14 days, and higher daily dosage. Histologically, the bile ducts may be reduced in number and size, the epithelium shows degeneration and cholestasis is dominant. Jaundice tends to be prolonged, with 10–30% of individuals experiencing jaundice for more than 6 months. In these cases, the histological findings are more typical of paucity of the smaller bile ducts, with portal tract inflammation centered around the bile ducts, progressing to vanishing bile duct syndrome. Cases of ALF secondary to flucloxacillin exposure have been reported.

Notably, genome-wide association studies have found that patients with the HLA-B*5701 allele on chromosome 6 have an 80-fold increased risk of flucloaxacillin-induced liver injury. However in adult studies, of the 8% of the population that carries this allele, only 1 in 10,000 patients are estimated to develop DILI after flucloxacillin treatment. Although genetic studies may support the diagnosis of liver injury, patients with the HLA-B*5701 allele are not prohibited from treatment with flucloxacillin nor are genetic studies required before treatment.

Oxacillin is a penicillinase-resistant penicillin in the same family as flucloxacillin, but the risk of cholestatic hepatotoxicity is approximately half that of flucloxacillin. Jaundice typically appears after 1–4 weeks of therapy or within 1 week of discontinuing the drug. Spontaneous recovery occurs within 3 months.

The ureidopenicillins (mezlocillin, azlocillin, piperacillin) cause a two-fold to three-fold increase in serum aminotransferase levels in 19.3% of patients taking these medications for a prolonged period of time (e.g., in the treatment of osteomyelitis). The severity of the reaction correlates with the cumulative dose and/or duration of therapy.

Cephalosporins

Cephalosporin-induced hepatotoxicity is rare accounting for 1–3% of DILI, with case reports primarily in adults. In these patients, cefazolin accounted for 58% of cases typically given as a one-time dose in the perioperative period. Cefazolin-induced liver injury typically presents with a cholestatic or mixed pattern with jaundice and pruritus in the vast majority of patients (95%) arising 1–3 weeks after one-time dosing and self-resolving after discontinuation of the medication.

Patients treated with other cephalosporins (cephalexin, cefadroxil, cefuroxime, cefaclor, ceftriaxone, cefdinir, and cefotaxime) presented similarly with a cholestatic or mixed pattern of injury typically 1–4 weeks after initiation of the medication. In infants, ceftriaxone is associated with a transient cholestasis. Hypersensitivity features (fever, rash, serum eosinophilia) may occur in a minority of patients.

Tetracycline antibiotics

The tetracycline class of antibiotics, which includes tetracycline, doxycycline, and minocycline, are used for skin and soft tissue infections as well as rickettsial disease. In adolescents, the most common indication is acne. Tetracyclines are not prescribed in children <8 years old as they can cause permanent tooth discoloration and retardation of bone growth.

The tetracyclines interfere with the mitochondrial oxidation of fatty acids, which results in an increased concentration of precursor free fatty acids in the liver. It is thought that both precursor free fatty acids and their oxidation metabolites are mitochondrial toxins, ultimately resulting in hepatocellular damage.

With tetracycline-induced hepatotoxicity, typical clinical features of acute hepatitis are experienced. Some 4–6 days into therapy, the patient experiences nausea, vomiting, abdominal pain and mild jaundice. The laboratory profile reveals:

- Aminotransferase levels as high as 10 times the upper limit of normal.
- Histological features demonstrate microvesicular steatosis, minimal portal mononuclear inflammation, and hepatocellular necrosis (see Figure 12.7). Although chronic cholestasis is far less common, there have been reports of tetracycline and doxycycline-associated vanishing bile duct syndrome.

Tetracycline

In children, hepatotoxicity has been described with intravenous tetracycline associated with jaundice and fatty infiltration on liver biopsy. However, only the oral route of administration is currently recommended for children. Rare reports of hepatotoxicity with oral tetracycline causing acute fatty liver have been described in adults, primarily in pregnant women (now a known contraindication), though these findings have not been described in children.

Doxycycline

Doxycycline is less likely to cause hepatotoxicity than tetracycline. Presentation following doxycycline-induced liver injury is typically 1–2 weeks after initial exposure with a mixed hepatocellular/cholestatic pattern. Hypersensitivity reactions have been described (fever, rash, peripheral eosinophilia) suggesting an immunoallergic mechanism of DILI. Resolution of doxycycline-induced hepatotoxicity occurs on average 4–6 weeks after medication withdrawal, though may be up to 6 months in patients with a cholestatic pattern of injury. Despite structural similarities to minocycline, doxycycline is not known to cause drug-induced autoimmune hepatitis.

Minocycline

The clinical presentation of minocycline-induced hepatotoxicity deserves special mention as three unique presentations can occur with this medication:

- An acute hypersensitivity reaction within days to weeks of starting the medication, characterized by fever, rash, lymphadenopathy, and associated peripheral eosinophilia with evidence of hepatocellular hepatitis.
- Autoimmune hepatitis (see Figure 12.3).
- Chronic hepatitis resulting in cirrhosis.

Of the tetracycline antibiotics, minocycline is the most likely to cause liver injury as it is metabolized in the liver and injury may be due to the formation of a reactive iminoquinone metabolite [14]. In a pediatric study utilizing the DILIN, minocycline was the most commonly implicated drug to cause hepatotoxicity (based on laboratory evidence) accounting for 13% of all cases of DILI in children and was also associated with autoantibodies [1].

Minocycline-induced drug injury can be differentiated by the time to presentation. The earlier presentation occurs 1–3 months after drug initiation and is more commonly associated with immunoallergic reactions (fever, rash, serum eosinophilia). Rarely, ALF has resulted from this reaction. Minocycline-induced autoimmune hepatitis has a prolonged latency period with a median of 569 days (range 196–647) from the time of drug initiation to clinical presentation [1]. This presentation is more common in young women and may be complicated by polyarthritis, drug-induced systemic lupus erythematosus, rash, and hypergammaglobulinemia.

The diagnosis of minocycline-induced autoimmune hepatitis is suspected in patients with a:

- Positive antinuclear antibody or antismooth muscle antibody (70–80%) or positive anti-liver kidney microsomal antibody (3–4%) though antimitochondrial antibody is negative.
- Hepatocellular pattern of liver injury (elevated AST and ALT, typically with normal alkaline phosphatase).
- Hypergammaglobulinemia with elevation of serum IgG (>1.5 times the upper limit of normal).

- Characteristic histopathology findings include chronic hepatitis with portal and pan-lobular plasma cell-rich inflammation, interface hepatitis with no biliary involvement. The features may be indistinguishable from autoimmune hepatitis without the relevant clinical history as eosinophils on histology are rare [14].

Treatment for minocycline-induced autoimmune hepatitis is immediate withdrawal of the drug, but additional treatment with immunosuppression (corticosteroids and azathioprine) may be required. Once minocycline has been discontinued, the symptoms and hepatitis usually resolve within 3 months. Unlike patients with classic autoimmune hepatitis (see Chapter 11), immunosuppressive medications can be discontinued following normalization of liver enzymes [14]. However, these patients should continue to be monitored for recurrence of liver laboratory abnormalities, which would necessitate restarting immunosuppressive therapy.

Case reports of progression to chronic liver disease as well as fulminant hepatic failure requiring liver transplantation have been reported in adolescents with minocycline-induced liver injury [1, 3].

Regarding prevention, adolescents on chronic minocycline should have liver enzymes, antinuclear antibody and erythrocyte sedimentation rate prior to the initiation of minocycline and every 2–3 months thereafter [14].

Nitrofurantoin

Nitrofurantoin is used either as prophylaxis or treatment for urinary tract infections. Hepatotoxicity is rare and presentation is often a hepatocellular or mixed hepatocellular and cholestatic pattern, frequently accompanied by rash and fever suggesting an immunoallergic mechanism of drug injury. Recovery is rapid following discontinuation of this medication. ALF has not yet been described in children and mortality in children only occurs with nitrofurantoin-induced pulmonary toxicity.

Macrolide antibiotics

The class of macrolide antibiotics includes azithromycin, erythromycin, roxithromycin, and clarithromycin. Hepatotoxicity occurs 10–21 days after the start of therapy in children. Fever is a presenting sign in approximately 50% of patients with serum eosinophilia in two-thirds of patients suggesting an immunoallergic mechanism of drug injury. Clinical symptoms may also include right upper quadrant pain, fever, nausea, and jaundice. Laboratory findings are often mixed hepatocellular and cholestatic with elevated serum bilirubin and alkaline phosphatase in 50% of patients and jaundice and pruritus in 20% of patients. Histopathology typically shows centrilobular cholestasis with mild hepatocellular necrosis in conjunction with intense portal and lobular inflammatory infiltrates, predominantly with eosinophils.

Most patients recover completely within weeks of discontinuation of the macrolide antibiotic. There have been reports of fatal liver injury, or injury requiring liver transplantation, in both children and adults, accentuating the potential severity of the hepatotoxicity. Following an initial hepatotoxic liver injury with a macrolide antibiotic, the entire class should be avoided as re-exposure leads to a rapid onset of hepatotoxic symptoms within 12 h, irrespective of the interval since the initial reaction.

Azithromycin

In a pediatric study utilizing the DILIN, azithromycin accounted for 10% of cases of DILI in children and was associated with autoantibodies [1]. In this study, liver histopathology noted an acute intrahepatic cholestasis with duct paucity, similar to adult findings where azithromycin is known to cause vanishing bile duct syndrome [1].

Erythromycin

Erythromycin increases the activity of cytochrome P450 3A and is metabolized via demethylation and oxidation into unstable intermediates that may cause cellular injury. The risk of erythromycin-induced hepatotoxicity increases with age in children [15]. Liver histopathology reveals pan-lobular degeneration with cholestasis. Treatment is withdrawal of erythromycin and improvement of clinical and laboratory studies may take 6–8 weeks with full recovery expected.

Clarithromycin

Clarithromycin related liver injury typically occurs 1–3 weeks after the start of treatment and presents with a cholestatic pattern. Recovery typically occurs 4–8 weeks after discontinuation of the medication though vanishing bile duct syndrome has been reported in adults.

Roxithromycin

Roxithromycin-induced liver injury presents on average 5 days after the onset of treatment with both hepatocellular and cholestatic injury. There is one case report of a 5 year old with weakly positive antinuclear antibodies who progressed to fulminant hepatic failure requiring liver transplantation after roxithromycin exposure.

Sulfonamide antibiotics

The most common hepatotoxicity experienced with sulfonamides is asymptomatic transient elevation of the transaminases (as high as 10% of patients), which is only detected by laboratory profiling. Clinically apparent hepatitis requiring hospitalization is less common, but is estimated to occur in the range of one in 100,000–280,000 prescriptions. Patients infected with human immunodeficiency virus (HIV), however, are at higher risk, as use of the sulfonamide antibiotics results in hepatotoxicity in 20% of patients. This is thought to be due to HIV-induced increased oxidation by their P450

enzymes and hence increased production of the toxic hydroxylamine metabolite.

Of the sulfonamide antibiotic class, trimethoprim–sulfamethoxazole is the most common drug to cause liver injury in children. Sulfamethoxazole is conjugated in the liver to the reactive metabolite hydroxylamine and accounts primarily for the incidence of DILI. However, patients have also redeveloped hepatotoxicity after therapy was changed from trimethroprim–sulfamethoxazole to trimethoprim alone.

In a pediatric study utilizing the DILIN, sulfamethoxazole accounted for 3% of cases of drug-induced hepatoxicity [1]. Clinical symptoms include hypersensitivity features (fever, rash, serum eosinophilia) and jaundice between 1 and 41 days after starting the medication, sometimes associated with pancreatitis, renal insufficiency, and lymphadenopathy. Centrilobular cholestasis usually dominates the histological findings, with only mild to moderate lymphocytic portal inflammation and minimal eosinophils and neutrophils. Although less common, it should be noted that granulomatous hepatitis and massive hepatocellular necrosis have been reported. Vanishing bile duct syndrome has also been described.

Hepatotoxicity resolves in the majority of patients 2–8 weeks after discontinuation of the medication except in cases of severe cholestasis when resolution is delayed. In an observational cohort study utilizing the PALF study group, 0.3% of patients had ALF attributable to trimethoprim–sulfamethoxazole exposure [3]. Fulminant liver failure leading to liver transplantation or death has been described in two pediatric case reports. Sulfonamide antibiotics as a class are contraindicated in children that develop hepatotoxicity with trimethroprim–sulfamethoxazole and re-exposure to the offending medication may lead to the rapid onset of fatal hepatotoxicity.

Clindamycin

Clindamycin causes an asymptomatic rise in serum aminotransferases in as many as 50% of recipients. However, symptomatic hepatitis or cholestasis is limited to individual case reports.

Antifungal medications

The most commonly used antifungal medications include ketoconazole, fluconazole, itraconazole, voriconazole, and amphotericin B. Overall, these medications are relatively safe with regard to hepatotoxicity. However, patients who are immunosuppressed and systemically ill are at higher risk for invasive fungal disease and also at risk for hepatotoxicity.

Ketoconazole

Ketoconazole carries the highest risk for hepatotoxicity, with an incidence of between 1.3 per 1000 and 1 in 3000. Although recurrence of hepatotoxicity with re-exposure is common, the symptoms are neither consistent nor immediate, arguing against an immunoallergic mechanism of injury.

Asymptomatic elevation of the transaminases will develop in 2–17% of users after weeks of therapy, but most cases will resolve spontaneously despite continued use. Approximately 3% of patients receiving ketoconazole develop symptomatic hepatitis. In this situation, recovery typically occurs 3 months after the medication is discontinued. However, fatal hepatitis and ALF have been reported.

The laboratory abnormalities suggest a hepatocellular pattern in more than half of the cases (54%), and a cholestatic (16%) or mixed pattern (25%) in the rest. Liver histology shows patchy and centrilobular necrosis, with portal mononuclear cellular infiltration.

Although it is not possible to predict the development of toxicity, the likelihood increases with prolonged use. Consequently, bi-weekly laboratory monitoring of biochemical liver function is suggested in patients on ketoconazole for longer than 10 days. Asymptomatic laboratory elevation should be re-checked 1 week later and the development of significant laboratory elevation or symptoms should precipitate immediate discontinuation of the medication.

Fluconazole

Fluconazole is a commonly used antifungal and transient mild elevations of hepatic enzymes are observed in 5% of patients. Fluconazole-induced drug injury is typically in a hepatocellular pattern arising in the first few weeks of therapy. However, a cholestatic pattern of injury appears to be more common in preterm neonates. Conjugated hyperbilirubinemia was found in 43% of extremely low birthweight neonates on fluconazole prophylaxis (compared to 9% of neonates not on prophylaxis). Histological findings with fluconazole-induced serious hepatotoxicity range from a mixed hepatocellular–cholestatic pattern to diffuse necrosis.

Fluconazole-induced liver injury in children is more likely in children receiving intravenous medication compared to the oral formulation and those on treatment doses rather than prophylaxis. Drug-related deaths secondary to antifungal agents have not been documented in children. Recovery after withdrawal of the medication may take up to 2–3 months.

Itraconazole

The relative risk of developing acute hepatitis due to itraconazole is 17.7 (95% CI, 2.6–72.6), with asymptomatic elevation of biochemical liver function tests occurring in 1–7% of patients. The pattern is usually hepatocellular, but cholestasis is also observed. Laboratory abnormalities resolve after cessation of the medication, although ALF has been reported.

Voriconazole

Hepatotoxicity with voriconazole is rare but occurs more frequently than with fluconazole. In children treated with voriconazole for invasive fungal infection, elevated

transaminase or bilirubin levels occurred in 14% of patients (median ALT 186, AST 216, alkaline phosphatase 946, total bilirubin 3.5). Presentation is with a hepatocellular or cholestatic pattern occurring 4 weeks following voriconazole initiation. Resolution of hepatotoxicity occurs between 6–10 weeks after the initial drug injury. Notably, laboratory evidence of hepatotoxicity has been noted to resolve in 50% of children despite continuation of voriconazole. In a pediatric case report, voriconazole hepatotoxicity was reported in a 10 year old with HIV on antiretroviral therapy who ultimately succumbed to liver failure. Both voriconazole and protease inhibitors are substrates of the cytochrome P450 enzymes, specifically CYP 2C19, and in the case of this patient antiretroviral levels became abnormally elevated after initiation of voriconazole further highlighting the risk of drug–drug interactions that may lead to hepatotoxicity.

Amphotericin B

Amphotericin B-induced hepatotoxicity is rare and only described as individual cases in children with significant underlying disease processes. In reported cases, an asymptomatic elevation of transaminases and alkaline phosphatase occurred 10 days to 3 weeks into therapy and all laboratory values returned to normal after discontinuation of the medication. The most prominent histological finding in the liver was centrilobular focal fatty infiltration.

Antiepileptic medications

Abnormal serum liver enzyme values are commonly associated with the antiepileptic medications. However, as children on these medications frequently have underlying metabolic conditions or serious seizures, it is important to distinguish whether the enzyme elevations are due to drug toxicity, underlying disease, a "tolerable" side effect, or progressive liver insufficiency. Prolonged convulsions may lead to hypoxic/ischemic liver damage and must be considered in the differential diagnosis of liver dysfunction.

Although elevation of liver laboratory values has been reported with many antiepileptic medications (chloral hydrate, clonazepam, diazepam, mephenytoin, primidone, and sultiame), significant hepatotoxicity has only been reported for valproic acid, carbamazepine, oxcarbazepine, phenytoin, phenobarbital, lamotrigine, felbamate, and topiramate.

Valproic acid

Utilizing the World Health Organization individual case safety report database to analyze suspected adverse drug reactions, valproic acid accounted for 3.2% of all pediatric cases of DILI [15]. In a pediatric study with a smaller sample size utilizing the DILIN, valproic acid accounted for 7% of cases of drug-induced hepatoxicity [1].

The estimated risk for liver failure increases in combination with other antiepileptic medications and inversely with age. In children under 2 years of age, the risk of liver failure is 1 in 8000 in children treated with valproate alone and increases to 1 in 550 when combined with other antiepileptic medications. The risk decreases to 1 in 6000–12,000 for children aged 3–10 years and to less than 1 in 50,000 in children older than 10 years.

Apart from age, other risk factors include fatty acid oxidation defects, urea cycle disorders and mitochondrial diseases. Specifically, a defect in the mitochondrial DNA polymerase γ gene (*POLG1*) is a risk factor for valproic acid-induced hepatotoxicity. Children with Alpers syndrome with mitochondrial mutations of *POLG1*, have developmental regression, seizures, and pre-terminal liver failure and subsequently death prior to 10 years of age. Liver failure in these patients can be precipitated by treatment with valproic acid at any stage, typically 2–3 months after medication initiation [16]. Though there are no current consensus guidelines, suggested recommendations are: evaluation of the *POLG1* gene in children (particularly in those <3 years old) with intractable seizures, especially if there is a history of developmental regression, prior to initiation of valproic acid therapy [16]. Conversely, sequencing of the *POLG1* gene should be pursued in children with valproate-induced hepatotoxicity.

Although asymptomatic laboratory elevation is common and may occur in 20% of patients, the onset of hepatitis is a sign of potentially serious hepatotoxicity. Routine monitoring of liver function may not distinguish those with impending liver failure from those with minor enzyme elevation and fulminant failure can occur without any preceding biochemical abnormality and may progress despite discontinuation of valproate. The onset of hepatotoxicity is typically within 5 months of the start of treatment and manifests as ALF due to hepatocellular necrosis, usually with macrovesicular and sometimes extensive microvesicular fatty change in the liver. Children with valproic acid-induced ALF accounted for 0.9% of patients in the first 348 patients of the pediatric acute liver failure study group [3].

The most common presenting symptoms in patients with fatal valproate hepatotoxicity are: diminished level of consciousness, jaundice, vomiting, bleeding, increased frequency of convulsions, anorexia, edema, and mild pancreatitis.

If symptoms develop, the drug should be immediately discontinued as liver failure may be reversible. Carnitine may improve hepatic survival and should be administered intravenous with a loading dose of 100 mg/kg to be given over 30 min (to a maximum dose of 6 g) followed by 15 mg/kg/dose (to a maximum of 3 g/dose) every 4 h until there is clinical improvement [17]. Treatment is otherwise supportive, as for fulminant hepatic failure.

The role of transplantation is controversial as children with valproate-induced ALF had a 20% 1-year post-liver transplant survival compared to 69% in children with other drug-induced liver failure with no long-term survivors [17]. It is likely that the progressive decline in neurological function in these patients was due to undiagnosed Alpers syndrome and thus liver transplantation is contraindicated [17].

Aromatic anticonvulsants: carbamazepine, oxcarbazepine, phenytoin, and phenobarbital

In patients with liver injury secondary to aromatic anticonvulsants, re-challenge is contraindicated and other aromatic anticonvulsants (phenytoin, phenobarbital, oxcarbazepine in particular) should be avoided due to cross-reactivity. While lamotrigine is not an aromatic anticonvulsant, hypersensitivity features as well as cross-reactivity have been described and this medication should also be avoided in these patients.

Carbamazepine

Hepatotoxicity from carbamazepine and related drugs (e.g., oxcarbazepine) is due to disturbance of glutathione metabolism. Carbamazepine accounts for 2–3% of pediatric cases of DILI and injury occurs in a hepatocellular or cholestatic pattern within the first few weeks of therapy [1, 6, 15]. Later presentations up to 4–6 months have also been described.

The majority of cases present with hypersensitivity features including fever, rash, facial edema, and serum eosinophilia; Stevens–Johnson syndrome has also been noted [4]. Other symptoms may include rigors, weakness, jaundice, and hepatic discomfort, resembling cholangitis. Serum immunoglobulins, especially immunoglobulin E (IgE) may be raised, while complement levels may be decreased. Histological changes reflect both hepatocellular damage and cholestasis, though vanishing bile duct syndrome and granulomatous hepatitis have also been described (see Figures 12.5 and 12.6).

In children, poorer outcomes are associated with a hepatocellular pattern of injury and longer duration of therapy (30 weeks compared to 4 weeks). Improvement typically occurs 5–7 days after discontinuation of the medication and case reports in children describe improvement with corticosteroids and ursodeoxycholic acid in severe cases. Progression to liver failure necessitating liver transplantation or resulting in death has been reported in children.

Oxcarbazepine

Oxcarbazepine is a less frequent cause of DILI than carbamazepine though presents similarly, often with hypersensitivity features and a hepatocellular or cholestatic pattern in the first few weeks of therapy [6]. Liver biopsy findings are similar to carbamazepine and resolution of symptoms is typically 5–7 days after withdrawal of the medication. Progression to liver failure has rarely been reported [6].

Phenytoin

Hepatotoxicity from phenytoin is uncommon, but benign elevation of GGT and alkaline phosphatase as a result of hepatic enzyme induction is not uncommon. Acute or cholestatic hepatitis with features of allergy – including fever, rash, Stevens–Johnson syndrome, and eosinophilia with lymphadenopathy and splenomegaly may occur 2–8 weeks after starting the medication. Histology may reveal the presence of granulomas. Recovery is expected after discontinuation of the drug within 1–2 months, but fatal outcomes have been reported when the drug is continued despite the onset of hepatotoxicity. Re-challenge with phenytoin is not recommended as fatal outcomes have been reported.

Phenobarbital

Phenobarbital is a barbiturate with anticonvulsant properties and relatively few reports of significant hepatotoxicity. Hepatotoxicity occurs with hypersensitivity features including fever, rash, and eosinophilia while hepatocellular and cholestatic features are less common. Significant hepatotoxicity is rare, but can be fatal.

Lamotrigine

Hepatotoxicity from lamotrigine is rare, but can be fatal and typically presents with hypersensitivity features including fever, rash, and eosinophilia within several weeks of initiation of the medication. An LKM-positive hepatitis has also been reported. Children taking valproic acid may be more susceptible to lamotrigine-induced hepatotoxicity. The pattern of liver injury is hepatocellular with rare progression to ALF. One case report of vanishing bile duct syndrome exists in the pediatric literature.

While lamotrigine is not an aromatic anticonvulsant, hypersensitivity features as well as cross-reactivity have been described and carbamazepine, oxcarbazepine, phenytoin, and phenobarbital should also be avoided.

Felbamate

Felbamate is an effective treatment for Lennox–Gastaut syndrome, but causes aplastic anemia and ALF in between 1 in 10,000 and 1 in 20,000 patients treated of whom 60% will have a fatal outcome. Due to this substantial risk, frequent monitoring of the biochemical liver laboratory values before and during therapy is recommended. Therapy should be discontinued at the first sign of transaminase elevation.

Topiramate

Hepatotoxicity from topiramate is exceedingly rare; however the risk increases when topiramate is added to long-term valproic acid therapy. Case reports describe the occurrence of transaminitis and hyperammonemia with encephalopathy after topiramate was added to a stable valproic acid regimen with rapid improvement after discontinuation of topiramate.

Anesthetic agents

Halothane

Although hepatotoxicity from halothane in children is relatively uncommon (an incidence of 1 in 82,000 exposures has been suggested) there are reports of serious hepatitis and liver failure and the same increased risk exists when the halothane is administered with multiple other anesthetics and after multiple exposures within a short period of time. Consequently, the Committee on Safety of Medicine in the UK has produced guidelines for the usage of halothane

discouraging its use if there have been prior episodes of pyrexia or jaundice after halothane administration and warning against repeating the drug within 3 months in any patient. As the use of halothane has decreased over the decades, so has the incidence of hepatotoxicity.

Halothane is stored in adipose tissue and subsequently released, which may contribute to the higher risk of toxicity among the obese and those who have repeated exposures within a short period of time. The underlying mechanism for liver damage is the formation of trifluoroacetylated proteins by cytochrome P450 (CYP 450). Approximately 20% of halothane is metabolized by CYP 450, predominantly CYP 2E1, to the unstable intermediate trifluoroacetyl chloride. This intermediate binds to liver proteins, causing hepatocellular necrosis. In some patients, there is an immune response with antibody production against CYP 2E1. Although these antibodies may further damage the hepatocytes, their presence also serves as a diagnostic tool in the diagnosis of halothane-induced hepatitis.

The clinical features include an allergic-type reaction with pyrexia associated with rigors approximately 1–2 weeks after the first exposure to halothane. Symptoms such as malaise and right hypochondrial abdominal pain have been described. Jaundice appears within 10–28 days. These symptoms are commonly accentuated and hastened when the halothane exposure is in combination with other anesthetics.

The laboratory profile reveals elevated transaminases and frequently an elevated eosinophil count. Histologically, the liver is comparable with the findings in viral hepatitis with leukocyte infiltration of the sinusoids, fatty changes, and granulomas. Extensive hepatocellular necrosis can also be seen in severe toxicity (see Figure 12.2).

Halothane-induced ALF carries a poor prognosis. However, those who only have mild hepatitis recover spontaneously and do well as long as repeat exposure is avoided. In one report of seven children who developed halothane-induced hepatitis and antibodies, six survived. The child who died developed ALF from the drug.

Enflurane, isoflurane, desflurane, and sevoflurane

As with halothane, hepatotoxicity has been reported with comparable anesthetics, although usually to a less severe extent. The clinical presentation is similar and the pathogenesis is also due to an immune response directed against the P450-created trifluoroacetylated proteins. Cross-sensitization to other volatile anesthetic agents may occur therefore children with previous reactions should be cautioned against additional exposures.

Sulfasalazine and related compounds

Liver injury associated with sulfasalazine is uncommon and relates to a hypersensitivity reaction to the sulfapyridine moiety with fever, rash, lymphadenopathy, and eosinophilia occurring within 2–3 weeks of treatment. ALF, although documented, is rare [18]. Patients with DILI secondary to sulfasalazine should avoid this medication in the future and additionally have other sulfonamides listed as an allergy.

Sulfasalazine, olsalazine, and balsalazide are prodrugs of mesalazine, a 5-aminosalyclate. Unlike sulfasalazine, however, olsalazine, balsalazide, and mesalazine all lack the sulfapyridine moiety and therefore have an improved hepatotoxicity profile; though cholestasis, chronic hepatitis, and granulomatous hepatitis have been reported.

Immunosuppressive medications

Azathioprine

Azathioprine is a prodrug of mercaptopurine and can give rise to a broad spectrum of liver dysfunction including:

- Asymptomatic liver enzyme elevation.
- Hepatitis and/or cholestasis and bile duct injury.
- Vascular injury (especially after renal transplantation).
- Sinusoidal dilation, peri-sinusoidal fibrosis.
- Peliosis (blood-filled cavities without endothelial lining).
- Veno-occlusive disease.

As indications for azathioprine include immunosuppression post-organ transplantation, chronic inflammatory disorders, and autoimmune disease, liver damage may occur in a complex setting and azathioprine may initially be overlooked as the potential cause. The onset may be months to years after the start of treatment, although the hepatic and cholestatic features may manifest earlier. Disease progression occurs with continued treatment. A therapeutic trial of azathioprine withdrawal may be necessary to establish or exclude its role.

Methotrexate

Methotrexate is a folic acid antagonist and has a therapeutic role in the treatment of leukemia, solid tumors, psoriasis, and rheumatoid arthritis. It is a dose-dependent hepatotoxin, which has a fibrogenic effect possibly by activation of hepatic Ito cells. The total dose administered is the most important predictor of fibrosis, although the dosing schedule may also be relevant as low-dose weekly pulsed therapy appears to carry a lower risk of significant fibrosis.

Other risk factors for methotrexate toxicity include alcohol ingestion and pre-existing liver disease; treatment with methotrexate should be avoided in these situations. In addition, as methotrexate is renally excreted, impairment of renal function including decreased renal blood flow due to NSAID administration will contribute to the risk of hepatotoxicity.

Liver enzyme monitoring in pediatric inflammatory bowel disease (IBD) patients is recommended at baseline, two times a week for the first 4 weeks then every 3 months. Minor elevations in transaminases occur in approximately 10% of children with IBD in a pooled meta-analysis and may require

dose reduction or medication discontinuation [19]. In an observational cohort study utilizing the PALF study group, 0.3% of patients had ALF attributable to methotrexate [3].

Hepatic fibrosis is usually asymptomatic, unless accompanied by manifestations of portal hypertension. Extensive fibrosis may be present with normal liver function, but serial monitoring is still recommended. A liver biopsy should be performed to assess for hepatic fibrosis if:

- Liver enzymes are either significantly elevated or persistently abnormal despite decreased dosage or medication discontinuation [19].
- After a cumulative dosage of 4 g [19].
- After 2 years of treatment.

In the presence of fibrosis, the risks and benefits of continued treatment have to be considered as there may be some reversal of fibrosis after stopping treatment.

Ciclosporin

Ciclosporin may rarely be associated with hyperbilirubinemia and cholestasis, both of which typically resolve after discontinuation of the medication. ALF secondary to ciclosporin has not been described in children.

Cytotoxic therapy

Cytotoxic chemotherapy and sinusoidal obstruction syndrome are discussed in Chapter 22.

Propylthiouracil

Using data from the United Network for Organ Sharing database (between 1990 and 2002), propylthiouracil was found to account for 10% of pediatric liver transplants secondary to DILI [9]. Currently, propylthiouracil is no longer recommended for the treatment of Graves disease in children as the rate of ALF was found to be as high as 1 in 2000 to 1 in 4000 in children receiving this medication [20]. In 2010, the Food and Drug Administration in the US added a "black box warning" describing the risk of ALF and associated mortality in children receiving propylthiouracil.

H_2-antagonists and proton-pump inhibitors

Both cimetidine and ranitidine are associated with raised aminotransferase activity, which is common, transient, and may reverse even when therapy is continued. Significant hepatocellular or cholestatic injury is rare in children and hypersensitivity reactions are unusual.

Cimetidine is a reversible inhibitor of cytochrome P450, depending on host variation and other drugs, therefore drug effects may be potentiated and the dose should be reduced accordingly. Ranitidine has a lower affinity for cytochrome P450, therefore interaction with other drugs is less marked.

Omeprazole has similar adverse effects to H_2-blockers in type and frequency with reversible elevation in aminotransferase levels reported. Other proton-pump inhibitors, including lansoprazole and pantoprazole, are structurally similar and considered likely to have similar pharmacokinetics, metabolism, and interactions.

Treatment for attention deficit hyperactivity disorder: atomoxetine

Atomoxetine is a primary therapy for ADHD. The medication was first introduced in 2002 and by 2004 the label had changed to include severe liver injury as an adverse event. Similar to minocycline, atomoxetine may have a prolonged latency period from the time of drug initiation until laboratory evidence of liver injury (median 510 days, range 117–699 days), though earlier presentations have been described [1, 21]. The pattern of injury is typically hepatocellular and resolves with withdrawal of the medication; re-challenge with atomoxetine is not recommended [1, 21]. One pediatric case report noted the presence of autoantibodies with liver histology resembling an autoimmune hepatitis that improved after treatment with immunosuppressive medications [21].

Recreational drugs

The relative ease of home preparation of recreational drugs has both facilitated their use and increased the potential for toxic contamination. As with the herbal medications discussed later, recreational drugs are frequently impure or contaminated with other potential toxins and are not regulated to control their concentration and content and toxicities are not documented. Consequently, the rates of hepatotoxicity with these drugs are all subject to inaccuracies.

Cannabis

Cannabis (*C. sativa*: marijuana; *C. indica*: hashish) are plants with more than 400 chemical compounds including the Δ-9-tetrahydrocannabinol that leads to the attractiveness of this drug for its analgesic and recreational affects. An association with hepatotoxicity is uncommon, but a clear toxicity analysis is difficult given the high frequency with which these drugs are used in association with alcohol or other drugs, and the rate of viral hepatitis that is common among parenteral drug users who also frequently use cannabis. A Brazilian community-based study evaluated the rates of AST, ALT, and alkaline phosphatase elevation among non-parenteral seronegative illicit drug users. In the group using marijuana alone, AST, ALT, and alkaline phosphatase were elevated in 42.3%, 34.6%, and 53.8% of users, respectively.

Box 12.1 Herbal medications reported to cause hepatotoxicity.

- *Amanita phalloides*
- Pyrrolizidine alkaloids
  - *Crotalaria*
  - *Heliotropium*
  - *Senecio*
  - *Symphytum officinale*
  - *Symphytum longilobus*
  - Mate ("Paraguay tea")
- Chinese herbal medicines
  - Ba Jiao Lian (*Dysosma pleianthum*)
  - Bol Gol Zhee (*Fructus psoraleae*)
  - Chi R Yun (*Breynia officinalis*)
  - Jin Bu Huan (*Lycopodium serratum*)
  - Ma huang (ephedra alkaloid, banned from sale in the USA in 2004)
  - Sho Saiko To
  - *Paeonia* spp.
  - *Dictamnus* spp.
  - Lingzhi (*Ganoderma lucidum*)
  - Shou Wu Pian (*Polygonum multiflorum*)
  - *N*-nitroso-fenluramine (marketed as Chaso and Onshido)
- Germander
  - *Teucrium chamaedrys*
  - *Teucrium polium*
- Atractylosides
  - *Atractylis gummifera*
  - *Callilepsis laureola*
- Chaparral
  - *Larrea tridentate*
- Anthraquinones
  - Senna (*Cassia angustifolia*)
  - *Cascara sagrada*
  - Noni (*Morinda citrifolia*)
- Miscellaneous
  - Mistletoe (*Viscum album*)
  - Valerian (*Valeriana officinalis*)
  - Skullcap (*Scutellaria* spp.)
  - Pennyroyal (*Mentha pulegium*)
  - Margosa oil (*Azadirachta indica*)
  - Sassafras (*Sassafras albidum*)
  - Kava (*Piper methysticum*)
  - Greater celandine (*Chelidonium majus*)
  - Usnic acid (*Usnea* spp.)

Amphetamines: MDMA (Ecstasy), methamphetamine

The amphetamines and their derivatives can cause neurotoxicity and hepatotoxicity. The clinical presentation typically resembles viral hepatitis and histological features similarly resemble acute viral hepatitis. Cases of ALF have also been described, resulting in a fatal outcome or need for transplantation.

Cocaine

Cocaine is metabolized by cytochrome P450 enzymes to a metabolite that is damaging to hepatocytes and can lead to severe liver injury that may be associated with rhabdomyolysis leading to renal failure or rarely ALF.

Herbal medications

Herbal medications are increasingly used in the US and Europe by consumers who are under the misconception that they are natural and hence must be safe. In fact, these products are commonly unpurified extracts from plants and do not fall under the normal oversight and regulatory systems used to scrutinize allopathic pharmacological products. The herbal medications that have been reported to cause hepatotoxicity are listed in Box 12.1.

In general, the pathogenesis of liver toxicity in most of the herbal medications is unknown. This is in large part because they are mixtures of multiple compounds, not all of which are known. In addition, their wide availability over the counter allows for unregulated usage and potentially harmful medication combinations; some of these herbal remedies induce the P450 enzyme system and could enhance the hepatotoxicity of conventional medications.

The first step in identifying an herbal medication as etiological in the development of hepatotoxicity is obtaining an exposure history. Many patients do not volunteer the information that they are taking herbal preparations. Furthermore, like many allopathic medications, significant indirect exposure is possible. For instance, exposure to an infant transplacentally or via breast milk has been reported with pyrrolizidine alkaloids and other compounds, causing fatal fetal veno-occlusive disease or obstructive jaundice.

Many of the herbal medications have been described as causing liver damage with characteristic histological features. These patterns are the same as seen with conventional medication toxicity, but may help differentiate between herbal causes when there are multiple exposures. For instance, the pyrrolizidine alkaloids cause veno-occlusive disease, *Chelidonium majus* causes a cholestatic hepatitis, *Larrea tridentata* and pennyroyal result in hepatocyte necrosis in zone 3 and kava can induce a non-specific hepatitis.

When it is suspected that hepatotoxicity is the result of herbal exposure, immediate withdrawal of that medication is recommended. Subsequent resolution of the hepatotoxicity strengthens the causal suspicion and re-exposure is contraindicated.

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CHAPTER 13

Viral Hepatitis

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Key points

- Hepatitis A and E viruses commonly cause acute hepatitis. In many countries, the prevalence of hepatitis A is decreasing, while hepatitis E is increasingly being recognized.
- Globally, hepatitis B and C are responsible for the majority of cases of chronic liver disease.
- An effective vaccine is available to prevent hepatitis B. The World Health Organization has advocated all countries to adopt hepatitis B vaccination as part of the childhood vaccination schedule.
- Though no vaccine is available for hepatitis C, treatment with direct acting antiviral agents are now available with high efficacy.
- Many viral infections also affect the liver as part of the systemic infection. Reactivation of latent viruses, such as those in the herpesvirus family, in immunocompromised patients may present as hepatitis.

A number of viruses primarily affect the liver causing hepatitis; others may affect the liver as part of systemic involvement. Viral hepatitis may present as acute, chronic, or fulminant hepatitis with liver failure. The clinical presentation varies according to the infecting virus, the presence of extrahepatic disease, and the host immune status. The main biochemical findings are raised aminotransferases with or without jaundice. Viral hepatitis may also mimic non-infective disorders, including autoimmune hepatitis, and thus the differential diagnosis in children is wide. In this chapter, the molecular characteristics, epidemiology, pathogenesis, clinical features, investigation, diagnosis, and management of viral hepatitis are considered.

Enteric transmitted viral hepatitis

Hepatitis A

Virology and pathogenesis

Hepatitis A virus (HAV) is a small spherical non-enveloped virus classified under the family Picornaviridae, genus *Hepatovirus*. The viral genome is a single-stranded positive-sense RNA. Six genotypes (I–VI) were described of which three were human (I, II, and III) and three simian strains (IV, V, and VI). Despite the nucleotide differences between genotypes, there is only one serotype, making the universal application of diagnostic tests and the production of effective vaccines possible. The virus grows slowly in tissue culture, but in sufficient quantity for the production of an inactivated vaccine.

HAV attaches to hepatocytes via a mucin-like glycoprotein receptor. The uncoated genome acts as a messenger RNA (mRNA). It is translated into a polyprotein, which is cleaved into four structural proteins (VP1–4) and seven non-structural proteins, including the enzyme for viral RNA synthesis. The liver is the primary site of HAV replication, though there may be an intermediate phase of gastrointestinal replication. HAV is not directly cytopathic to the liver. Rather, liver damage and subsequent recovery from infection are mediated by the host's immune response. HAV-specific $CD8^+$ cytotoxic T cells, $CD4^+$ T cells and natural killer cells all play a role. Most patients recover completely with the development of neutralizing antibodies. However, a small percentage has fulminant hepatitis due to extensive hepatic necrosis following an overwhelming immune response. There is no chronic infection associated with HAV.

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Epidemiology

Poor sanitation and personal hygiene, and consumption of contaminated food and water, are the commonest causes of HAV transmission. Sporadic HAV infection is often due to person-to-person spread. Outbreaks can occur as a result of spread within family or the common consumption of contaminated food. The contamination event could have occurred at any point during the growing, harvesting, processing, distributing, or serving of the food.

HAV used to be the commonest cause of acute viral hepatitis. In resource-poor countries, almost all children acquire the infection before the age of 10 years and children are known to be effective transmitters of HAV within households. However, with improvement in sanitation and personal hygiene, the prevalence of HAV infection in developed countries has decreased significantly. In the US, the incidence of acute hepatitis A decreased from 12.8 to 0.9 per 100,000 over the last 30 years; while in England and Wales, the annual notifications of acute hepatitis A have fallen more than five-fold in 10 years. This decrease in prevalence is associated with a shift in the age when the infection is acquired. As children with HAV infection tends to be asymptomatic or mild, this change in epidemiology has paradoxically led to an increase in incidence of symptomatic cases seen in adults.

Clinical presentation and natural history

Hepatitis A is an acute, necroinflammatory disease of the liver with an incubation period of 2–6 weeks.

Typical clinical manifestations

The clinical spectrum of HAV infection ranges from asymptomatic infection to fulminant hepatitis. The severity of illness is age dependent. In children, hepatitis A is usually asymptomatic; in contrast, infection in >70% of adults presents with jaundice and high levels of serum aminotransferases.

The onset of hepatitis A is characterized by:

- Prodromal symptoms including anorexia, nausea, malaise, and fever. In children, gastrointestinal symptoms such as diarrhea and vomiting may predominate.
- Jaundice, dark urine and pale stools follow within a few days.
- Mild to moderate tender hepatomegaly is often detected.
- Splenomegaly and posterior cervical lymphadenopathy may occur.
- Rarely, extrahepatic manifestations such as arthritis and vasculitis may accompany the acute illness.
- Serum aminotransferase rise rapidly during the prodromal period.
- Serum bilirubin levels peak later and decline less rapidly than serum aminotransferases. The degree of elevation of transaminases and bilirubin levels does not correlate with the severity of the illness.
- The prothrombin time is usually normal. Persistently abnormal coagulation is an indication for referral to a specialist center as it may indicate the development of fulminant hepatitis.

Jaundice persists for less than 2 weeks in the majority of cases. Clinical illness and laboratory abnormalities recover within 2 months from onset of illness. Children almost universally recover from HAV infections.

Atypical clinical manifestations

Atypical presentation with prolonged cholestasis, fulminant hepatitis, relapsing hepatitis, pleural effusion, and ascites has been reported to occur in 14%.

Prolonged cholestasis. This is characterized by pruritus, loose stools, fatigue, and weight loss in addition to prolonged cholestasis. Histological features include intralobular cholestasis and portal tract infiltrates, associated with dystrophy and paucity of bile ducts.

Fulminant hepatitis. This is a rare complication, with a reported incidence of 0.015–0.5%. Low serum HAV RNA and high bilirubin levels are associated with fulminant hepatitis, which may suggest that HAV-related fulminant hepatitis is due to a host immune response rather than a direct viral effect.

Relapsing hepatitis. This is characterized by a biphasic or relapsing peak of serum aminotransferases in 25% of symptomatic cases. Complete recovery is usual.

Pleural effusion and ascites. These are rare complications that resolve spontaneously.

Extrahepatic manifestations

They are rare and may include: autoimmune hemolytic anemia, aplastic anemia, pure red cell aplasia, acute reactive arthritis, acute pancreatitis, acalculous cholecystitis, Guillain–Barré syndrome, mesangioproliferative glomerulonephritis, and acute renal failure.

Laboratory diagnosis

The two main diagnostic tests for HAV are anti-HAV immunoglobulin M (IgM) and anti-HAV IgG (or total antibody). Anti-HAV IgM peaks during the acute phase of acute hepatitis A and persists for 4–6 months. An isolated weakly reactive anti-HAV IgM results should always be interpreted with caution as false positive results are common. A high IgG level in the presence of weak IgM reactivity would raise doubts on the specificity of the IgM result. A negative anti-HAV IgM result may not exclude acute hepatitis A if the test is performed very early, in which case a repeat sample is required.

The presence of detectable anti-HAV IgG (or total antibody) without anti-HAV IgM suggests immunity to HAV either from previous infection or from vaccination. HAV RNA detection and sequencing are not widely available as diagnostic tests, though these may be useful for epidemiological investigations.

Management of hepatitis A

Management of HAV infection is mainly supportive and includes rest, nutritional support, adequate hydration, and the use of antiemetics and antipyretics. Cases complicated by fulminant hepatic failure and hepatic encephalopathy should be transferred to a center where liver transplantation is available. In cases of prolonged cholestasis, fat-soluble vitamins should be provided, together with antipruritic treatment. Corticosteroids may shorten the duration of cholestasis, but should be used with caution as persistently elevated aminotransferase with progressive liver fibrosis has been reported in a patient treated with steroids [1].

HAV immunization is recommended in all children with chronic liver disease due the increased risk of fulminant hepatic failure and increased mortality with acute hepatitis A infection.

Prevention of hepatitis A

Global strategies to minimize epidemics of HAV infection include improving sanitation and providing education to improve standards of basic hygiene. HAV can also be prevented by immunization, which could be passive or active.

Passive immunization

Human normal immunoglobulin (HNIG), given by intramuscular injection within 14 days of exposure, offers up to 3–6 months of protection against HAV. With increasing evidence of the efficacy of post-exposure immunization using HAV vaccine alone, the role of HNIG has become more secondary.

Active immunization

Two types of HAV vaccines have been developed: inactivated cell cultured based vaccines are used in Western countries and live-attenuated vaccines used primarily in China, India, and Asia. A combined HAV/HBV and HAV/typhoid vaccines are also available. Studies have shown that seroprotective levels of neutralizing antibody developed by 14 days after vaccination with inactivated vaccine. A randomized controlled trial showed non-inferior result of the vaccine compared to HNIG when used within 14 days of exposure [2].

Protective levels of antibodies are achieved and persist for at least 1 year after a single dose of inactivated HAV vaccine. A second dose given after an interval of 6–12 months may increase the duration of protection to up to 10 years. It is not necessary to perform post-vaccination antibody test as the serology assays are not sufficiently sensitive to detect the low but protective level of antibody. HAV vaccine is not licensed for children <1 year of age. In addition to contacts of cases of acute hepatitis A, HAV vaccination is also recommended as pre-exposure prophylaxis for individuals at high risk of acquiring hepatitis A. Some countries, such as the USA, have incorporated HAV vaccine into routine childhood vaccination program.

Hepatitis E virus

Virology and pathogenesis

Hepatitis E virus (HEV) is a small non-enveloped virus with a positive-sense, single-stranded RNA genome. It is classified under the family Hepeviridae and genus *Hepevirus*. There are four HEV genotypes, though all are of the same serotype. Genotypes 1 and 2 infect only human and non-human primates. The main host of genotypes 3 and 4 is swine, but human and other animals such as deer and rabbits could also be infected. Geographically, genotype 1 is mainly found in resource-poor countries in the Far East, South Asia, and Africa; and genotype 2 in Central America and Africa. Genotype 3 is widespread in the swine population of Western countries, whereas genotype 4 is found in the Far East.

Ingested HEV migrates through the gut mucosa to the liver and enters hepatocytes by binding to a yet unidentified receptor. After translation of the structural and non-structural proteins, new virions are assembled and released through biliary secretions and excreted in feces.

Epidemiology

There are two epidemiological patterns of HEV. Genotypes 1 and 2 are common in developing countries and often transmitted by contaminated water. Epidemics typically occur after the rainy season. A striking and unexplained feature is the high frequency of fulminant disease in pregnant women, with a mortality rate as high as 20% during the third trimester. Vertical and perinatal transmissions have been described, with significant morbidity and mortality.

The pattern of HEV infection in Western countries is very different. While some cases are associated with travel to endemic areas, many are autochthonous and caused by genotype 3 infection. Symptomatic disease is mainly seen in older males over 55 years of age. Recent studies suggest that HEV has overtaken HAV as the commonest cause of acute hepatitis in industrialized countries. In the UK, it is estimated that the annual attack rate is 0.1–0.2% and with up to 60,000 cases per year in England. Pigs are implicated as the reservoir as >80% of pigs in the UK are seropositive for HEV and the genomic sequences of human cases were closely related to those seen in the local pig population.

HEV can also be transmitted through blood donation from an asymptomatic or pre-symptomatic donor [3]. As many blood product recipients are immunocompromised, this could potentially result in severe and chronic hepatitis.

Clinical features

Acute hepatitis E

In highly endemic areas (HEV) infection is considered the most common cause of acute viral hepatitis. The infection varies in severity, ranging from subclinical to fulminant hepatitis.

The clinical presentation of HEV infection has broad similarities to (HAV) infection, with most cases being subclinical; however it can be symptomatic in up to 20% of cases mostly in youths and adults (14–40 years old).

The incubation period ranges from 2 to 9 weeks, with symptomatic illness lasting up to 4 weeks. The presenting symptoms are non-specific and include fever, flu-like myalgia, arthralgia, anorexia, hepatomegaly, and vomiting. Patients may also present with symptoms of acute hepatitis. Clinical symptoms are usually accompanied by increased levels of aminotransferases and bilirubin.

Acute HEV is generally self-limiting in immunocompetent children.

Chronic HEV infection

Chronic HEV infection in immunocompromised individuals is an emerging and significant clinical problem. This group is thought to be the main population at risk for chronic hepatitis E which can result in progressive liver fibrosis, cirrhosis, and subsequent decompensated liver disease requiring liver transplantation [4].

There have reports of chronic HEV infection in children following solid organ transplant. HEV seroprevalence was reported as 3.2% in one of the studies evaluating 124 pediatric recipients of solid organ transplant. Liver transplant patients are at increased risk of chronic hepatitis when they acquire HEV infection. Therefore HEV infection has to be considered in the differential diagnosis of graft hepatitis in liver transplant recipients, even in a low endemic country.

Both acute and chronic HEV infections are associated with extrahepatic manifestations which may include acute pancreatitis, encephalitis, Guillain–Barré syndrome, hemolysis, prolonged cholestasis, bilateral brachial neuritis, and proximal myopathy.

Laboratory diagnosis

HEV specific IgG and IgM antibody tests are available as diagnostic tests. HEV IgM peaks during the first 4 weeks of infection and remain positive for 4–5 months. HEV IgG peaks between week 2 and 4 and may remain detectable for several years. The use of HEV RNA assays can be a useful adjunct when the serological result is inconclusive. It is particularly useful during early infection and in the investigation of immunocompromised individuals who are not able to mount a serological response.

Management

Acute HEV is a self-limited disease. There is no specific treatment for acute HEV and only supportive care is indicated. However, in patients with poor prognostic factors such as immunosuppressed status or underlying liver disease, a short course of ribavirin has been shown to produce complete recovery and avoid the need for liver transplantation in case reports and small series.

In solid organ transplant recipients with HEV infection, reducing the doses of immunosuppressive drugs that are aimed at T cells (mainly calcineurin inhibitors) has been proposed as the first-line therapeutic approach. This may result in spontaneous HEV clearance but with the risk of rejection.

Pegylated interferon-α-2a (PEG-IFN-α-2a) has been used as monotherapy for liver transplant recipients with chronic HEV. It is contraindicated in kidney, lung, and heart transplant recipients because of the high risk of rejection. In these cases, ribavirin could be an option as the first line of treatment for chronic HEV since it seems to be well tolerated, safe, and capable of inducing a sustained virological response (SVR). Recommended duration of such therapy is 2–3 months [5].

Prevention

In developing countries, access to clean water is the key to prevent HEV transmission. To prevent genotype 3 HEV in developed countries, improved animal husbandry is required to reduce infection in swine population. Food hygiene should be enhanced and only fully cooked pork products should be consumed. Blood donor screening of HEV should be considered.

Two recombinant vaccines based on HEV genotype 1 have been on trials which showed a high level of efficacy [6]. They may prove useful in outbreak control, but their role against genotype 3 infection in developed countries is not clear.

Parenterally transmitted viral hepatitis

Hepatitis B virus

Virology and pathogenesis

Hepatitis B virus (HBV) is a member of the Hepadnaviridae family which consists of a group of species-specific enveloped hepatotropic DNA viruses infecting various vertebral hosts, including mammals and birds. Viruses of this family carry a viral polymerase with reverse transcriptase property and have a unique lifecycle involving an RNA intermediate and a reverse transcription step. The genome of HBV is a partially double-stranded, partially single-stranded circular DNA of 3200 nucleotides. HBV infects human as well as great apes such as chimpanzees, orang-utans, and gibbons, though each primate species is infected by a distinct variant of HBV. In humans, up to 10 different genotypes (A–J) have been described based on a genetic divergence of >8% over the entire genome. Some genotypes have a wide geographical distribution but some are relatively restricted in its distribution (Table 13.1).

The virion of HBV, also known as the Dane particle, is a 42-nm diameter spherical structure. It consists of an outer coat of hepatitis B surface antigen (HBsAg) and an inner core of hepatitis B core antigen (HBcAg). Typically, HBV

Table 13.1 Geographical distribution of HBV genotypes.

Genotype	Geographical distribution
A	Europe, North America, Australia, Africa
B	East and South East Asia, Alaska
C	East and South East Asia, Pacific
D	Mediterranean, Africa, Europe, West Asia
E	West Africa
F	Central and South America
G	Europe, North America
H	Central America
I (proposed)	Vietnam, Laos
J (proposed)	Japan

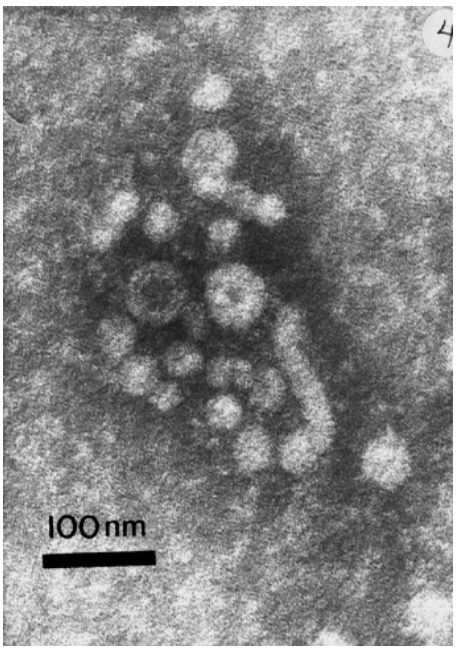

Figure 13.1 Electron micrograph of HBV, showing the double-shelled virus particles and small spherical and tubular subviral particles.

overproduces its outer coat and the excess HBsAg accumulates and aggregates into small 22-nm diameter subviral particles and filaments (Figure 13.1). All HBV genotypes carry a common antigenic epitope, the "a" determinant, on HBsAg. Antibodies raised against this determinant provide cross-protection against infection by all genotypes.

The functional receptor for hepatitis B on the cell surface of hepatocytes is recently identified as the transmembrane transporter protein sodium taurocholate co-transporting polypeptide. The pre-S1 domain of the large HBsAg molecule is thought to bind to this receptor to gain cell entry. Once uncoated, the virion core migrates to the nucleus and the viral polymerase carried in the virion closes the gap and the incomplete single-stranded part of the DNA genome to form a covalent closed circular DNA (cccDNA) molecule. This cccDNA acts a mini-chromosome, provides a stable reservoir for viral replication and serves as a template for the production of several subgenomic mRNA species and a full-length pregenomic RNA. Viral integration into host chromosome can occur during chronic infection and could contribute to oncogenesis, but unlike human immunodeficiency virus (HIV), integration per se is not required in the lifecycle of HBV.

Viral proteins are transcribed from four overlapping open reading frames (ORF) of the viral genome: ORF-C (HBcAg and HBeAg), ORF-S (HBsAg: three domains pre-S1, pre-S2, and S), ORF-P (viral polymerase), and ORF-X (X protein, a transactivator).

The pregenomic RNA is packed into particles with polymerase enzyme and surrounded by core protein. RNA is reverse transcribed into DNA, and some of these progeny genomes cycle back into the nucleus to form further cccDNA to maintain the pool of infected hepatocytes. The majority of the core particles are then coated with HBsAg.

The pregenomic RNA has a stem loop structure in the precore region that codes for a secretory protein HBeAg. The role of HBeAg is not entirely clear as it is not part of the virion and not essential for viral replication. It is believed that HBeAg may have a role in inducing immune tolerance, particular in young infants exposed to maternal HBeAg in utero. In the early phase of chronic infection, the presence of HBeAg is usually correlated with a high HBV DNA viral load and can therefore be used as a surrogate marker for viral replication. A mutation in the precore region position 1896 from G to A is encouraged in some genotypes during chronic infection, as it increases the stability of the hairpin structure. However, this mutation also leads to a premature termination of the translation of HBeAg, resulting in seroconversion from HBeAg to anti-HBe, while with persistent viral replication. A similar effect can be achieved through mutations in the basal core promoter (BCP) region. Precore and BCP mutations often coexist in HBeAg-negative variants.

Most acute HBV infections in older children and adults are self-limiting, and the virus is cleared with resultant long-term immunity. People who recovered tend to have strong T-cell responses to HBV. The elimination of infected cells containing cccDNA is important in clearing the infection from the liver. If these cells are not cleared, they remain a source of virus and are important in the development of persistent infection.

In young children and those who are immunocompromised, a state of immune tolerance is often developed. This allows the virus to replicate heavily in the hepatocytes without any immune resistance. When the tolerance is broken in early adulthood or during immune reconstitution, the cellular immune response to the presence of HBV in the hepatocyte is responsible for the liver damage. Some people eventually managed to suppress the virus and enter into a state of inactive infection whereas some have persistent liver damage due to ineffective attempts to clear the virus, either the wild type virus

(HBeAg-positive chronic hepatitis B; CHB) or the HBeAg-negative variants (HBeAg-negative CHB). Clinical outcome is determined by a complex interplay between HBV replication with the immune response of the host.

HBV is not a cytopathic virus. Immunopathology leads to liver damage, ranged from minimal inflammatory infiltrate to piecemeal necrosis, and from mild fibrosis to established cirrhosis (Figure 13.2). An overwhelming immune response is thought to be the cause of fulminant HBV infection, and a burst of aggressive immune activity could be responsible for the flares seen in exacerbation of chronic disease. Pathognomonic features of HBV are the detection by immunohistochemistry of HBcAg in the nuclei and cytoplasm of hepatocytes, and the presence of "ground-glass" hepatocytes due to the abundance of HBsAg in the endoplasmic reticulum (Figure 13.3).

Chronic HBV infection is strongly associated with hepatocellular carcinoma (HCC). Up to 80% of HCC worldwide is believed to be caused by HBV. While cirrhosis is a significant factor in the development of HCC in CHB, a proportion of HCC occurs in the absence of underlying cirrhosis.

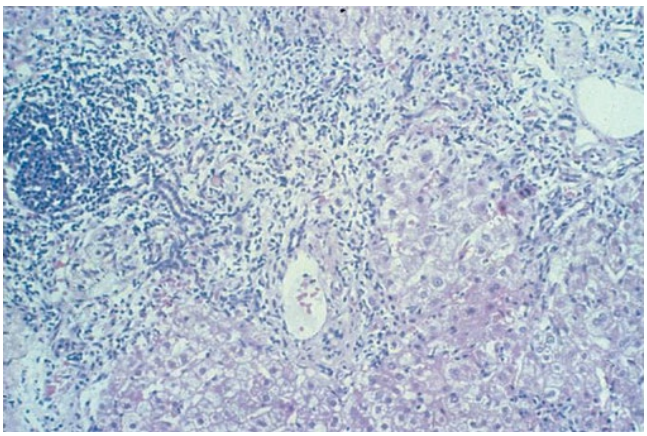

Figure 13.2 Chronic hepatitis B is characterized histologically by portal inflammation, consisting predominantly of lymphocytes and plasma cells. It may be limited to the portal tract or may spill out into the lobule.

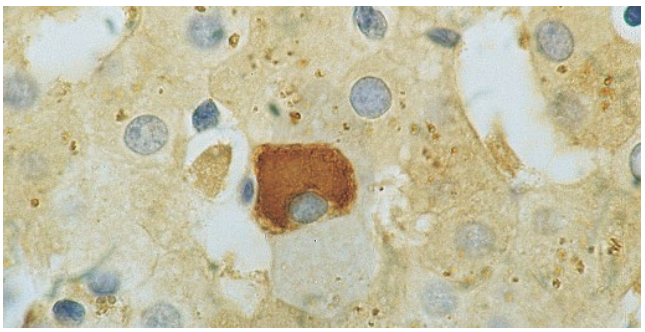

Figure 13.3 Hepatitis B infection may be suggested by the detection of hepatitis B surface antigen. (Orcein, original magnification × 1000.)

Epidemiology

Globally, it is estimated that over 350 million people are chronically infected with HBV. HBsAg prevalence is highest in China, South East Asia, Africa, Pacific Islands, some areas of the Middle East, and the Amazon basin (5–10%). Intermediate rates of chronic infections are also found in the south-central and southwest Asia, eastern and southern Europe, Russia, and Central and South America (2–7%). Less than 2% of the population in Western Europe and North America is chronically infected [7].

Most infection in highly endemic areas is acquired through mother-to-child transmission. Transmission may occur through placental tears, trauma during delivery, and contact of the infant mucous membranes with infected maternal fluids. Intrauterine transmission may occur, but this does not appear to be a major route. Mothers who are HBeAg positive have the highest infectivity and without prophylaxis, a 70–90% risk of transmitting infection to their offspring. Those who are anti-HBe positive have a lower risk of transmitting infection. However, if the offspring of these individuals are infected, they are at risk of developing fulminant hepatitis due to a mutant virus.

As HBV is present in infected individuals in high concentrations in blood, serum and serous exudates, semen, vaginal fluid, and saliva, horizontal transmission is also common through parenteral and sexual exposure routes. Although HBV is also found in low concentrations in feces and breast milk, these are not associated with a significant risk of transmission. People at high risk include those requiring frequent transfusions (e.g., with hemolytic anemia, thalassemia, hemophilia), those with high-risk sexual behavior, intravenous drug users, and carers of infected individuals (including health-care workers). In some countries, the use of unsafe medical injections also contributes to HBV transmission.

Environmental transmission in childhood may occur by exposure to infected body fluids through a broken skin surface, either directly by biting or by accidental contamination. Household transmission of HBV from infected family members may occur; up to 40% of children who are born to infected mothers but not infected at birth acquire infection in the first 5 years of life. An increased risk of environmental transmission also occurs in residential institutions and hemodialysis units with inadequate infection control procedures.

Clinical presentations

Acute hepatitis B

The incubation period of acute hepatitis B can range from 1 to 6 months after viral exposure. The majority of children with acute hepatitis B infection have a mild, asymptomatic, and subclinical illness. Clinical symptoms and signs of hepatitis, most commonly constitutional symptoms, usually precedes the onset of jaundice. During this phase, serum alanine aminotransferase (ALT) levels rise and high levels of

Table 13.2 Serological markers of HBV infection.

HBsAg	Anti-HBs (>10 IU/L)	Anti-HBc	Anti-HBc IgM	HBeAg	Anti-HBe	HBV DNA*	Scenarios
+	–	+	++	+	–	+++	Acute HBV infection
–	–	+	+	–	–	+	Seroconversion window after acute HBV infection
–	+/–	+	–	–	+/–	–	Recovered hepatitis B with HBsAg clearance
–	+	–	–	–	–	–	Immunity due to vaccination
+	–	+	+/–	+	–	++++	HBeAg-positive chronic hepatitis B
+	–	+	+/–	–	+	++/+++	HBeAg-negative chronic hepatitis B
+	–	+	–	–	+	+	Chronic inactive hepatitis B
–	–	+	–	–	+/–	+	Occult hepatitis B

*HBV DNA levels: – undetectable <20 IU/mL; + <2000 IU/mL; ++ 2000–20,000 IU/mL; +++ >20,000 IU/mL; ++++ >200,000 IU/mL.

HBsAg and HBV DNA are detectable. Viral levels decrease during the icteric phase. In convalescence, jaundice resolves but constitutional symptoms may last for weeks or even months. During the convalescence phase, HBsAg is cleared followed by the disappearance of detectable HBV DNA from serum. Serological markers of acute hepatitis B infection are summarized in Table 13.2.

Fulminant hepatic failure requiring liver transplant may occur in up to 1% of infected patients.

Mild or asymptomatic acute infection is more likely to progress to chronicity and those requiring liver transplantation for fulminant infection are less likely to have recurrence in the transplanted liver.

Chronic hepatitis B infection

CHB is defined as persistence of HBsAg for >6 months. Ninety per cent of infected neonates and 25–50% of acutely infected children between the ages of 1 and 5 years will develop chronic infection. The risk of developing chronic infection is less in adolescents and adults, <5% of symptomatic and 5–10% of asymptomatic infected teenagers and adults will develop CHB.

Children with CHB generally have a mild disease and are mostly asymptomatic with normal growth and physical examination. Non-specific symptoms as fatigue and anorexia may occur and diagnosis is usually made by screening those known to be at risk.

HBV infections can be associated with extrahepatic manifestations in 1–10% of patients as a result of the host's immune reaction to the viral infection. These manifestations include:

- Serum sickness like syndrome and reactive arthritis. The serum sickness like syndrome occurs in the setting of acute hepatitis B and presents with fever, skin rash, and polyarteritis which subsides shortly after the onset of jaundice. Clearance of the virus leads to rapid resolution of the illness.
- Vasculitis (mainly polyarteritis nodosa). Immune-mediated vascular injury can involve large, medium, and small vessels. Multisystem involvement is common, including arthritis, renal disease (proteinuria and hematuria), heart disease (pericarditis and congestive heart failure), hypertension, gastrointestinal disease (acute abdominal pain and bleeding), and skin vasculitic lesions.
- Membranous glomerulonephritis is the most common form of renal involvement in HBV infection. Liver disease may be mild or absent and spontaneous remission is achieved in 30–60% of patients.
- Papular acrodermatitis of childhood (Gianotti–Crosti syndrome). Skin lesions are non-pruritic maculopapular and erythematous involving the face and extremities. The rash may last 15–20 days and can either precede or follow the onset of jaundice in acute hepatitis B. Hepatomegaly and generalized lymphadenopathy were also described.

New HBV antiviral drugs should improve the prognosis of vascular and renal involvement.

Natural history and outcome

CHB is a dynamic process marked by four phases which determine the risk of disease progression and responsiveness to therapy (Table 13.3).

Immune tolerant phase

The majority of perinatally infected children are in this phase, which may last for decades. Transplacental transfer of maternal HBeAg is believed to have a role in inducing T-cell tolerance which explains the higher rate of chronicity in infants born to HBeAg-positive mothers.

It is characterized by a normal serum levels of ALT, high HBV DNA levels (reflecting high levels of viral replication), and both HBsAg and HBeAg are detectable in the serum. Histologically there is minimal liver necroinflammation and fibrosis. Children are highly contagious during this phase.

The current available antiviral treatment options are not effective in achieving viral clearance during this phase and hence risk increasing the risk of HBV resistance. Therefore children are not routinely treated in the immune tolerant phase and are only offered treatment as part of clinical trials.

Immune clearance phase

Viral mutants (precore mutants) that are not capable of secreting HBeAg emerge. As a result of the decline in HBeAg, a shift from the HBeAg-specific T-cell tolerance to T-cell

Table 13.3 Natural history and phases of chronic HBV infection.

Phase	HBeAg	HBeAb	Serum ALT	HBV DNA	Liver inflammation/fibrosis
Immune tolerance	P	N	Normal	Very high (≥200,000)	Absent/minimal
Immune clearance	N	P/N	Elevated/ fluctuating	Decreased levels (2000–200,000)	Moderate to severe
Inactive HBV carrier	N	P	Normal	Undetectable (<20 IU/mL) Or low (<2000 IU/mL)	Absent/minimal inflammation Fibrosis may regress
Reactivation	N	P	Elevated	>2000 IU/mL	Active inflammation

N, negative; P, positive.

activation occurs and initiates the immune clearance phase. This immune response results in an inflammatory process within the liver reflected by increased or fluctuating ALT levels and moderate necroinflammation histologically. Significant inflammation and cirrhosis are rare histological findings in children. This immune active phase leads to a decline in HBV DNA levels and seroconversion to anti-HBe antibodies. ALT levels significantly increase before HBeAg clearance and may remain elevated for 6 months after seroconversion. Spontaneous seroconversion occurs earlier and more frequently in children who have acquired HBV horizontally than in those infected perinatally. This phase usually occurs after puberty and is delayed in children with genotype C.

Children continue to be asymptomatic, however, the longer children remain in this phase, the more likely they are to develop chronic liver damage.

Inactive hepatitis B virus carrier phase

This phase, which follows seroconversion to anti-HBe antibody, is characterized by low or undetectable HBV DNA levels and normal serum ALT levels. Children in this phase have non-specific or minimal fibrosis. A small percentage of patients (0.6% per year) may clear (HBsAg) during this phase.

Reactivation phase

This can occur in 5% of anti-HBe-positive children, usually secondary to developing viral mutation. This mutant virus is able to escape the immune detection, leading to persistent viral replication, increase in HBV-DNA and ALT levels and the development of active hepatitis in patients who are HBeAg negative.

Laboratory diagnosis

HBsAg and HBeAg are the first markers to be detected during acute infection, even before the onset of symptoms. At the time of onset, anti-HBc can be detected, mostly as IgM antibody (IgM anti-HBc). However, IgM anti-HBc can also be detected at low levels by sensitive methods during persistent infection and is therefore not always a reliable marker of an acute infection. During clearance of the acute infection leading to recovery, HBeAg is replaced by anti-HBe, and HBsAg replaced by anti-HBs. There is a potential window period during the seroconversion phase when HBsAg, HBeAg, anti-HBs, and anti-HBe are all not detectable and the only positive detectable markers are IgM and IgG anti-HBc and HBV DNA.

In chronic infection, the initial serological response is as for an acute infection. In the absence of symptoms, however, this phase may not be detected. Ongoing viral replication is accompanied by persistence of HBeAg, although loss of HBeAg and anti-HBe seroconversion may occur despite ongoing chronic infection due to the presence of HBeAg-negative variants. In some immunocompromised patients (such as HIV infection), anti-HBc may not be produced and therefore cannot be used as a marker for the absence of HBV infection. HBsAg should always be used as the primary screening test for such individuals. Table 13.2 lists some of the possible combination of serology results in various clinical scenarios.

Quantitative measurement of HBV DNA (viral load) is a useful indicator of viral replication. Current available commercial real-time polymerase chain reaction (PCR) assays are highly sensitive with detection limit as low as 20 IU/mL and linear range up to 10^9 IU/mL. As a result, HBV DNA viral load can also be used to assess response to treatment. Quantitative HBsAg assay is also available. As the concentration of serum HBsAg could reflect the level of transcription activity in the liver, it could be used as a surrogate marker for the amount of transcriptionally active cccDNA in hepatocytes. Hence, HBsAg level monitoring can be used to guide interferon therapy and may also help to identify inactive disease in HBeAg-negative individuals.

Occult HBV infection is described when HBsAg is not detectable using standard techniques. In some cases, this could be due to vaccine escape surface antigen mutants. The mutated HBsAg is present but not detected by the laboratory tests. Some immunoassays, particularly those dependent on monoclonal antibodies, are more prone to false negative HBsAg due to escape mutants. However, in most cases of occult HBV infection, the absence of HBsAg is genuine and the diagnosis is made by recognizing HBsAg-negative, anti-HBc-positive

Table 13.4 Suggested intervals of monitoring children with chronic HBV (see text for abbreviations).

Phase of disease	Parameter monitored	Frequency of monitoring
At diagnosis	LFTs, AFP, HBV DNA, HBV serology	Baseline
Immune tolerance	LFTs, HBeAg, HBeAb, HBV DNA, AFP	Every 6–12 months
Immune clearance	LFTs, HBeAg, HBeAb, HBV DNA, AFP	Every 3 months
Inactive carrier	LFTs, HBV DNA	Every 6–12 months

serology, and confirmed by detecting HBV DNA. For this reason, all potential liver transplant donations should also be screened for anti-HBc, to avoid the risk of transplanting a liver with intracellular cccDNA within the hepatocytes, which would reactivate to cause an active infection after transplantation in the presence of immunosuppression.

Liver biopsy is not required for diagnosis, but may be useful pre-treatment or if there are clinical complications.

Management of hepatitis B virus infection

Management of children with CHB requires expert multidisciplinary input, support, counseling in addition to screening and immunization of other family members.

Routine review should include standard liver function tests (LFTs), α-fetoprotein (AFP), HBV serology, HBV DNA, and abdominal ultrasound scan for monitoring of disease progression, and/or HCC (Table 13.4). It is recommended to perform an ultrasound scan for HCC surveillance yearly or more frequently depending on the stage of fibrosis. Liver biopsy is not required for monitoring disease. The use of transient elastography (FibroScan®) may be useful in detecting the development of fibrosis or cirrhosis, but it has not yet been validated in children.

Why, when, and who to treat

The main goals of therapy are to prevent disease progression, reduce the risk of developing cirrhosis and HCC, and to reduce the pool of carriers. This can be achieved by suppressing viral replication, reducing liver inflammation, and achieving sustained HBeAg seroconversion. The ultimate goal is HBsAg loss; however this is difficult to achieve with current therapeutic options.

A decision to treat should take into account several factors including the age and gender of the child, ALT and HBV DNA levels, liver histology, the efficacy of current antivirals and their side effects, existing co-morbidities, and family history of liver disease or (HCC).

Predictors of favourable treatment response include elevated ALT levels, low HBV DNA levels and horizontally acquired infection.

Treatment should be considered in children with persistently elevated ALT levels (>1.5 upper limit of normal) and high HBV DNA levels. The cut-off level of HBV DNA has not been yet defined in children however values of >2000 IU/mL have been used in adults.

Liver biopsy for assessing the degree of inflammation and the stage of fibrosis is recommended prior to initiating treatment. At least moderate inflammation or fibrosis is required to ensure better response to treatment, however children with a family history of HCC should be considered for treatment even with mild inflammation or fibrosis in the liver [8, 9].

Current treatment options

Drugs currently available for the treatment of CHB in children include interferon-α (IFN-α), and nucleos(t)ide analogues.

Interferon-α. It is the first drug to be approved for treating children with CHB. The recommended dose to treat children aged 2 years or older is 5–10 million units/m^2 subcutaneously three times weekly for 24 weeks. The efficacy of IFN ranges from 20 to 40% with improved response rate in patients with horizontally transmitted infection and elevated ALT levels. The benefit of prednisolone priming is unproven but it may increase the spontaneous remission rate and reduce time to seroconversion.

Pegylated interferon-α. Pegylated interferon-α (PEG-IFN-α), in which the addition of a polyethylene glycol (PEG) moiety increases its half-life and reduces the frequency of injection to once-weekly rather than 3 times/week, has not yet been approved for HBV treatment in children but clinical trials in children are ongoing. Studies in adults demonstrated highest HBeAg seroconversion rate with 48-week treatment regimen and a similar safety profile to IFN.

Side effects of IFN-α and PEG-IFN are similar and include:

- Fever.
- "Flu like" symptoms.
- Bone marrow suppression.
- Gastrointestinal symptoms.
- Autoimmune thyroid disease.
- Neuropsychiatric effects, particularly depression.

IFN is contraindicated in children with decompensated liver disease, cytopenia, severe renal or cardiac disorders, and autoimmune disease. The advantage of IFN and PEG-IFN is that they have a finite duration of treatment and are not associated with the development of viral resistance.

Oral nucleos(t)ide analogues. Compared to IFN, oral nucleos(t)ide analogues (NAs) are easily administered with less significant side effects. However, the absence of finite treatment duration and the development of antiviral resistant mutations with long-term treatment limits their use. The recent approval of new NAs with higher resistance barrier has means they can be considered as first-line treatment option in children and adolescents.

It is essential that all patients who receive NAs therapy for CHB are monitored every 3 months for virological breakthrough (HBV DNA level increase of more than 1 $\log_{10}$ IU/mL) during treatment with early adaptation of treatment if virological breakthrough is detected. In addition monitoring of durability of response and viral relapse after discontinuation of therapy is recommended. The exact duration of NA treatment has not been established, however it is recommended to continue treatment for a minimum of 12 months after HBe seroconversion and undetectable HBV DNA.

Lamivudine. Lamivudine is an oral nucleoside analogue approved for treating children over 3 years of age. The recommended dose is 3 mg/kg/day (maximum 100 mg/day), administered orally once daily. In a large multicenter randomized, double-blind, placebo-controlled trial in HBeAg-positive children, virological response (HBeAg seroconversion and undetectable HBV DNA levels) was achieved in 23% after 52 weeks of treatment which increased to 35% in children with elevated ALT levels at the start of therapy.

The main limitation of lamivudine is relapse following discontinuation of treatment and the development of mutation within the YMDD motif of HBV polymerase. The YMDD variant emerges in 15–20% after 1 year of treatment and more than 80% patients were found to develop resistance after 48 months of treatment. It is therefore recommended to discontinue treatment if suppression of viral replication is not achieved within 6 months. Lamivudine is safe and well tolerated by children. No significant side effects were reported after 3 years of treatment.

Adefovir dipivoxil. Adefovir (ADV) is a purine analogue that is approved for treating children who are ≥12 years of age. It inhibits viral replication by binding to DNA polymerase. In a large randomized controlled trial, virological response was achieved in 23% of children in the 12 to <18-year age group. No statistically significant difference compared to placebo was reported in the younger age group. ADV is generally well tolerated. Nephrotoxicity has been reported in adults but not in children and is reversible if the treatment is rapidly stopped. The rate of selection for ADV-resistant virus is lower than lamivudine; however a 30% resistance rate at 5 years has been reported.

Entecavir. Entecavir is a carbocyclic analogue 2′ deoxyguanosine and a potent and selective inhibitor of HBV replication in vitro. It has been approved for CHB treatment in adolescents aged 16 years or older. The recommended dose is 0.5 mg once daily.

Entecavir was proven in adults to be more effective than lamividune and ADV in treating HBeAg-positive and HBeAg-negative CHB. A phase III clinical trial in children is underway.

Entecavir has a high barrier to resistance with only 0.4% resistance rate reported from adult studies. Resistence is more likely to occur in patients with lamivudine resistance and a higher treatment dose (1 mg/day) is recommended.

Telbivudine. Telbivudine is a nucleoside analogue with a potent antiviral activity. Resistance rate is lower than lamivudine but higher than ADV. A phase I trial is ongoing to assess the safety of telbuvidine in children with CHB.

Tenofovir. Tenofovir disoproxil fumarate (TDF) is a nucleotide analogue with an activity against HBV DNA polymerase. It is approved for children ≥12 years. Studies in adults have shown that TDF is superior to ADV in treating HBeAg-positive and HBeAg-negative patients. In a recent study assessing the efficacy and tolerability of tenofovir in adolescents with CHB, tenofovir was shown to be effective and well tolerated. Viral suppression was achieved in 89% of treated adolescents compared to 0% in the placebo group.

Unlike entecavir, tenofovir is effective against lamivudine-resistant mutant strains.

Combination therapy

Drugs used in combination should have additive/synergistic effects and preferably different mechanisms of action. Combinations of NAs are unlikely to be more effective than therapy with single NA and can have antagonistic effects.

In adults the combination of PEG-IFN and lamivudine led to an improvement in viral suppression, but no difference in seroconversion rates. There are a few studies of PEG-IFN in children with HBV with or without lamivudine but the results are inconclusive. A recent study by Chu *et al.* [10], has demonstrated more effective virological response with add-on ADV to lamivudine in children with lamivudine resistance.

Immunosuppressed children

All children receiving chemotherapy or immunosuppressive therapy (corticosteroids, rituximab, and other monoclonal antibodies) should be screened for HBV prior to treatment. Seronegative patients should be immunized against hepatitis B.

Children with CHB or HBsAg-negative, anti-HBc-positive, and detectable HBV DNA should receive NAs therapy during immunosuppressive treatment and for at least 6 months after cessation of immunosuppression. The most experience with pre-emptive therapy has been with lamivudine which reduces the risk of HBV reactivation. Lamivudine is sufficient in children receiving immune suppression for a short duration and if they have low HBV DNA levels (<2000 IU/mL). In children with prolonged or repeated cycles of immune suppression therapy and those with high HBV DNA it is recommended to use NAs with high genetic barrier to resistance (entecavir or tenofovir).

Children with HBsAg-negative, anti-HBc-positive, and undetectable HBV DNA should be monitored closely (every 1–3 months) during immunosuppression therapy and NAs treatment should be started upon confirmation of HBV reactivation.

Table 13.5 Current UK recommendations for immunization against HBV in neonates (HBIG also advocated for acute hepatitis B during pregnancy or babies born to HBsAg-positive mother with birth weight <1500 g).

Maternal hepatitis B status				Given to baby at birth	
HBsAg	**HBeAg**	**Anti-HBe**	**HBV DNA**	**HBV vaccine**	**HBIG**
+	+	–	±	Yes	Yes
+	–	–	±	Yes	Yes
+	Unknown	Unknown	Unknown	Yes	Yes
+	–	+	≥1,000,000 IU/mL	Yes	Yes
+	–	+	<1,000,000 IU/mL or unknown	Yes	No
–	NT	NT	NT	No*	No

*HBV vaccine should also be given to babies with other risk factors of acquiring HBV infection (e.g., close family contact).
NT, test not required.

Lamivudine prophylaxis is also recommended for anti-HBc-positive children undergoing bone marrow or stem cell transplantation [11, 12].

Prevention

Hepatitis B vaccine is one of the most successful vaccines developed. Since its introduction in Taiwan in 1984, the prevalence of HBsAg in children decreased from 10% to 0.5% between 1984 and 2009 [13]. The incidence of HCC in children and adolescents was reduced by 70%. The current HBV vaccine is based on recombinant HBsAg. The standard immunization schedule consists of a three-dose regimen usually spaced at 0, 1, and 6 months, though a rapid schedule of 0, 1, and 2 months with a booster dose at 1 year is frequently used in neonates born to infected mother. Anti-HBs level of >10 IU/L is induced after successful immunization. The effect of a successful immunization is believed to be longlasting, though vaccine non-response could occur in about 5% of individuals, particular in older men, dialysis patients and immunocompromised individuals. The vaccine response rate in children is usually good. HBV transmission to the newborn can be successfully prevented by immunization starting at birth, with an effective response rate of up to 97%.

Many countries have adopted universal childhood HBV immunization as economic and epidemiological evaluations indicated that this is cost effective. However, some countries, including the UK and some Scandinavian countries, have adopted a targeted vaccination strategy based on risk. The burden of CHB in these countries is likely to be heavily influenced by adult migration rather than indigenous childhood acquisition. However, the availability of combination vaccines covering diphtheria, tetanus, pertussis, HBV, polio, and *Haemophilus influenzae* type b (DTaP-HB-IPV-Hib) may alter the cost effectiveness argument.

In the absence of universal immunization, at-risk infants need to be identified for selective immunization. A combination of hepatitis B immunoglobulin (HBIG) together with vaccine provide more protection in comparison with vaccine alone, particularly in babies born to the higher risk HBeAg-positive mothers. In countries where HBIG can be easily and safely resourced, a combination of HBIG and vaccine at birth with a further two or three doses of vaccine later provides optimal protection, irrespective of maternal HBeAg status. However, a vaccine-alone protocol starting at birth was almost as effective as the combination and may be more appropriate for countries with high endemicity and low resources. In countries such as the UK, the use of HBIG in combination with vaccine is based on risk assessment during pregnancy (Table 13.5). However, the complicated administration protocol means that currently in the UK, only about 75% of infants receive a full course on time, leading to HBV infection of these infants.

Children with chronic HBV infection are at risk of social isolation and stigmatization if inappropriate guidance is given to their families, carers, health workers, and other professionals. As there is no obligation for parents to inform schools and nurseries of their child's HBV status, the same precautions should apply in dealing with blood/body fluid spillage of all children. Staff at schools and other institutions, as well as health-care workers, should adhere to the principle of universal Infection control precautions. During hospital admission, children with HBV infection do not need to be nursed in isolation. At home and in school, children's social and sporting activities should not be restricted on account of their HBV status. Immunization should be offered to all non-immune family members.

Liver transplantation

Liver transplantation is rarely required in childhood except for fulminant hepatitis. The recurrence of hepatitis B is unusual following transplantation for acute fulminant hepatitis but common following transplantation for CHB due to persistence of HBV in extrahepatic sites. It is therefore recommended to use a combination of oral lamivudine and anti-HBV HBIG to prevent recurrence in the graft.

Hepatitis D virus

Virology and pathogenesis

Hepatitis D or delta virus (HDV) is a defective virus that can only replicate in the presence of HBV. It is a small RNA virus with a circular single-stranded RNA genome bound within a delta-specific antigen and coated with the envelope protein of HBV HBsAg. There are at least eight genotypes of HDV: type 1 is found worldwide; type 2 and 4 is in the Far East; type 3 in the Amazon region of South America; and types 5–8 in sub-Saharan Africa.

As HDV is coated by HBsAg, it enters hepatocytes through the HBV receptor. HDV does not encode its own viral RNA polymerase and utilize host RNA polymerase II for replication.

Epidemiology

The route of transmission is parenteral, with infection occurring either at the same time (coinfection) or subsequent to HBV acquisition (superinfection). It was initially thought that HDV infection in Western countries is mainly associated with intravenous drug use, but recent studies have shown that migration from endemic areas, such as Eastern Europe and sub-Saharan Africa, is a significant factor in the epidemiology [14]. Perinatal acquisition of HDV in infant is rare and can be effectively prevented through hepatitis B vaccination at birth.

Clinical presentation and natural history

Coinfection

HDV coinfection often presents as acute hepatitis with clinical manifestations similar to acute HBV infection. Clinical presentation ranges from mild to severe and occasionally fulminant hepatitis. HDV tends to worsen acute HBV course of infection.

HBV/HDV coinfection usually resolves with complete recovery. The rate of progression to chronic hepatitis is similar to that of isolated HBV infection.

Superinfection

Superinfection may present as an exacerbation of pre-existing HBV infection or as a new hepatitis in a previously asymptomatic HBV carrier. In the majority of cases, superinfection results in chronic delta hepatitis and is characterized by a more severe form of chronic hepatitis. Chronic delta hepatitis is characterized by rapid progression to cirrhosis in 85% within few years. It also carries an increased risk developing HCC.

Laboratory diagnosis

Testing for HDV is only necessary when HBsAg is positive. The first-line screening test is usually anti-HDV IgG. HDV antigen and anti-HDV IgM are secondary markers and if present, may indicate the presence of viral activity. However, quantitative HDV RNA is the best test to monitor viral activity. HDV infection is often associated with suppression of HBV activity, with a low HBV viral load in the presence of ongoing liver damage.

Treatment

Anti-HBV therapies that can eliminate HBsAg should lead to clearance of associated HDV infection.

Acute HDV infection

Treatment is mainly supportive with close monitoring for possible fulminant hepatitis for which liver transplantation is the only treatment.

Chronic HDV infection

The only approved treatment for HDV is IFN-α. High doses (5 million units daily or 9–10 million units three times weekly) for over a year are required. PEG-IFN-α has also been used with reported sustained viral response (negative HDV RNA 6 months after completing treatment) of 20–43%.

Better response rates were achieved in the treatment of naive patients and those with low γ-glutamyltransferase levels. Relapse is common and may occur more than 1 year after cessation of treatment.

Other agents have been evaluated including lamivudine and ADV without significant clinical efficacy [15].

Prevention

The prevention of HBV infection will also prevent HDV infection. Identification of HBV-infected mothers and immunization of their babies against HBV prevent HDV infection in children.

Hepatitis C virus

Virology and pathogenesis

Hepatitis C virus (HCV) is an enveloped positive-sense, single-stranded RNA virus. It is a member of the Flaviviridae family and classified under the genus *Hepacivirus*. The RNA genome codes for a large polyprotein of >3000 amino acids. Flanking the coding region is a 5′ and 3′ untranslated region (UTR). During and after translation, the polyprotein is cleaved by proteases into 10 proteins (Table 13.6)

Viral entry involves interaction of the E2 glycoprotein with a number of cellular proteins, which are highly expressed in hepatocytes, which explain its tropism. HCV can also enter into lymphocytes through interaction with CD81, thus explaining extrahepatic features and immune dysregulation sometimes seen in chronic HCV infection. After release of the viral genome, production of the polyprotein is initiated through binding of ribosomes to the internal ribosomal entry site (IRES) site in the 5′UTR. NS4B and NS5A together form a membranous web in which the NS5B polymerase replicates the RNA genome to produce both positive- and negative-sense RNA strands (10 : 1 ratio). The excessively produced positive sense RNA is encapsidated by the core

Table 13.6 Arrangement of the HCV genome and the functions of each encoded gene, listing from the 5′ to 3′ end (see text for abbreviations).

Name of the genome region	Name of protein (if any)	Functions
5′ UTR	–	Contain internal ribosomal entry site (IRES) for initiation of translation
Core	Nucleocapsid protein	Structural protein, packaging function
E1	Envelope glycoprotein	Viral envelope proteins for cell attachment and entry
E2	Envelope glycoprotein	Viral envelope proteins for cell attachment and entry
P7	Viroporin	Ion channel
NS2	Membrane-anchored cysteine protease	Cleaves NS2 and NS3
NS3	Serine protease-helicase	Cleaves most of the non-structural proteins
NS4A	NS3 protease cofactor	Cofactor for NS3
NS4B	Membrane remodeling protein	Formation of replication complex
NS5A	Phosphoprotein	Interaction with host proteins to form a polymerase complex
NS5B	RNA-dependent RNA polymerase	Replication of RNA genome
3′ UTR	–	Important for RNA replication

protein and coated with E1 and E2 glycoproteins before release extracellularly.

A remarkable feature of HCV is the degree of genetic diversity. Based on sequence variations, seven confirmed genotypes and 67 subtypes were described (Table 13.7). There are sequence variations among HCV within an infected individual at any one time, resulting in the presence of quasi-species. Some HCV genotypes have a restricted geographic distribution, but worldwide, genotypes 1 and 3 are the commonest. In the UK, genotype 1 and genotype 3 together account for 90% of the infection. Recombinant genotypes have been described. The commonest recombinant form (RF2k/1b) was reported in Europe which consists of a genotype 2k-like sequence at the 5′ end, and a genotype 1b-like sequence at the 3′ end. Discrepant genotyping results could be reported in such viral strains depending on the region of the RNA genome used for genotyping.

There is no evidence that HCV is directly cytopathic to hepatocytes and there is no direct correlation between the level of viremia and the level of liver damage. Immune-mediated damage, as with hepatitis B, is thought to be important. A strong virus-specific CD8 T-cell response is associated with spontaneous viral clearance after acute infection, whereas chronic infection is associated with weak, oligo/mono-specific CD4 and CD8 T-cell response. A single nucleotide polymorphism (SNP) around the human IL-28B (*IFNλ*) gene in chromosome 19 was found to have a

Table 13.7 Geographical distribution of HCV genotypes.

Genotype	Geographical distribution
1 (7 subtypes)	America, Europe, Africa
2 (11 subtypes)	Europe, South East Asia
3 (6 subtypes)	Worldwide, south Asia, South East Asia
4 (17 subtypes)	Egypt, Central Africa, Middle East
5 (1 subtype)	South Africa
6 (24 subtypes)	Far East
7 (1 subtype)	Central Africa

significant association with spontaneous clearance and the respond to interferon treatment in the more difficult to treat genotype 1 patients.

The histological features of chronic HCV infection are characterized by the presence of hepatic steatosis, bile duct injury, and portal lymphoid aggregates. In chronic HCV infection, changes in the liver can range from mild non-specific changes to end-stage liver disease with cirrhosis and HCC.

Histological grading of liver biopsies is based on: necroinflammatory activity and fibrosis. Two popular grading systems are in use: Ishak (modified Knodell score) and METAVIR scores. Fibrosis (0–6 for Ishak; 0–4 for METAVIR) predicts progression to irreversible liver disease better than necroinflammation. Non-invasive methods for diagnosis of fibrosis are used in adults and include the use of serum biomarkers such as AST/ALT ratio, AST-to-platelet ratio index (APRI), enhanced liver fibrosis (ELF) score, and imaging techniques such as transient hepatic elastography (TE, FibroScan).

Epidemiology and transmission

HCV infection is a global health problem, with an estimated 150 million people chronically infected worldwide. In the UK, it is estimated that around 214,000 individuals have chronic HCV infection. Intravenous drug use is the most important risk factors. In some countries, unsafe medical use of needles and poor infection control procedures in medical facilities such as dialysis units contribute to transmission. HCV can be transmitted sexually, though this is more common in men who have sex with men.

In children, HCV infection used to be prevalent in recipients of contaminated blood products – particularly in those with hemophilia, leukemia, and thalassemia. Due to the success in the screening of blood products and organ donors for HCV, transfusion- or transplant-related HCV is virtually eliminated. Currently, most new cases of HCV infection in childhood are due to vertical transmission from infected mothers, which occur in 5–10% of deliveries. However, the risk of transmission is increased by coexistent maternal HIV infection and high maternal HCV RNA viral load. In the UK and Ireland, the vertical transmission rate was 6.7% overall, and 3.8 times higher in HIV-coinfected than in HIV-negative

women. The seroprevalence of HCV infection in pregnant women in the UK is estimated to be 0.2%, with an estimated 70 infants acquiring infection each year. As there is currently no antenatal screening of HCV infection, at-risk pregnancies and infected children are missed.

Mother-to-child transmission of HCV may occur in utero or at the time of delivery. There is conflicting evidence regarding the benefit of delivery by cesarean section. There is no evidence of an increased risk of transmission through breastfeeding.

Clinical presentation (hepatic and extrahepatic)

Acute hepatitis C

Acute hepatitis C infection is defined as acute hepatitis within 6 months following acquisition of the infection associated with detectable serum HCV RNA. Acute hepatitis (jaundice and ALT elevation) is the presenting feature in only 15–30% of acute hepatitis C infections and the majority of cases are asymptomatic and clinically undetected.

In vertically transmitted infection, acute neonatal hepatitis, is defined as detectable HCV RNA in infant's blood for up to 6 months. This may be associated with elevated transaminase levels and often resolves during early infancy. The treatment response for acute hepatitis C is significantly better than chronic infection. It is therefore important to identify a transmission as soon as possible so that treatment can be started at an early stage. This can be achieved by better awareness of acute hepatitis C infection, a thorough follow-up of those who had an exposure such as health-care-workers sustaining a sharps injury from an HCV-infected source, and an infant acquiring infection from the mother.

Chronic hepatitis C

Chronic hepatitis C (CHC) is defined as detectable HCV RNA for at least 6 months. The chronic infection rate of perinatally acquired HCV infection is as high as 80%.

CHC in infancy and childhood is usually asymptomatic, although non-specific symptoms or mild hepatomegaly may be apparent. Aggressive disease with progression to cirrhosis and end-stage liver disease has been described in childhood but is uncommon. Most vertically infected infants will have raised aminotransferase enzymes, particularly in the first few years of life, which do not correlate with clinical severity.

Many extrahepatic disorders have been associated with CHC. Autoimmune disorders, including membranoproliferative glomerulonephritis, autoimmune thyroiditis, and porphyria cutanea tarda have been reported. Unlike adults, cryoglobulinemia and lymphoma are rare. A diagnosis of HCV infection should be suspected in the following clinical settings:

- Children born to infected mothers.
- Exposure to potentially infected blood products or contaminated equipment, usually in resource-poor countries.
- Children offered for adoption, particularly from endemic areas.
- Children with unexplained abnormal aminotransferase levels.
- Acute hepatitis.

Natural history and outcome

The natural history of CHC may be influenced by the route of acquisition, age at the time of infection, genotype, and co-morbidities.

Spontaneous resolution of HCV infection is defined by sustained disappearance of HCV RNA from the serum, accompanied by normalization of the aminotransferase enzymes. Spontaneous resolution in children varies between 10 and 40% with lower conversion rates in the perinatally infected group. The European Paediatric Hepatitis C Virus Network evaluated 266 children with vertically acquired HCV infection and reported a clearance rate of 21–25% over a median follow-up period of 4.2 years. Spontaneous viral clearance in vertically infected children occurs mainly in the first 2 years of life but some may have spontaneous resolution as late as 7 years. Spontaneous viral clearance is dependent on genotype with higher spontaneous clearance rates in children infected with genotype 3.

Histological findings in CHC in children are not as severe as in adults. Inflammation is often mild and fibrosis is absent or mild in the majority of cases. In children, fibrosis is a slow progressive process, hence its severity relates to the duration of infection. Progression of fibrosis is also dependent on associated risk factors as obesity and alcohol consumption or co-morbidity such as previous chemotherapy or thalassemia. Progression of fibrosis is not linear and it is thus not a reliable prognostic indicator.

Despite the favorable prognosis in the first two decades of life, approximately 5% of children will develop evidence of severe fibrosis or cirrhosis. HCC is extremely uncommon in children with CHC. A few liver transplant centers have reported children requiring liver transplantation as a result of progressive CHC [16].

Laboratory diagnosis

Most initial screening tests for HCV depend on the detection of antibody to hepatitis C (anti-HCV). Anti-HCV is often negative during the acute phase of infection and the value of antibody testing alone is limited by the following:

- Anti-HCV may persist for many years after clearance of viremia and resolution of infection.
- Anti-HCV may be absent in patients with HCV infection who are immunocompromised.
- Maternal anti-HCV may persist in the newborn for up to 18 months.

The presence of viremia can be detected by testing for HCV core antigen or HCV RNA. HCV RNA is more sensitive than the antigen test, and both are useful in the diagnosis of HCV infection before anti-HCV has become detectable. Due to a relatively prolonged incubation period of up to 3 months, testing for HCV RNA after an exposure needs to be deferred.

In infants, a negative HCV RNA test at 1 month provides a high predictive value for absence of transmission and a negative test at 3 months excludes transmission. Quantitative measurement of HCV RNA (viral load) is useful for monitoring the response to antiviral therapy (see later) or for evaluating natural seroconversion. Sequencing of the antiviral targets in the HCV genome to detect resistance associated mutations is possible. However, there may not be any clinical need for this, as so far there is no evidence of a significant problem of drug resistance developed against the use of the new direct acting agents. For genotype 1a, a polymorphism Q80K in the NS3 protease might reduce the response rate to the protease inhibitor simeprevir. It is therefore recommended that patients infected with genotype 1a who are being considered for a treatment regimen that contains simeprevir should be tested for the presence of Q80K.

Management

Children with CHC should be seen annually. Assessment should include:

- Clinical signs and symptoms.
- Disease progression/resolution (LFTs, HCV RNA, AFP, ultrasound).
- Co-morbidity.
- Discussion about the timing of treatment.
- Counseling families regarding the risk of transmission and treatment options.

Liver biopsy should only be considered in cases of unexplained clinical hepatic decompensation and in children who are being considered for antiviral treatment.

All children with HCV should undergo vaccination against HAV and HBV.

Why, when, and who to treat

Treatment allows definitive resolution of the infection and elimination of the social stigma associated with HCV infection. Although HCV infection does not impair the quality of life in most affected children, it can lead to cognitive impairment and is associated with high caregiver stress, particularly in cases of mother-to-child transmission.

The primary aim of treatment is to prevent chronic liver disease, prevent the development of HCC, and improve quality of life by achieving an SVR. Treatment should be delayed until the age of 3 years in children infected at birth in view of the possibility of spontaneous seroconversion.

Treatment should be considered in children who remain infected, demonstrate persistently elevated aminotransferases, or those with evidence of progressive liver disease.

It is important to provide families with information on possible treatment outcomes in order to tailor the treatment plan based on the individual's circumstances and potential response. To date, HCV genotype has been the strongest predictor of treatment response as treatment is more successful in patients with genotypes 2 and 3. Lower SVR were reported in children with high baseline viral load (>500,000 IU/mL) and who have acquired the infection vertically. Viral response during the early weeks of treatment (4 and 12 weeks) is considered a strong predictor of SVR. Another predictor of treatment response identified from studies in adults, are SNPs in proximity to the *IL28B* gene. SVR rates are higher in patients with C/C genotype of the rs12979860 SNP and the T/T genotype of another SNP rs8099917.

Current treatment options

IFN-α monotherapy has a better response in children compared with adults with reported SVR up to 36% compared to 10–15% in adults. As with reports from adult studies, the addition of ribavirin resulted in a better SVR rates in children. These early data meant that the current standard of care for children is combination therapy with longer-acting PEG-IFN-α and ribavirin which has been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of chronic HCV in children aged 3–17 years. IFN is not recommended for the treatment of viral hepatitis in children <2 years of age.

In the UK, consensus among the three national pediatric liver centers is to use combination therapy of PEG-IFN-α and ribavirin. The recommended treatment regimen is PEG-IFN-α-2b at a dose of 60 μg/m^2 or PEG-IFN-α-2a at a dose 180 μg/1.73 m^2 subcutaneously once weekly in combination with ribavirin 15 mg/kg/day orally in two divided doses for 48 weeks in children infected with genotypes 1 or 4 and 24 weeks in genotypes 2 or 3.

Several studies reported overall SVR rates of 46–76% in children treated with PEG-IFN and ribavirin. Response rates depend on viral genotypes, with a higher response of >90% among children with genotypes 2 and 3. Conversely, higher mean body mass index (BMI) and liver steatosis are associated with poor response. The improved response rate in children compared to adults might be related to a lower frequency of inflammation and fibrosis, shorter duration of disease, and absent co-morbidities. Careful medical and psychological monitoring by the provider and a supportive and motivated family is essential for the success of treatment.

Side effects of therapy

PEG-IFN and ribavirin are generally well tolerated by children.

- Constitutional symptoms are almost universal in all treated children and are similar to those described (above) for interferon therapy.
- IFN-α induced bone marrow suppression is a common side effect requiring dose reductions and discontinuation of treatment for neutropenia.
- Anemia is mild and generally no hematopoietic factors are required.
- Detectable antithyroid antibodies are common and clinical thyroid disease may occur, which rarely becomes permanent.

- Weight loss and changes in linear growth velocity occur in children on the combination therapy. Most patients regain their original weight and experience increase in growth velocity following discontinuation of treatment. Treatment should be avoided in puberty and during growth spurts.
- Mood disturbances, including irritability, insomnia, and somnolence are a problem, particularly in adolescents. More severe side effects, including depression and suicidal ideation, are more common in those with pre-existing mood disturbances or those receiving treatment during adolescence.

Successful treatment is defined as undetectable HCV RNA 6 months after completion of the treatment course (SVR), as relapses usually occur within 6 months of cessation of therapy. Patients who are spontaneously clear of HCV or are successfully treated will remain anti-HCV positive, but HCV RNA negative [16, 17].

Future treatment strategies

Development of new therapies for HCV has been driven by increased understanding of the molecular pathways of its lifecycle. Despite the significant advances in therapies available for HCV in adults, little data are available on these therapies in children as clinical trials are in progress.

The main targets of the new therapies are HCV encoded proteins and host encoded proteins which are vital for viral replication. Initial therapy focussed on two NS3/NS4A protease inhibitors (telaprevir and boceprevir) which were approved for treating adults with genotype 1 in combination with PEG-IFN and ribavirin. SVR treatment in naive patients using the triple therapy regimen was achieved in 80%; however, the complexity of this regimen and the significant side effects affected patient compliance. The most common side effects with boceprevir are anemia, neutropenia, and dysgeusia (altered taste sensation). Severe skin rash has been reported in association with telaprevir and has resulted in discontinuation of treatment in a number of treated patients.

Another group of antiviral therapies under investigation includes polymerase inhibitors (anti-NS5B). Sofosbuvir is an HCV NS5B polymerase inhibitor with similar activity against all HCV genotypes. Studies on sofosbuvir in combination with PEG-IFN and ribavirin or in combination with other direct antiviral therapies have shown higher response rates over a shorter duration of treatment (12–24 weeks) for all HCV genotypes.

Other viral proteins being explored as possible targets for antiviral therapy include NS5A and core protein. Promising host targets include cyclophilin A and miR122. Ciclosporin A, a cyclophilin A inhibitor, is an effective inhibitor of HCV replication in vitro. MiR122 is a micro RNA expressed in the liver and facilitates HCV replication. A nucleic acid inhibitor of miR122 (miravirsen) is a potent inhibitor of HCV replication both in animal models and in humans.

With the new direct-acting agents, many studies have used 12 weeks after cessation of therapy to define SVR (SVR12) [18–20].

Prevention

There is currently no vaccine available to prevent HCV infection. The issues of vaccine development in HCV are very similar to that of HIV as both have multiple genotypes, the presence of quasi-species, and a lack of neutralizing antibodies. In addition, there are no in-vitro culture systems or suitable animal models. Recent developments include an HCV culture model, which is infectious for chimpanzees. Vaccine efficacy data in chimpanzees indicate that it appears feasible to impede progression to chronic infection. Based on this knowledge, a human vaccine strategy has been developed [21].

Coinfection

Coinfection with HBV has a negative effect on the natural history of HCV infection Patients coinfected with HCV and HIV have higher rates of viral persistence, higher viral load, and lower response to combination therapy. It is therefore recommended that coinfected patients should receive the combination therapy of PEG-IFN and ribavirin for 48 weeks irrespective of genotype.

Liver transplantation

Liver transplantation in children for end-stage liver disease due to HCV is uncommon. In a large study evaluating outcomes in children transplanted for hepatitis C, patient and allograft survival rates at 5 years were reported as 72% and 55%, respectively. There is a high risk of HCV recurrence post-liver transplant which can now be prevented with the new direct acting antiviral drugs.

Herpesviruses

The herpesviruses are a family of icosahedral double-stranded DNA viruses (Table 13.8). They can persist in the host after primary infection and the latent virus can reactivate when the host is immunosuppressed. Hepatitis can be a manifestation of systemic primary herpesvirus infection or during reactivation.

Laboratory diagnosis

HSV1 and HSV2

Herpes simplex virus type 1 (HSV1) can be isolated easily using cell culture. Rapid diagnosis is achieved by direct detection in lesions using immunofluorescence for antigen or molecular techniques for viral DNA. Patients with HSV hepatitis are likely to have detectable HSV DNA in serum.

Table 13.8 Human viruses in the family of Herpesviridae.

Viruses (common abbreviation)	Subfamily	Common primary infection site	Common latent sites	Context of liver involvement
Herpes simplex virus type 1 (HSV1)	α	Oral mucosa	Neurons in sensory ganglions, e.g., the trigeminal ganglion	Neonatal disseminated infection associated with hepatic dysfunction and fulminant hepatitis Immunosuppressed patients
Herpes simplex virus type 2 (HSV2)	α	Genital mucosa	Neurons in sensory ganglions, e.g., the sacral ganglion	Neonatal disseminated infection associated with hepatic dysfunction and fulminant hepatitis Immunosuppressed patients
Varicella-zoster virus (VZV)	α	Cutaneous epithelial cells (chickenpox)	Neurons in sensory ganglions, e.g., the trigeminal ganglion, dorsal root ganglions	During systemic infection (both immunocompetent and immunosuppressed)
Cytomegalovirus (CMV)	β	Initial mucosal sites, spread to salivary glands, kidneys, endothelial cells and mononuclear cells	Lymphocytes, salivary gland, kidney	Primary infection (infectious mononucleosis-like syndrome); congenital infection Immunosuppressed patients Infection of transplanted liver from CMV-positive donor
Human herpesvirus 6A and 6B (HHV6A/HHV6B)	β	Tonsillar lymphocytic cells, olfactory cells	T lymphocytes, salivary gland	Immunosuppressed patients
Human herpesvirus 7 (HHV7)	β	Tonsilar lymphocytic cells, olfactory cells	T lymphocytes, salivary gland	Immunosuppressed patients
Epstein–Barr virus (EBV)	ɣ	Oropharynx and tonsilar epithelial cells, B lymphocytes	B lymphocytes	Primary infection (infectious mononucleosis/glandular fever); EBV-driven lymphoproliferative disorder and EBV-associated malignancy in immunosuppressed patients
Human herpesvirus 8/Kaposi's sarcoma-associated herpesvirus (HHV8/KSHV)	ɣ	Oral epithelial cells, endothelial cells, B lymphocytes, monocytes, dendritic cells	B lymphocytes	Multicentric Castleman disease

Serology is of little value in diagnosis, though it may help to differentiate between primary infection and reactivation in cases of maternal genital herpes during pregnancy.

VZV

Varicella-zoster virus (VZV) grows slowly in cell culture. Diagnosis of chickenpox or shingles can be made by detection of VZV in vesicle fluid by immunofluorescence or by molecular techniques. VZV DNA can also be detected in serum and cerebrospinal fluid during active infection. VZV IgG is a useful marker for the determination of susceptibility to VZV.

CMV

The characteristic histological feature of cytomegalovirus (CMV) infection is the so-called "owl's eye" inclusion bodies in infected cells. The presence of CMV antigen can be confirmed by immunostaining of biopsy material using monoclonal antibody.

CMV-specific IgM is produced during primary infection as well as reactivation, though usually at a lower level. CMV IgG avidity test may help to distinguish between recent (low avidity) from past (high avidity) infections. Tests for CMV early antigen or pp65 matrix protein are available, but these have largely been replaced by molecular technique such as PCR for detection of CMV DNA. Quantitative PCR can be used to monitor viral load during surveillance and follow-up of therapy. To diagnose congenital CMV, the best sample is urine or mouth swab taken within the first 3 weeks of life.

HHV6A/HHV6B/HHV7

Diagnostic tests for human herpesvirus 6 (HHV6) and HHV7 are not widely available. They can be detected by using viral-specific PCR. However, the detection of HHV6 DNA is complicated by the fact that between 1 and 2% of the population has germline integration of HHV6 genome in chromosome. There is no pathology associated with HHV6 integration. Hence, detection of HHV6 DNA in body fluid does not necessarily indicate an active infection, but could be confusing for clinicians.

EBV

The diagnosis of primary Epstein–Barr virus (EBV) infection is mainly serological. IgG and IgM antibody to EBV viral capsid antigen (VCA) is usually present at clinical presentation. However, non-specific EBV VCA IgM is common and EBV VCA IgM can also be positive during reactivation. Antibody to Epstein–Barr nuclear antigen (EBNA) is usually the last antibody to develop during primary infection and can therefore be useful for diagnosis when it is absent in the presence of detectable EBV VCA IgM. Primary EBV infection can also be associated with the development of heterophile antibodies against sheep or horse red blood cells (Paul-Bunnell or monospot test). Adults are more likely to be heterophile antibody-positive during primary EBV infection, whereas up to 50% of children are negative.

Detection of EBV-encoded products – including latent membrane protein (LMP), EBNA, and EBV-encoded RNA (EBER) by immunohistochemistry, and detection of EBV genome by in situ hybridization, allow specific detection of EBV in infected tissues. PCR detection and quantitation of EBV DNA in blood samples allow the monitoring of viral load, which is of particular value in organ-transplant recipients.

HHV8

A serological test for HHV8 is not widely available. HHV8 antigen can be detected in tissue samples using immunohistological staining techniques. Quantitative HHV8 DNA PCR can help to measure viral load in patient with HHV8-driven lymphoproliferative disease or Castleman syndrome.

Treatment of herpesvirus infections

Infections in the immunocompetent host are mild and self-limiting and no treatment is required. Only in severe or disseminated cases or in the presence of immunosuppression antiviral therapy is indicated.

Antiviral agents effective against herpes viruses include:

- Aciclovir and valaciclovir.
- Ganciclovir and valganciclovir.
- Foscarnet.
- Cidofovir.

Aciclovir

Aciclovir, a nucleoside analogue, is safe and relatively non-toxic, and has the best therapeutic index of all currently available antiviral agents. Its activation by phosphorylation to aciclovir triphosphate is catalyzed by a virus-encoded thymidine kinase, and thus only occurs in infected cells.

The susceptibility of herpesviruses to aciclovir varies with HSV1 and HSV2 and VZV having good responses to aciclovir and CMV with a very limited susceptibility.

Aciclovir is effective against EBV replication, but has no effect on latent virus. It may therefore reduce viral shedding in infectious mononucleosis, but has no effect on the symptoms or course. Aciclovir is not recommended for treating HHV6, HHV7, and HHV8.

Valaciclovir

Valaciclovir is an ester of aciclovir with valine, with good absorption and bioavailability. Clinical application is limited by palatability and the lack of an oral suspension.

Ganciclovir

Ganciclovir is 100 times more active than aciclovir in its action against CMV replication. Ganciclovir is the first antiviral agent approved for the treatment of CMV disease. It is more toxic than aciclovir, with 25% of patients experiencing reversible bone marrow suppression, therefore treatment should be reserved for specific indications, which include prophylaxis against CMV in organ-transplant recipients and CMV disease in immunocompromised hosts. IV ganciclovir is recommended for at least 2–3 weeks followed by oral valganciclovir in treating CMV infection in post-transplant patients. The optimal duration of treatment is not well defined but quantitative CMV DNA level should be monitored as a marker of response, and treatment should be continued for at least 1 week after viral load is reduced to levels below detection. There is growing evidence that treating neonates with symptomatic congenital CMV infection, with either ganciclovir or valganciclovir, may improve hearing and developmental outcomes in the longer term. Oral ganciclovir is poorly absorbed and has poor bioavailability.

Valganciclovir

As for aciclovir, ganciclovir linked to the amino acid valine increases its bioavailability 10-fold. It has the advantages of a once-daily regimen and provides greater systemic exposure to ganciclovir than oral ganciclovir.

Foscarnet

Foscarnet acts by directly binding to pyrophosphate binding sites of DNA polymerases, leading to non-competitive inhibition. As it does not require activation by cellular or viral kinases, it is of value in ganciclovir-resistant CMV disease. Its usefulness, however, is limited by its toxicity, with major adverse effects being renal impairment, electrolyte disturbance, and seizures.

Cidofovir

Cidofovir is an acyclic nucleoside phosphonate with activity against all the herpesviruses and also against adenovirus. It has been successfully used for CMV disease with resistance to ganciclovir or foscarnet, and in invasive adenovirus disease. It has significant nephrotoxicity and is therefore not recommended as first-line treatment. It has a potential role in treating herpesvirus and adenovirus infections in immunosuppressed patients when there is resistance to first-line agents.

Common childhood viral infection which may be associated with hepatitis

Some common childhood viral infection could present with features of hepatitis as part of its manifestation. This include measles virus, rubella virus, parvovirus B19 (erythrovirus), adenoviruses, enteroviruses, and parechoviruses (Table 13.9).

Laboratory diagnosis

Serological assays for both IgG and IgM antibodies to measles, rubella, and parvovirus B19 are available commercially. Serology testing of oral fluid can also be used to provide diagnostic and public health information. As with all IgM assays, weakly reactive samples without IgG require a follow-up sample for confirmation. The detection of viral nucleic acid (measles RNA, rubella RNA, and parvovirus B19 DNA) in mouth swabs or blood can be a useful diagnostic tool, especially in early infection before the development of serological respond. Persistent infection with parvovirus B19 could occur in immunocompromised patients and viral activity can be monitored using serum DNA viral load testing,

Serology tests are less useful in adenoviruses, enteroviruses, and parechoviruses. Though they can be isolated in tissue culture, the main state of diagnosis nowadays is by detection of viral nucleic acid. Parechoviruses were previously classified as enteroviruses. This has changed because of the genetic differences between the two virus groups. Molecular tests for enterovirus RNA do not detect parechovirus RNA. Hence, a specific test for parechovirus is required for its diagnosis.

Table 13.9 List of common childhood viral infections that could be associated with liver function derangement.

Viruses	Viral family (nature of nucleic acid)	Primary illness	Context of liver involvement	Liver involvement
Measles virus	Paramyxoviridae (RNA)	Measles	Part of measles	Brief transient elevation of transaminases Cholestasis (rare)
Rubella virus	Togaviridae (RNA)	Rubella	Congenital rubella syndrome	Hepatitis, hepatomegaly, jaundice or massive hepatic necrosis
Parvovirus B19 (erythrovirus)	Parvovirudae (DNA)	Erythema infectiosum (fifth disease, slapped cheek syndrome)	Part of erythema infectiosum In patient with aplastic crisis In immunocompromised patients	Acute hepatitis Fulminant liver failure
Adenoviruses	Adenoviridae (DNA)	Upper and lower respiratory tract infection, keratoconjunctivitis, gastroenteritis	In immunocompromised patients	Acute hepatitis Fulminant liver failure
Enteroviruses (include polio, coxsackie A and B, echo, and numbered enteroviruses)	Picornaviridae (RNA)	Neonatal sepsis, central nervous system infection, myocarditis, gastroenteritis	Neonates	Hepatitis ± coagulopathy Fulminant liver failure
Parechoviruses (numerous subtypes)	Picornaviridae (RNA)	Neonatal sepsis (particularly type 3), central nervous system infection, gastroenteritis	Neonates	Hepatitis

Table 13.10 Some of the commoner causes of viral hemorrhagic fever.

Viruses	Virus family (nature of nucleic acid)	Host in nature	Insect vectors	Geographical regions
Dengue virus	Flaviviridae (RNA)	No animal reservoir	*Aedes* mosquitoes	Africa, central and South America, the Caribbean, eastern Mediterranean, South East Asia and the Western Pacific
Yellow fever virus	Flaviviridae (RNA)	Monkeys	*Aedes* mosquitoes	Africa and Latin America
Lassa virus	Arenaviridae (RNA)	Multimammate rats	None	West Africa
Crimean–Congo hemorrhagic fever virus	Bunyaviridae (RNA)	Livestock animals	Ticks	Africa, the Balkans, the Middle East and Asian countries south of the 50th parallel north
Ebola and Marburg viruses	Filoviridae (RNA)	Bats	None	West, central, and South Africa

Treatment

Treatment is mainly supportive. Cidofovir may be of value in immunosuppressed children with adenovirus infection. Immunoglobulin has been used as a therapeutic agent for neonates with enterovirus disease; however, clinical efficacy has not been proven. Specific antiviral therapy for enteroviruses is being trialled.

Prevention

Measles and rubella can be effectively prevented by the measles, mumps, and rubella (MMR) vaccine.

Viral hemorrhagic fever viruses

These viruses are grouped together because they cause a similar syndrome of viral hemorrhagic fever which is associated with multiorgan failure and severe hepatocellular necrosis (Table 13.10).

Laboratory diagnosis

Apart from dengue virus and yellow fever virus (UK Advisory Committee on Dangerous Pathogens (ACDP) hazard group 3), most other viral hemorrhagic viruses have to be managed under high levels of biocontainment (hazard group 4). Specific reference laboratories are therefore required for their diagnosis. Dependent on the stages of the illness, a combination of molecular tests for viral RNA and serology tests can be used to make the diagnosis.

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CHAPTER 14

Congenital and Structural Abnormalities of the Liver

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Key points

- Ductal plate malformations result from persistence of ductal plate remnants postnatally resulting in abnormal bile ducts surrounded by abundant, dense extracellular matrix leading to congenital hepatic fibrosis.
- There is a range of abnormalities depending on the level of the biliary tree affected:
  - Congenital hepatic fibrosis: microscopic dilatations of the intrahepatic biliary ducts.
  - Caroli syndrome: macroscopically visible liver cysts in continuity with bile ducts.
  - Caroli disease: liver cysts which are contiguous with biliary tree without the microscopic intrahepatic biliary duct dilatations.
- Congenital hepatic fibrosis can lead to portal hypertension, cholangitis secondary to biliary cysts or obstruction, biliary tract stone formation, or features of an associated ciliopathy including renal disease.
- Ciliopathies are genetic disorders caused by mutations in genes encoding ciliary proteins resulting in abnormal formation or function of cilia. Multiple organ systems are affected including cystic kidney disease, retinal, respiratory, skeletal, hepatic, and neurological defects, in addition to obesity, laterality defects, and polydactyly.
- Hepatorenal fibrocystic diseases include autosomal recessive polycystic kidney disease and Meckel–Gruber syndrome in which congenital hepatic fibrosis is always present to a certain degree. Progression of liver and kidney disease are independent and highly variable.
- Liver transplantation for liver failure is very rare in congenital hepatic fibrosis and the timing of transplantation is determined by the need for renal transplantation. Combined liver and kidney transplantation is indicated because of the risk of sepsis and recurrent cholangitis with immunosuppression post-isolated renal transplant.
- A multidisciplinary approach is essential for the management of ciliopathies and associated hepatic and multisystem manifestations.

This chapter focusses on the two major congenital and structural abnormalities of the liver: fibrocystic disorders and vascular anomalies. Other congenital anomalies including biliary atresia are discussed in Chapter 8.

Fibrocystic disorders

Embryology including ductal plate malformations

Liver development starts on the 18th day of gestation when the hepatic diverticulum forms as a thickening of the ventral floor of the distal foregut endoderm. The hepatic diverticulum divides into the solid cranial portion, which forms the hepatic parenchyma and intrahepatic biliary ducts, and the cystic caudal part, which forms the gallbladder, common bile duct, and cystic duct. Primitive hepatocytes that are in contact with the mesenchyme surrounding the hepatic portal veins form a structure known as the ductal plate which gives rise to the cholangiocytes lining the intrahepatic bile ducts as well as the periportal hepatocytes and adult hepatic progenitor cells [1]. The ductal plate is a transient structure along the branches of the portal vein. Hepatocytes in contact with the portal vein mesenchyme differentiate into primitive cholangiocytes and bile ducts. The ductal plate remodeling process involves a high rate of

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mesenchymal proliferation which separates the bile ducts and liver parenchyma combined with high rate of apoptosis of hepatocytes and intrahepatic cholangiocytes. Ductal plate malformations result from persistence of ductal plate remnants postnatally due to an imbalance between apoptosis and proliferation resulting in abnormal bile ducts surrounded by abundant, dense extracellular matrix [2]. In congenital hepatic fibrosis (CHF) ductal plate malformation predominantly involves interlobular bile ducts whereas in Caroli disease (CD) there is larger bile duct involvement.

Ciliopathies and liver disease

There are two types of cilia: motile and non-motile primary cilia. Ciliopathies are diseases caused by abnormalities in primary cilia.

Motile cilia are present on cell surfaces in large numbers and beat in rhythmic motion where their function is transport of substances along the epithelium, e.g., in respiratory epithelium motile cilia clear mucus. Disorders of motile cilia are known as primary ciliary dyskinesias and include features such as bronchiectasis, situs inversus, and infertility. Their structure involves a microtubule-based cytoskeleton called the axoneme consisting of nine outer microtubule doublets and two central microtubule singlets known as the 9 + 2 axoneme arrangement.

Primary, non-motile, cilia were first named by Sergei Sorokin in 1968 as a solitary organelle arising from the cell surface of most mammalian cell types during growth arrest. However, it was not until recently that the function of primary cilia as key coordinators of signaling pathways during development and in tissue homeostasis where cilia are critical in transducing "outside-in" signals. Primary cilia consist of an axoneme of nine doublet microtubules extending from a basal body which is derived from the centrioles. Centrioles are complex microtubule-based structures located within the cytoplasm and there is a complex system of anterograde as well as retrograde trafficking of vesicles along the ciliary intracellular cytoskeleton. Ciliary assembly and targeting is mediated by several multiprotein complexes that include intraflagellar transport (IFT) proteins and the BBSome which is a complex of several Bardet–Biedl syndrome (BBS) proteins [3]. The main function of primary cilia is to monitor the environment external to the cells including chemical, osmotic, and mechanical stimuli and transmit these signals via several pathways including Hedgehog, Wnt, platelet-derived growth factor receptor α (PDGFR-α), and integrin. It is through these signals that cilia can maintain cell polarity, mitotic spindle orientation and cell proliferation and thereby ensure optimal epithelial cell structure and function [4].

Ciliopathies are disorders caused by mutations in genes encoding ciliary proteins resulting in abnormal formation or function of cilia. Multiple organ system involvement is the norm in ciliopathies due to the ubiquitous nature of cilia and characteristically include cystic kidney disease, retinal, respiratory, skeletal, hepatic, and neurological defects, in addition to obesity, laterality defects, and polydactyly. The number of known mutations causing ciliopathies is currently over 95 but this is likely to be an underestimate as there are over 1000 identified proteins in the ciliary proteome. Genetic panel testing is becoming available.

In the liver, cholangiocytes are the only epithelial cell which contains cilia. They are important in early development and in the maintenance of normal epithelial function. Cilia on cholangiocytes are positioned on the apical plasma membrane where they can detect changes in bile flow, osmolality, and composition. This allows cholangiocytes to modify bile through secretory and absorptive processes which are dependent on osmotic gradients sensed by cilia.

The most common manifestations of ciliopathies in the liver, namely CHF, CD, or Caroli syndrome (CS), arise because of abnormal development leading to ductal plate abnormalities. Ductal plate malformations result in the persistence of ductal plate remnants postnatally with abnormal bile ducts being surrounded by dense extracellular matrix. There is a range of abnormalities depending on the level of the biliary tree affected: in CHF the smaller interlobular bile ducts whilst in CD there is larger bile duct involvement. This means that in CHF, there are dilatations of the intrahepatic biliary ducts which are microscopic whereas in CS, there are macroscopically visible liver cysts in continuity with bile ducts. CD is much rarer and refers to liver cysts which are contiguous with biliary tree without the microscopic intrahepatic biliary duct dilatations characteristic of CHF. However, this is a spectrum of disease which can vary between different members of the same affected family in which some members have CS and others CD.

Congenital hepatic fibrosis, Caroli disease, and Caroli syndrome

The diagnosis of CHF is defined histologically by ductal plate malformation, abnormal branching of the intrahepatic portal veins, and associated progressive fibrosis of the portal tracts.

Whilst a histological diagnosis of CHF can be made in infancy the clinical features may be subtle or develop at a variable rate with age. Hepatic function is usually well preserved.

As mentioned earlier, the difference between CHF, CD, and CS depends on the level of the biliary tree affected. In CHF the smaller interlobular bile ducts are affected whilst in CD there is larger bile duct involvement.

Clinical presentation of congenital hepatic fibrosis

Symptoms of CHF vary and are non-specific which makes the diagnosis difficult. The age of presentation also varies between infancy to later adult life although most cases present in childhood and adolescence.

CHF may also be asymptomatic and only detected in association with a recognized syndrome.

The main ways that CHF can present are portal hypertension, cholangitis secondary to biliary cysts or obstruction, biliary tract stone formation, or features of an associated disease (see later).

- *Portal hypertension and associated features of hepatomegaly, splenomegaly, hypersplenism, and gastrointestinal tract varices*. Portal hypertension is the main manifestation of CHF and is caused by increased vascular resistance in the portal blood flow through the liver due to the ductal plate malformation and associated fibrosis and venous abnormalities. The fibrosis and hence portal hypertension are progressive and clinical manifestations develop and worsen over time. These include: splenomegaly, hypersplenism, and portosystemic vascular collaterals which result in gastroesophageal and rectal varices and the consequent risk of gastrointestinal bleeding. Other manifestations of portal hypertension include pulmonary hypertension (portopulmonary hypertension) and vascular shunts in the pulmonary parenchyma (hepatopulmonary syndrome) [5, 6] (see also Chapters 22 and 27).

  In a recent comprehensive literature review [7], portal hypertension presented in 71–97% of individuals with CHF at a median age of 12 years (range between 0.3 and 41 years). At least a third of patients had some sequelae of portal hypertension with hypersplenism and esophageal varices being the commonest. Forty per cent of those with portal hypertension developed varices, 45% of whom had bleeding varices and 19.8% underwent portosystemic shunting. Ascites, hepatopulmonary syndrome, and encephalopathy were uncommon. Complications of portal hypertension were commoner in isolated CHF and CD/CS compared to autosomal recessive polycystic kidney disease (ARPKD).
- *Cholangitis*. This was reported in 70% of cases of CD/CS and 20% of ARPKD cases and in a few cases of isolated CHF. When cholangitis did occur it tended to be recurrent and it proved fatal in three of 23 children when it occurred after isolated renal transplantation.
- *Biliary stone formation and cholangiocarcinoma*. These have been reported in CD/CS. Twenty-one patients out of 1230 (1.71 %) developed hepatobiliary cancer of which 19 were cholangiocarcinoma with the mean age of diagnosis was 60.1 years.
- *Disorders associated with CHF* (Table 14.1). Most cases of CHF/CS are caused by a ciliopathy and so there may be specific clinical features including renal disease (hence also known as hepatorenal fibrocystic diseases), abnormalities of mid-/hindbrain, retinopathy, and skeletal dysplasia [8].

  Hepatorenal fibrocystic diseases include ARPKD and Meckel–Gruber syndrome (MKS) in which CHF is always present to a certain degree. Progression of liver and kidney disease are independent and highly variable.
- *Other*. Other hepatorenal fibrocystic diseases in which CHF occurs in variable frequencies include Joubert syndrome, BBS, oral–facial–digital syndrome 1, Ellis–van Creveld syndrome (EVC), Jeune syndrome (asphyxiating thoracic dystrophy), and renal hepatic pancreatic dysplasia. Therefore, it is important to screen and monitor patients for these manifestations. In autosomal dominant polycystic kidney disease (ADPKD), the liver manifestations are different to CHF as there may be multiple liver cysts similar to polycystic liver disease.

Diagnosis of congenital hepatic fibrosis

CHF should be suspected in all ciliopathies, especially ARPKD, MKS, and other fibrocystic hepatorenal diseases. As most of these conditions are autosomal recessive, a family history of consanguinity should be sought.

Clinical examination reveals firm smooth hepatomegaly with splenomegaly if portal hypertension has developed.

Investigations

- Abdominal ultrasound examination which classically shows increased heterogenicity of the liver. There may be visible cysts in CD or CS due to saccular or fusiform dilatations of the intrahepatic bile ducts. Biliary stones may be present.
- If there is portal hypertension, splenomegaly and varices or spontaneous portosystemic shunts may be visible. Abnormal portal vein development including hypoplasia or extrahepatic portal vein obstruction may occur. As CHF is associated with renal diseases, there may be abnormalities of the kidneys including cystic changes, increased echogenicity as well as other renal tract abnormalities which are discussed in more detail later.
- MRI is useful to evaluate for parenchymal liver and biliary tract abnormalities.
- Magnetic resonance cholangiopancreatography (MRCP) is useful to delineate cystic or fusiform dilatations and irregularities of the intrahepatic bile ducts as well as dilation of the extrahepatic bile ducts (Figure 14.1).
- The gold standard for diagnosis of CHF is a liver biopsy.

  The liver biopsy in CHF has the following features (Figure 14.2):
  - Normal hepatic parenchyma without abnormalities of the hepatocellular plates or intrahepatic cholestasis.
  - Abnormally formed abundant bile ducts in the portal tracts which are caused by an excess of embryonic bile duct structures remaining in their primitive ductal plate configuration. This is mistakenly called "bile duct proliferation."
  - Abnormal portal vein branches.
  - Periportal fibrosis without inflammation.
  - Portal tract–portal vein bridging fibrosis which differs from the portal tract–central vein fibrosis in other causes of cirrhosis.

Table 14.1 Hepatorenal fibrocystic diseases associated with congenital hepatic fibrosis (CHF).

Disease	Disease subgroup	Gene	Liver disease	Renal disease	Other features	Prevalence
ARPKD		PKHD1	CHF common	Cystic disease	None	1 in 20,000
Meckel–Gruber syndrome		MKS1, TMEM67, TMEM216, CEP290, CC2D2A, RPGRIP1L B9D1	CHF common	Cystic dysplasia	Encephalocele often posterior Postaxial polydactyly, other central nervous system anomalies, orofacial clefts	2.6 in 100,000
Nephronophthisis		NPHP1-18	Occasionally CHF	Cystic kidneys – normal or small in size	Yes in NPHP-related ciliopathies (NPHP-RC)	1 in 50,000–100,000
Joubert syndrome		TMEM67, CC2D2A, RPGRIP1L, CEP290	Occasionally CHF	Cystic kidneys – normal or small in size	Cerebellar and brainstem abnormality (molar tooth sign) hypotonia, abnormal breathing pattern, atypical eye movements, developmental delay, truncal ataxia hepatic fibrosis	1 in 100,000
Renal–hepatic–pancreatic dysplasia		NPHP3	CHF common	Cystic dysplastic kidneys	Fibrocystic dysplasia of pancreas, polydactyly, situs inversus	Less than 1 in 100,000
Skeletal ciliopathies						
Short-rib polydactyly syndromes	Jeune asphyxiating thoracic dysplasia		CHF occasional	NPHP	Shortened ribs, thoracic cage constriction, short tubular In Jeune asphyxiating thoracic dysplasia situs inversus	Rare
	Ellis–van Creveld (EVC) (chondroectodermal dysplasia) syndrome		CHF common	NPHP	Polydactyly, limb and rib shorterning, ectodermal dysplasia affecting hair, teeth, and nails. Cardiac abnormalities	Rare
Sensenbrenner syndrome (cranioectodermal dysplasia)		IFT122, WDR35, C14ORF179 and WDR19,	CHF common		Characteristic craniofacial appearance, craniosynsotosis, frontal bossing, dolichocephaly; metaphyseal dysplasia with narrow thorax, short fingers and proximal limbs, respiratory problems and cardiac defect	Rare
Oral–facial–digital (OFD) syndromes		OFD1 and OFD related genes	CHF occasionally		Oral cleft, pancreatic cysts	1 in 100,000
Bardet–Biedl syndrome		BBS1-18	CHF occasionally	Cystic dysplasia	Retinal dystrophy, obesity, polydactyly, learning difficulties, diabetes mellitus	1 in 100,000
Alström syndrome		ALMS1	CHF occasionally		Retinal dystrophy, pulmonary disease, obesity, sensorineural hearing loss, cardiomyopathy, diabetes mellitus	Rare – 950 cases worldwide
ADPKD		PKD1 and PKD2	CHF rare	Enlarged cystic kidneys	Hypertension, intracerebral aneurysms	1 in 1000

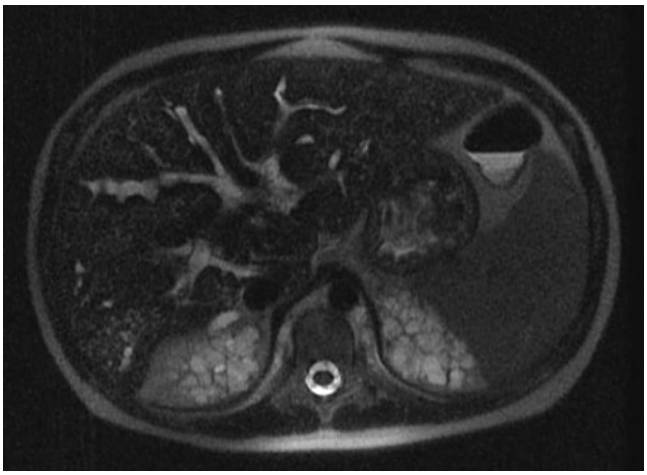


Figure 14.1 Magnetic resonance cholangiopancreatiocogram demonstrating multiple, tiny intrahepatic cysts, consistent with Caroli disease and hepatic fibrosis.

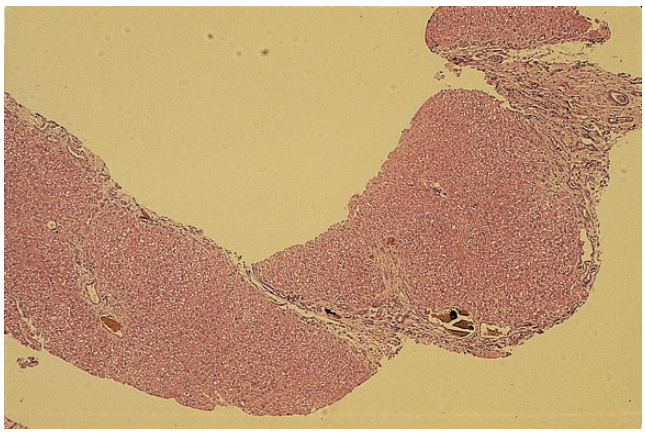


Figure 14.2 Histology of congenital hepatic fibrosis demonstrates widened portal tracts which are linked by broad bands of fibrous tissue. Bile ducts are prominent, dilated, and abnormal. Recurrent cholangitis may lead to biliary cirrhosis.

  - There may be inspissated bile in the lumen of some biliary ducts.
  - von Meyenburg complexes, which are multiple bile duct hamartomas, may be seen within dense fibrous stroma.
- Endoscopy may demonstrate esphageal and gastric varices and a portal gastropathy secondary to portal hypertension.
- Genetic analysis to identify the precise disorder (see Table 14.1).

Monitoring of liver disease

As CHF causes hypersplenism, splenomegaly as well as portal hypertension it is important to perform the following investigations on an annual basis:

- Full blood count – for signs of pancytopenia caused by hypersplenism.
- LFTs as well as monitoring of the synthetic liver function (clotting, glucose, etc.) as these all should be normal. Occasionally the ALP and GGT are mildly elevated in CS or if there is cholangitis.
- Ultrasound of liver and spleen as well as kidneys. Ultrasound is a very sensitive investigation to monitor splenic size, biliary tree abnormalities as well as abnormalities of the kidneys. It may also show varices.
- Upper gastrointestinal endoscopy should be considered where there is evidence of portal hypertension. This can provide useful information for families on the future risk of gastrointestinal bleeding and the need for prophylaxis for large esophageal varices. There is no definitive evidence that this is effective and a decision should be made on a case-by-case basis, including local endoscopic expertise and results, the family's view and the availability of emergency services in the event of gastrointestinal bleed.
- Oxygen saturations should be regularly monitored as they become abnormal if hepatopulmonary syndrome develops.
- Cardiovascular assessment with echocardiogram and cardiac angiography to determine pulmonary arterial pressures.
- Geneticist referral and counseling bearing in mind that some of the families may wish to have more children and ensuring that they are fully informed of their reproductive options is important.

Treatment of complications of liver disease

- Hypersplenism causes pancytopenia including low platelets which may lead to increased bruising, epistaxis and greater risk of variceal bleeding. Non-steroidal anti-inflammatory drugs (NSAIDs) are contraindicated as they will affect the function of platelets too. The neutropenia is usually not functional. Anemia may be a problem especially if renal anemia is also present. Contact sports are contraindicated if the spleen is very large, but splenic rupture is unusual. Splenectomy should not be performed as it will not treat portal hypertension and may complicate liver transplantation.
- Variceal bleeding is an important cause of morbidity and mortality in patients with CHF. The management of varices – including primary prophylaxis – is reviewed in detail in Chapter 21. The families of all children at risk of significant varices should be provided with written information about what to do in the event of gastrointestinal bleed.
- Cholangitis is a common complication in patients with CHF but especially those with CS and CD. The risk of ascending cholangitis is 6% for CHF and 65% for CS. Clinical features include jaundice and/or right upper quadrant pain but these are not always present. Treatment is with broad-spectrum antibiotics with good Gram-negative and anaerobic cover according to local antibiotic policies. As recurrent cholangitis is common prophylactic

antibiotics should be considered. If there is a requirement for renal transplantation in the setting of recurrent cholangitis, then a combined liver–kidney transplant should be considered as there is a greater risk of fatal sepsis post-renal transplant in such patients.

- Cholestasis may develop from the combination of ductal plate malformation and bile duct damage from recurrent episodes of ascending cholangitis. If severe, fat and fat-soluble vitamin malabsorption may cause nutritional problems and exacerbate bone disease especially if there is concurrent bone disease from renal failure.
- Pruritus may be problematic. Ursodeoxycholic acid (10–20 mg/kg) is helpful as a choleretic as is standard therapy (see Chapter 8).
- Biliary stones are a possible complication of CS and CD. Removal depends on their size, location, and number and should be done in a specialized center. Choleretics such as ursodeoxycholic acid may be beneficial in preventing cholestasis and biliary stones although there is no evidence to support this.
- Cholangiocarcinoma and hepatocellular carcinoma are rare complications of CS and CD which may be detected on surveillance ultrasound imaging.

Preventative management

Infection with hepatitis viruses may precipitate a crisis, vaccination against hepatitis A and B are recommended in patients with CHF/CS.

Initial management and surveillance of renal disease

Several conditions that cause CHF also cause renal disease. Therefore, it is important to screen for renal disease with renal ultrasound, serum creatinine and glomerular filtration rate (GFR) (measured or estimated using Schwartz formula), urine protein : creatinine ratio, and blood pressure monitoring.

Treatment of chronic kidney disease

Chronic kidney disease (CKD) is divided into five stages depending on the GFR:

- Stage 1 – GFR >90 mL/min/1.73 m^2.
- Stage 2 – GFR 60–89 mL/min/1.73 m^2.
- Stage 3 – GFR 30–59 mL/min/1.73 m^2.
- Stage 4 – GFR 15–29 mL/min/1.73 m^2.
- Stage 5 – GFR <15 mL/min/1.73 m^2.

Preparations for renal replacement therapy should commence when stage 4 CKD is reached. Renal osteodystrophy may occur as early as stage 2 CKD and is managed by phosphate restriction and administration of activated vitamin D supplements. Renal anemia is a particular feature of some of the fibrocystic diseases such as nephronophthisis and is managed by prescribing optimal iron therapy as well as erythopoietin.

Nutrition is extremely important in the management of children with CKD who have poor appetite, high energy demands, and are often catabolic. They may require nasogastric tube feeding to achieve optimal nutrition. Growth hormone therapy should be considered for those children whose height velocity is low despite optimal nutrition.

In terms of renal replacement therapy, pre-emptive renal transplantation is preferred. If this is not possible, then either hemodialysis and peritoneal dialysis should be offered depending on the patient and family. Peritoneal dialysis should not be used if there are concerns about recurrent transplantation or liver transplant is imminent.

Timing and indications for transplantation

The decision regarding transplantation is challenging and requires assessment by a skilled multidisciplinary and multispecialty team in a center with significant expertise. A useful algorithm is as follows (Figure 14.3).

In general, children rarely require liver transplantation for liver failure and the timing of transplantation is determined by the need for renal transplantation (see Chapter 33).

Combined liver and kidney transplantation is usually indicated because of the risk of sepsis and recurrent cholangitis increases with post-transplant immunosuppression and is a major cause of morbidity and mortality especially after isolated renal transplantation (see Chapter 33).

Outcome

A recent 20-year review of combined kidney and liver transplantation in 716 children with fibrocystic liver kidney disease, reported that 73 had an isolated liver transplant, 602 had a renal transplant and 41 a combined liver kidney transplant [9]. This reflects the variability in degree and rate of progression of liver and kidney disease in each individual patient.

Post-transplant mortality was as follows: 23% for liver, 10% for renal, and 12% for liver–kidney transplant.

Thus, whilst the issue of transplantation in CHF associated with kidney disease is a challenging one, the combination of better data regarding outcomes in association with the development of more useful criteria will be beneficial.

Future therapies

Future therapies are discussed after the conditions associated with CHF are reviewed.

Autosomal recessive polycystic kidney disease

ARPKD is caused by mutations in a single gene, *PKHD1*, which encodes fibrocystin/polyductin protein. This is a single-membrane spanning protein that is localized to the apical membrane, the primary apical cilium/basal body, and

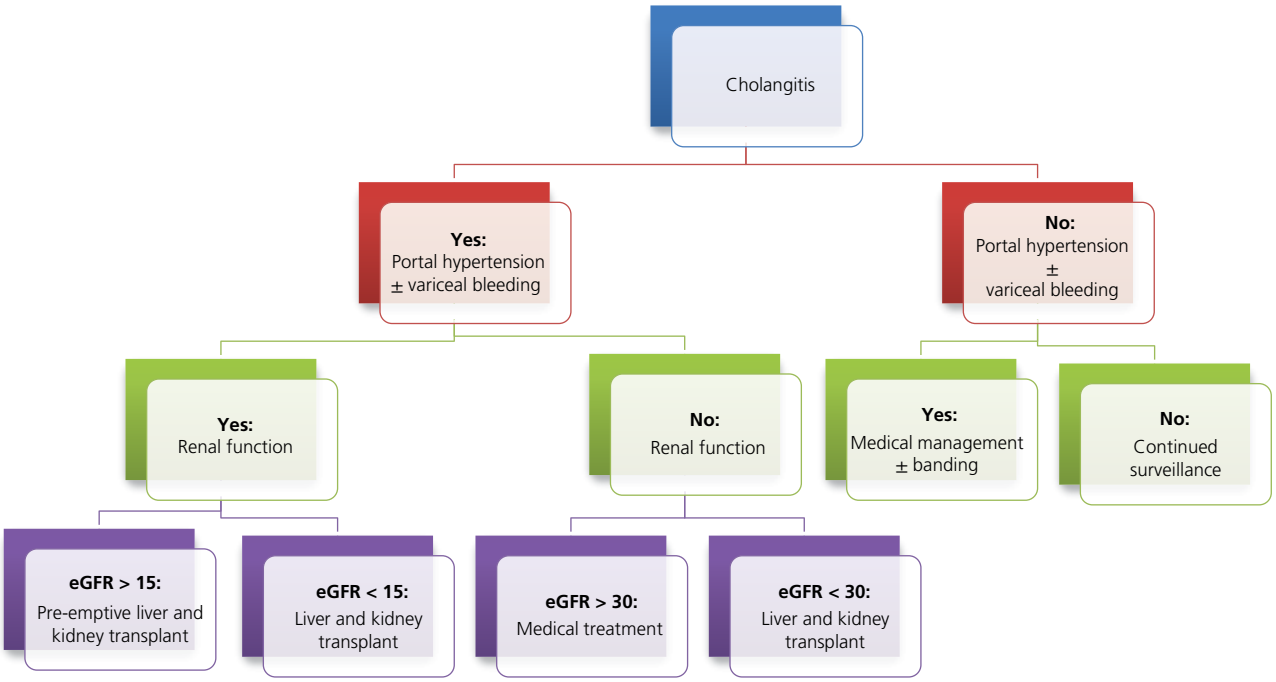


Figure 14.3 Algorithm for the management of hepatic and renal complications of fibrocystic hepatorenal disease. eGFR, estimate glomerular filtration rate.

the mitotic spindle. ARPKD is a severe, typically early-onset form of cystic disease that primarily involves the kidneys and biliary tract and typically has a high perinatal mortality rate of 30–40% caused by pulmonary hypoplasia. In the patients that survive infancy, the 10-year survival of those who live beyond the first year of life has improved to 82%. Fifteen-year survival is estimated at 67–79%, and may be improving.

Clinical features

Age and presenting features. The incidence of ARPKD is approximately 1 in 20,000 live births. Approximately half of patients present perinatally with enlarged cystic kidneys and one-third die in the neonatal period due to pulmonary hypoplasia. Hypertension is present in over two-thirds of patients and requires treatment with several antihypertensives.

The liver manifestations of CHF are mild in childhood and progress at a variable rate to portal hypertension with associated complications.

Patients who present in late adolescence or adulthood tend to present with hypertension and milder renal disease.

Diagnosis

The diagnosis of ARPKD is made on the characteristic clinical features, radiological findings which confirm the presence of cysts in the kidneys, and confirmed by genetic testing.

Prenatal testing for future pregnancies is a possibility.

Natural history and complications

The initial renal manifestations include systemic hypertension, hyponatremia, and renal impairment which lead to end-stage renal failure. Although most ARPKD patients progress to end-stage renal disease, the timing of this is highly variable and depends on the age at initial presentation: approximately 25% of patients diagnosed in the perinatal period will need renal replacement by the age of 11 years, whereas only 25% of those presenting after 1 month of age will need renal replacement by the age of 32 years.

Fewer than half of infants present with CHF and the onset is variable. Most children have features of CHF or CS.

A subset of patients with ARPKD with *PKHD1* mutations have a phenotype of isolated CHF with minimal or no kidney disease.

Management

It is important to monitor all ARPKD patients closely. Platelet counts, prothrombin time, splenic volume, and Doppler flow studies have been correlated with the severity of portal hypertension and should be serially monitored. Liver synthetic function is preserved and transaminases are generally normal, with infrequent abnormalities in serum alkaline phosphatase and γ-glutamyltransferase.

Medical management includes conventional treatment for preventing and treating cholangitis with prophylactic antibiotic; surveillance endoscopy; and treatment of portal hypertension.

Indications for liver transplantation

Combined kidney–liver transplantation is required when there is a combination of renal failure and either recurrent cholangitis or significant complications of portal hypertension, e.g., recurrent variceal bleeding or hepatopulmonary syndrome [10]. Liver transplantation has been required in about 7% of long-term survivors who had significant portal hypertension or recurrent cholangitis [11] (see Chapters 31 and 33).

Meckel–Gruber syndrome

MKS is an autosomal recessive disease with a reported prevalence of 2.6 per 100,000 births with only 1 in 5 being live births [12].

MKS shows significant genetic heterogeneity and several loci have been identified for MKS: *MKS1*, *TMEM67*, *TMEM CEP290*, *CC2D2A*, *RPGRIP1L*, and most recently *B9D1*. The underlying disease mechanism in MKS1 has revealed that absence of functional MKS1 results in de-regulation of multiple signaling pathways (Wnt, mTOR, and Hh) which leads to ectopic high cell proliferation in the brain and kidney of the mutant mouse model.

There is significant clinical overlap with Joubert syndrome and related disorders (JSRDs) and BBS.

Clinical features and diagnosis

A recent European Surveillance of Congenital Anomalies (EUROCAT) network review reported that the commonest clinical features include:

- Cystic kidneys (97.7%).
- Encephalocele which is often posterior (83.8%).
- Postaxial polydactyly (87.3%).
- Fibrocystic liver disease (65.5%).
- Other central nervous system anomalies (51.4%).
- Orofacial clefts (31.8%).

Various other anomalies were present in 64 (37%) patients. Over 90% of cases are antenatally detected and many pregnancies are terminated due to the central nervous system abnormalities.

Management and outcome

The management of CHF is discussed earlier. Patients with MKS require a multispecialty multidisciplinary team to manage all the other features of the condition in an integrated fashion including genetic counseling for the family who may be considering future pregnancies.

Nephronophthisis

Nephronophthisis (NPHP) is a form of ciliopathy, causing cystic kidneys which are either normal or small in size; cysts are mainly concentrated in the corticomedullary junction with a significant amount of tubulointerstitial fibrosis.

It is the most common genetic cause of end-stage renal failure in the first three decades of life. With an incidence of 0.9 cases per million people in the US, and 1 in 50,000 births in Canada. It has an autosomal recessive pattern of inheritance so it occurs more commonly in consanguineous families.

Mutations in *NPHP* genes cause defects in signaling mechanisms, including the non-canonical Wnt signaling pathway. There are an increasing number of genes causing nephonophthisis – so far 13 have been reported. Nephrocystins, which are the gene products of *NPHP*, are highly conserved in a variety of species from *Caenorhabditis elegans* and zebrafish. This has helped to elucidate the fact that multiple interactions between the different nephrocystins exist and outline the interconnectivity of the affected proteins and shared pathways.

Clinical features and diagnosis

There are three forms of NPHP which have different onsets of end-stage renal failure (ESRF): infantile, juvenile and adolescent NPHP which develop ESRF at the median ages of 1, 13, and 19 years, respectively. Presenting symptoms are insidious with polyuria, polydipsia, enuresis, and anemia.

About 15% of patients also have extrarenal manifestations and are said to have NPHP-related ciliopathies (NPHP-RC). These include retinitis pigmentosa (in Senior–Løken syndrome), liver fibrosis, ciliary dyskinesia, skeletal abnormalities, and cerebellar vermis aplasia (Joubert syndrome) [13].

NPHP2 is infantile type NPHP and is sometimes associated with situs inversus which can be explained by its relation with *inversin* gene. *NPHP1*, *NPHP3*, *NPHP4*, *NPHP5*, and *NPHP6* are associated with retinitis pigmentosa.

Management and outcome

Management of NPHP focusses on the management of chronic kidney disease and eventually renal replacement therapy. It is important to exclude or manage CHF if present. There is no disease recurrence in the renal graft post-renal transplant.

Joubert syndrome

Clinical features and diagnosis

Joubert syndrome is a group of heterogeneous conditions characterized by a distinctive cerebellar and brainstem abnormality known as the molar tooth sign and accompanying neurological symptoms of hypotonia, abnormal breathing pattern, atypical eye movements, developmental delay, truncal ataxia as well as NPHP, retinal dystrophy, and hepatic fibrosis [14].

The prevalence of Joubert syndrome is estimated to be 1 in 100,000. There is also considerable genetic heterogeneity

with more than 21 genes identified to date with complex genotype–phenotype correlation.

Liver involvement occurs in 80% in a group of patients with *TMEM67* gene mutations. Mutations in the genes *CC2D2A* or *RPGRIP1L* cause Joubert syndrome with liver involvement which is known as COACH syndrome (cerebellar vermis hypoplasia, oligophrenia, ataxia, coloboma, and hepatic fibrosis).

Mutations in the *CEP290* gene account for approximately 50% of Joubert syndrome patients with the cerebello–oculo–renal phenotype, also known as Senior–Løken syndrome, in which patients have juvenile NPH and retinopathy. However, there is significant variability even within the same family. Joubert syndrome can be subclassified according to the extent of the organs involved.

There is also considerable genotype and phenotype overlap with other ciliopathies including NPHP, MKS, BBS, oral–facial–digital syndrome and skeletal ciliopathies. This variability is likely to be explained by an oligogenic model of inheritance, in which mutations, rare variants, and polymorphisms at distinct loci interplay to modulate the expressivity of the ciliary phenotype. In fact, MKS and Joubert syndrome are allelic disorders with 11 genes so far known to cause both conditions. There are even reports of siblings with the same homozygous mutation who have either MKS or Joubert syndrome [15].

Management and outcome

Therefore, it is very important that patients with Joubert syndrome are assessed for other organ involvement and screened regularly for the potential development of other organ involvement. This includes regular ophthalmological surveillance of ocular movements, visual acuity, slit-lamp examination, fundoscopy, and electroretinogram.

Renal surveillance is also important as about 20–30 % of Joubert syndrome patients have NPHS which may slowly progress, initially only manifesting with loss of urine concentrating capacity and only later presenting with abnormal renal function, anemia, and abnormal ultrasound which shows small kidneys.

Renal–hepatic–pancreatic dysplasia

Renal–hepatic–pancreatic dysplasia was first described in 1959 by Ivemark *et al.* [16] as a triad of cystic dysplastic kidneys, and fibrocystic dysplasia of the liver and pancreas. It is caused by mutation in the *NPHP3* gene and other characteristics include situs inversus, polydactyly, and preauricular fistulae. Most cases are fatal in infancy but some have successfully undergone combined liver–kidney transplant. There is clinical overlap with MKS and other ciliopathies.

Skeletal ciliopathies

There are a group of ciliopathies which affect bone and cartilage development via pathways such as hedgehog resulting in chondrodysplasia in a variety of phenotypes. This group includes short-rib polydactyly syndrome, Sensenbrenner syndrome, and oro–facial–digital syndrome type 1.

Clinical features and diagnosis

The short-rib polydactyly group includes the lethal conditions short-rib polydactyly types I–IV as well as Jeune asphyxiating thoracic dysplasia (JATD) and EVC (chondroectodermal dysplasia) syndrome. In the two latter conditions, there are shortened ribs and thoracic cage constriction which can be fatal in the neonatal period, short tubular bones, and trident morphology of acetabulum.

In JATD there is NPH, CHF, liver cysts as well as occasionally situs inversus. EVC is a milder condition with polydactyly, limb and rib shortening but also ectodermal dysplasia affecting the hair, teeth, and nails. Liver and renal involvement are less commonly reported in EVC but cardiac abnormalities such as atrial septal defects are present in about 50% of cases.

Sensenbrenner syndrome is also known as cranioectodermal dysplasia which is a skeletal ciliopathy with a characteristic craniofacial appearance accompanied by craniosynsotosis, frontal bossing, and dolichocephaly; metaphyseal dysplasia with narrow thorax, short fingers and proximal limbs as well as nephronophthisis, CHF, respiratory problems, and occasionally cardiac malformations. Mutations in four genes, namely *IFT122*, *WDR35*, *C14ORF179*, and *WDR19*, have been found in Sensenbrenner syndrome and they encode ciliary IFT complex A proteins. There is genetic and phenotypic overlap with other skeletal dysplasias including short-rib polydactyly and EVC.

Another subgroup of skeletal ciliopathies is the oral–facial–digital syndromes which are characterized by abnormalities of the face, oral cavity, and digits. There are least 10 different oral–facial–digital syndromes of which type 1 is the most common. Type 1 is an X-linked condition which features polycystic kidneys but also the lesser known problems of fibrocystic disease of the liver and pancreas which need lifelong surveillance [17].

Management and outcome

The management of these skeletal dysplasias requires multidisciplinary and multispecialty coordinated care. If there is CHF and/or renal disease, management is as above. Prognosis depends on the combination of features but there are limited outcome data.

Bardet–Biedl syndrome

BBS is a ciliopathy with a wide range of features which vary in severity and in timing of onset. So far 18 genes have been described (*BBS1-18*) with seven BBS proteins (BBS1, −2, −4, −5, −7, −8, and −9) which form a stable complex called a BBSome whose function is to regulate the export and/or import of ciliary proteins [18]. There is significant variation in phenotype and triallelic inheritance occurs in BBS where the third mutation in one of the BBS genes causes the disease state or a more severe phenotype.

Clinical features and diagnosis

The diagnosis is based on at least four major features which include:

- Progressive retinal degeneration with rod–cone dystrophy.
- Obesity.
- Polydactyly.
- Genital abnormalities.
- Learning difficulties.
- Renal abnormalities which include cystic kidneys and end-stage renal failure.

Other features include:

- Diabetes mellitus.
- Dental abnormalities.
- Developmental and speech delay.
- Congenital heart disease.
- Ataxia.
- Brachydactyly or syndactyly.
- Anosmia.
- CHF.

Management and outcome

Patients with BBS need to be monitored for associated renal, hepatic, endocrine, and retinal disease.

Alström syndrome

Alström syndrome is another ciliopathy phenotypically similar to BBS. It is caused by mutations of the *ALMS1* gene which encodes a ciliary basal body protein ALMS1 which is thought to contribute to centriolar stability.

Clinical features and diagnosis

The phenotype of Alström syndrome includes retinal degeneration, renal, hepatic and pulmonary disease, childhood truncal obesity, sensorineural hearing loss, cardiomyopathy, and type 2 diabetes mellitus. The age of onset and phenotypic features can vary widely even within the same family. Retinal degeneration occurs from birth often leading to blindness in childhood with the first signs being nystagmus and photophobia. Dilated cardiomyopathy affects two-thirds of patients who are at risk of sudden cardiac death. Obesity and diabetes mellitus are early features. Slowly progressive renal disease, hypertension, and hepatic dysfunction are frequent. Intelligence is normal although there may be some motor delay.

Management and outcome

Patients with Alström syndrome require coordinated multidisciplinary and multispecialty coordinated care. Features and complications of CHF must be actively sought and proactively managed.

Autosomal dominant polycystic kidney disease

ADPKD is the most common ciliopathy and is caused by mutations in two genes: *PKD1* and *PKD2* which encode for polycystin-1 and polycystin-2, respectively. Although polycystic liver disease (PLD) is not associated with CHF, there are well-documented cases of CHF and portal hypertension in ADPKD with pathogenic variants in *PKD1*. In fact, CHF in these cases of ADPKD are very similar to CHF in ARPKD with portal hypertension being the main consequence. Interestingly, CHF was not inherited vertically from affected parent with ADPKD which implicates.

Clinical features and diagnosis

Classically there are numerous cysts in kidneys which are very enlarged. The onset of renal failure is in late adulthood although there are cases of children with ADPKD requiring renal replacement therapy. At least 10% of children with ADPKD have hypertension and this should be regularly monitored in all those at risk. Extrarenal features include cysts in the liver and pancreas as well as intracranial aneurysms and mitral valve prolapse.

PLD is the most common extrarenal manifestation of ADPKD and has become more prevalent due to improved renal survival and life expectancy. PLD causes hepatomegaly early in the disease course. Cyst volumes are greater in women and with increasing age and are associated with a lower quality of life. With advanced PLD, there is loss of liver parenchyma with abnormalities in LFTs, splenomegaly, and hypersplenism.

Management and outcome

Management is mainly focussed on renal aspects with emphasis on the management of hypertension and reducing the decline of renal function. Once end-stage renal failure is reached, management is as discussed above. There are several potential therapeutic agents targeting activation of the cyclic adenosine monophosphate (cAMP) signaling and the mammalian target of rapamycin (sirolimus) (mTOR) pathways, as well as dysregulation of the epidermal growth factor receptor (EGFR) axis which have been well described in human

ADPKD and murine models of PKD. Tolvaptan has been shown to slow down cyst development but is associated with a significant side effect profile.

Future therapies

Although there is no disease modifying treatment for ciliopathies, this may change in the future as many research groups are evaluating the treatment for ADPKD. Treatment targets have included activation of the cAMP signaling and the mTOR pathways, as well as dysregulation of the EGFR axis which have been well described in human ADPKD, ARPKD as well as animal PKD models. All these treatment targets are implicated in the pathogenesis of cilia dysfunction.

Several treatments have been beneficial in animal models but have not been as effective in human clinical trials in adults with established cysts and disease. Earlier treatment may prove more beneficial but there are complex issues in treating younger patients as the treatments may have undesirable effects on growth and development. Tovalptan, which antagonizes the vasopressin 2 receptor (V2R) and decreases intracellular cAMP has been approved for ADPKD while octreotide which is a somatostatin analogue is also beneficial in ADPKD.

Treatment for blindness in BBS models using pharmacological agents are also being investigated.

Gene therapy or stem cell treatments have the potential to target specific organs and correct the genetic defects on a cellular level. Several gene therapies have been successful in ciliopathy models which have rescued cilia mobility, vision, and olfaction. This technology may improve our understanding of ciliopathies and lead to other treatment avenues in the future.

Vascular disorders of the liver

Vascular anomalies are classified into tumors and malformations. Vascular malformations are divided into simple and combined, based on which vessel type(s) are involved. By far the commonest lesions are hepatic hemangiomas, which are considered benign vascular tumors [19]. While the majority of these are asymptomatic, clinical presentations may include hepatomegaly, heart failure, anemia, thrombocytopenia, hypothyroidism, and liver dysfunction. In addition there are a wide range of rarer vascular disorders that will be considered individually.

Hepatic hemangiomas are not a homogenous group, compromising two distinct pathologies and three distinct phenotypes: focal, multifocal, and diffuse [19] (Table 14.2).

Table 14.2 Types of hepatic vascular tumors.

Benign	Focal hepatic hemangioma
	Multifocal hepatic hemangioma
	Diffuse hepatic hemangioma
Malignant	Epithelioid hemangioendothelioma
	Hepatic angiosarcoma

Focal hepatic hemangioma

These usually show the biological behavior of congenital hemangiomas. Congenital hemangiomas develop prenatally and are fully grown at birth.

Based upon their natural history, two major subtypes of congenital hemangiomas have been recognized: rapidly involuting congenital hemangiomas (RICHs) and the rarer non-involuting congenital hemangiomas (NICHs). The majority of RICHs involute by 1 year of age whereas NICHs do not completely regress but grow in proportion with the child. These lesions do not express endothelial glucose transporter 1 (GLUT1), as opposed to multifocal lesions which strongly express this.

Focal hepatic hemangiomas have associated skin hemangioma in just over 50% of cases. At presentation it is important to distinguish the liver lesions from malignant causes such as hepatoblastoma, mesenchymal hamartoma, and angiosarcoma. Although they may be associated with high output cardiac failure, the natural history of this subtype may be benign, but intervention may be necessary as described later.

Multifocal and diffuse hepatic hemangioma (see also Chapter 28)

In contrast to focal hemangiomas, these lesions usually show the biological behavior of infantile hemangiomas. Infantile hemangiomas arise from proliferation of multipotent endothelial stem cells [20]. They express high levels of angiogenic proteins, including vascular endothelial growth factor (VEGF) and the characteristic GLUT1-positive cells which emphasizes their biological distinction from congenital hemangiomas [21]. They also express type 3 iodothyronine deiodinase which inactivates thyroxine and can result in severe hypothyroidism [22]. These lesions evolve through three phases: proliferation, involution, and involuted. The proliferative phase usually lasts from shortly after birth until 1 year of age. Involution begins as proliferation ends, generally at about 1 year, and usually lasts for 4–6 years. In the involuted state, the hemangioma is replaced by fibrofatty tissue.

Predisposing factors for infantile hemangiomas include white race, female sex, prematurity, low birth weight, and multiple pregnancy.

Infantile hemangiomas are not visible at birth, but are obvious within the first 1–2 weeks of life. They grow rapidly, reaching 80% of their final size by 3 months and are fully grown by 9 months. Most will have involuted maximally by 4 years of age.

Hepatic lesions are more common in those with multiple skin lesions and are very rare in the absence of skin lesions.

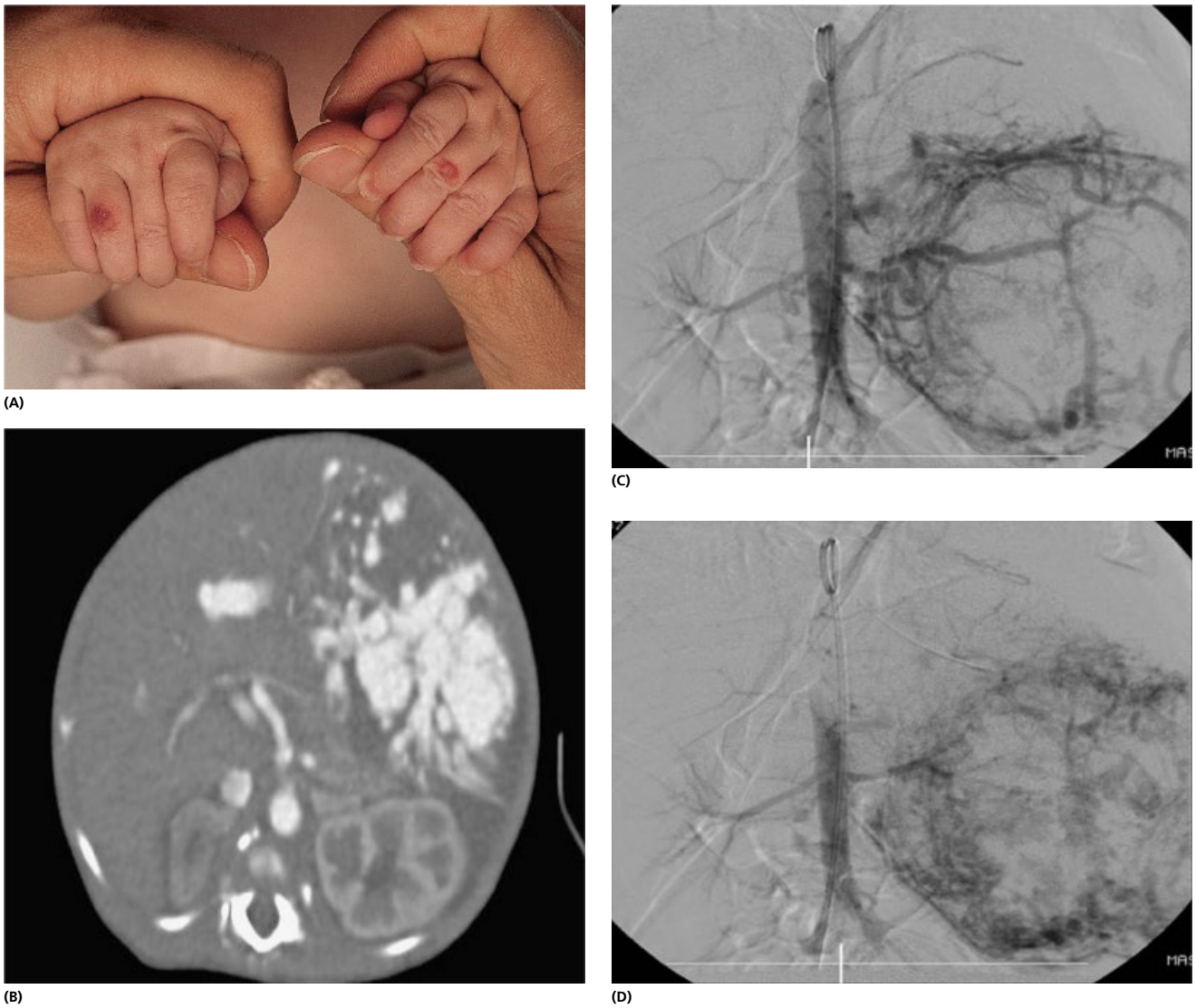


Figure 14.4 Cutaneous hemangiomatas are common and usually regress within 6 months (A). They may be associated with multiple hemangiomatas leading to high output cardiac failure. This CT angiogram in a 12-week-old infant demonstrates a large hepatic hemangioma. (B) Treatment includes cyanoacrylate glue embolization using a pigtail catheter in lower thoracic aorta showing intense hypervascularity of the hepatic hemangioma before (C) and reduction in blood flow following successful embolization (D). Hepatic artery ligation or liver transplantation are alternative treatments.

About 16% of children with five or more skin lesions will have hepatic hemagiomas [23].

Multifocal hepatic hemangiomas are individual spherical lesions separated by normal liver tissue. In contrast, diffuse hepatic hemangiomas replace large amounts of hepatic parenchyma with innumerable centripetal enhancing lesions visible on CT. The multifocal lesion are more likely to be associated with high output cardiac failure, whereas the diffuse lesions are marked by a large mass effect, liver failure, and hypothyroidism.

Clinical features

Congenital hemangiomas may be detected on antenatal ultrasound as they are fully developed by birth.

Children with cutaneous hemangiomas should undergo a screening abdominal ultrasound if there is hepatomegaly or more than five cutaneous lesions.

Most patients with symptomatic hepatic hemangioma present before 2 months of age and all by 6 months. Hepatomegaly and heart failure are the commonest sign but anemia, abdominal compartment syndrome, and liver failure can occur (Figure 14.4).

Diagnostic approach

- Baseline laboratory tests should include blood count, coagulation profile, conventional LFTs, and α-fetoprotein (AFP).
- Thyroid function tests should be checked if the child has diffuse and multifocal lesions.

- Ultrasound will define the number and nature of lesions. Hemangiomas classically appear as heterogeneous, septated lesions with a mixture of hypoechoic, isoechoic, and hyperreflective areas. In addition, evidence of arteriovenous shunting may be visible.
- CT demonstrates that hemangiomas are hypodense with uniform or centripetal enhancement.
- MRI highlights that they are low-intensity lesions on T1-weighted images and intense on T2-weighted images. With gadolinium contrast, hemangiomas demonstrate peripheral enhancement, followed by delayed filling of the whole lesion.
- An echocardiogram should be undertaken if there is clinical evidence of heart failure or radiological evidence of shunting.
- B-type natriuretic peptide (BNP) is a sensitive indicator of vascular overload. Serial measures of BNP can be very useful in monitoring and titrating treatment.

It is crucial to distinguish focal lesions from lesions such hepatoblastoma, mesenchymal hamartoma, and angiosarcoma. A helpful sign is finding multifocal or diffuse lesions in the liver in the absence of cutaneous hemangiomas. This may be a manifestation of malignancy such as metastatic neuroblastoma.

If baseline investigations are not definitive or if a focal lesion does not resolve after a short period of monitoring, biopsy will be required. This should be done percutaneously using image guidance in a center with facilities to deal with bleeding complications. Angiography is rarely used for diagnostic purposes but is increasingly used to deliver endotherapy (see later) therapeutically.

Treatment

This is best planned in a multidisciplinary setting with access to specialist pediatrics, endocrinology, cardiology, interventional radiology, and surgery.

Asymptomatic lesions do not require treatment as most focal hemangiomas resolve spontaneously.

Indications for treatment are related to shunt size and include:

- Cardiac failure.
- Abdominal pain and increasing hepatomegaly.
- Mass effect causing abdominal compartment syndrome.
- Respiratory embarrassment.
- Liver failure.

Pharmacotherapy

Propranolol has become the drug of choice for symptomatic multifocal or diffuse hepatic hemangiomas, following encouraging experience in treating cutaneous hemangiomas. Propranolol has an antiangiogenic effect on endothelial stem cell proliferation, but the speed of action suggest some of its effect may be vasoactive [24]. Propranolol inhibits the growth of lesions and induces more rapid involution. A small meta-analysis showed it to be significantly more effective than other drug treatments [25].

Treatment should be commenced as an inpatient in the first instance. The usual starting dose is 1 mg/kg/day given twice daily and if tolerated, titrated up to 3 mg/kg/day. Treatment once started is usually required for the first year of life, with subsequent gradual weaning with close clinical and radiological monitoring.

Prior to the adoption of propranolol, corticosteroids were the drug of choice. Prednisolone 2 mg/kg/day was usual, with subsequent weaning being guided by clinical response. This should now be reserved for patients intolerant or resistant to propranolol. Interferon-α is no longer considered appropriate while vincristine has been used in selected resistant cases. There is theoretical and experimental grounds to suggest that the antiproliferative effect of mTOR inhibitors could modify this disease, but there is as yet no clinical experience [21].

Thyroxine replacement may be indicated, and should be anticipated in diffuse lesions. Frequent monitoring and titration may be required, and this can usually be discontinued as the lesions involute.

Embolization

Embolization, by interventional radiology (see Chapter 5) is usually reserved for failure of medical treatment for multifocal or large symptomatic focal lesions, but may be indicated earlier in patients with severe heart failure. There is a risk of hepatic infarction and secondary sepsis and preventative antibiotics and antifungal treatment may be required.

Surgical treatment

Surgery is less commonly required in current practice. Hepatic artery ligation may have a role where interventional radiology is unavailable. In selected cases where there is a discrete symptomatic lesion, partial hepatectomy may be considered. A decompressive laparotomy may be helpful if a diffuse lesion results in an abdominal compartment syndrome. Liver transplantation may occasionally be required for large lesions (see also Chapters 27 and 28).

Epithelioid hemangioendothelioma

This is a rare and unpredictable tumor of vascular origin. It is commonest in young adult women but can occur in children [26]. Most present with upper abdominal pain and an abdominal mass but occasionally with jaundice or Budd–Chiari syndrome. AFP tends to be normal.

Diagnosis

Lesions have central vascularity on ultrasound while cross-sectional imaging shows a diffuse mass with multiple peripheral hepatic lesions with capsular retraction, peripheral contrast enhancement and calcification. Definitive diagnosis usually requires histology which shows positive factor VIII and CD31 staining.

Management and outcome

The tumor shows variable biological activity ranging from long-term stability to rapid progression. Chemotherapy does not have an established role and the best option is complete surgical resection where possible. Liver transplantation has been useful in adults but less effective in children [27].

Hepatic angiosarcoma

This is very rare in children. The usual presentation of hepatic angiosarcoma is as an abdominal mass and reported risk factors include radiation exposure. However, presentation with a phenotype similar to infantile hemangioma including cutaneous hemangioma has been reported [28]. The distinguishing feature in these cases was the lack of any response to treatment.

Biopsy is necessary for diagnosis and typically these lesions express endothelial markers including von Willebrand factor, CD34, CD31, and VEGF.

The outlook is poor as these tumors are usually diagnosed late. There is some evidence of response to bevacizumab and sorafenib. Complete resection or liver transplantation may have a role in selected cases.

Hepatic arteriovenous malformations

(see also Chapter 27)

These vascular anomalies are abnormal communications between the hepatic artery and portal or hepatic vein. They may occur as an isolated congenital anomaly, but more commonly complicate liver trauma or biopsy. Depending on the site of the fistula, increased arterial flow may result in portal hypertension or high output heart failure. Hepatic ischemia and hemobilia may also occur.

The most common presentation is with portal hypertension with gastrointestinal bleeding and abdominal pain. Infants are more likely to present with high output heart failure.

Ultrasound may reveal dilation of portal vein with arterial waveforms. Cross-sectional imaging shows enhancement of the portal vein during arterial phase imaging.

In the presence of symptoms endovascular transcatheter arterial embolization is the initial treatment of choice, although recurrence rates are high. In selected cases segmental resection may be necessary and in rare cases, if portal hypertension is advanced, liver transplantation may be necessary [29].

Portovenous fistulae

Most of these fistulae are congenital and are often symptomatic. They may be found serendipitously due to raised galactose levels on newborn screening. Ultrasound is usually sufficient for confirming the diagnosis. The prognosis is good, with most resolving within 2 years. Persistent fistulae are associated with the complications of any portosystemic shunt including hepatic nodules, hepatopulmonary syndrome, or hepatic encephalopathy. As a result, lesions persisting after 2 years should be treated with endovascular occlusion.

Hereditary hemorrhagic telangiectasia

This is an autosomal dominant inherited disorder with a high penetrance, characterized by multiple mucocutaneous telangiectases, recurrent nasal and gastrointestinal bleeding, and large arteriovenous malformations in the liver, lungs, and brain. It is usually caused by mutations in genes that code for the transforming growth factor β superfamily. Abnormal angiogenesis is seen, but how this causes arteriovenous malformations to develop is still unclear [30].

Hepatic abnormalities are seen in more than 50% of cases, but these are only clinically relevant in about 20%. Clinical manifestations in addition to telangiectasia and epistaxis include heart failure, portal hypertension, and ischemic biliary disease.

In symptomatic liver involvement there is usually hepatosplenomegaly and a liver bruit. Most will have abnormal liver function with elevated alkaline phosphatase or γ-glutamyltransferase. In the clinical setting finding hepatic arteriovenous malformations is usually diagnostic. Focal nodular hyperplasia and secondary biliary abnormalities are common radiological findings.

Treatment is essentially symptomatic. Recently, bevacizumab has been shown to reduce cardiac output and to ameliorate severe ischemic cholangiopathy due to hereditary hemorrhagic telangiectasia (HHT). Liver transplantation has been successful where indicated [31].

Peliosis hepatis

This is a rare condition that may lead to liver rupture and hemorrhage. Blood-filled cavities develop throughout the liver probably due to sinusoidal endothelial disruption. It is associated with infections, immunosuppressive drugs, cystic fibrosis, hereditary hemorrhagic telangiectasia, and myotubular myopathy.

Peliosis hepatis is usually an incidental imaging finding and is asymptomatic unless they bleed.

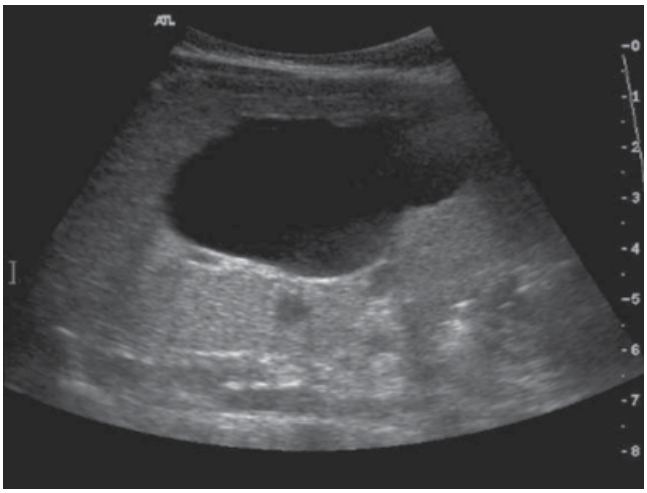
Ultrasonography reveals cystic lesions. On unenhanced CT, lesions are hypodense to liver parenchyma. Peliotic lesions are classically low intensity on MRI T1 images and bright on T2-weighted images.

Management in the acute presentation may require surgical control of bleeding. Subsequently, or in elective cases at high risk of bleeding, hepatic artery embolization is the treatment of choice [31].

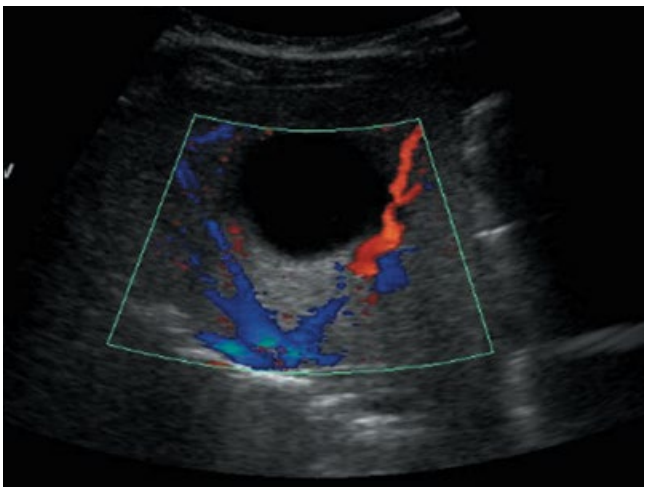
Other hepatic cysts

Solitary cysts

Solitary simple cysts occur at all ages without symptoms (Figure 14.5A,B). They may be discovered incidentally by antenatal ultrasound or other radiological studies or at autopsy. They most frequently involve the right lobe of the liver. The cysts usually contain serous fluid and are lined by atrophic biliary epithelium. Multiple cysts develop with



(A)



(B)

Figure 14.5 Abdominal ultrasound indicating single hepatic cyst with an irregular border (A). Using Doppler ultrasound, the medial hepatic vein can be seen on the lateral border and the portal vein and hepatic artery are seen on the medial border (B). These are often asymptomatic, but can be aspirated under radiological control if symptoms arise.

potassium deficiency, toxic renal injury, metabolic disease, and congenital disorders. Management is usually conservative. Observation alone is recommended unless there are symptoms such as abdominal pain from bleeding into the cyst or rapid enlargement. Radiological or surgical aspiration is rarely required. If they become infected, antibiotic therapy is recommended with lipid-soluble antibiotics such as trimethoprim–sulfamethoxazole or ciprofloxacin.

Traumatic cysts

Traumatic cysts are thought to occur after intrahepatic hemorrhage from abdominal trauma. The blood is resorbed and bile fills the space, creating a cyst. They are asymptomatic but may occasionally present with anorexia or abdominal pain from distension or rupture of the cyst. Rarely, they may become infected. Treatment includes antibiotics, and aspiration or drainage of the cysts if symptoms are severe.

Cysts secondary to infarction

These cysts occur after focal vascular insufficiency of the liver and have been observed in liver transplant recipients following occlusion of the hepatic artery. They are lined by endothelium and contain bile. They are asymptomatic but may occasionally become infected. They are usually discovered incidentally on CT or ultrasound.

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CHAPTER 15

Non-Alcoholic Steatohepatitis in Childhood

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Key points

- Non-alcoholic fatty liver disease (NAFLD) has rapidly become the most common cause of chronic hepatopathies in children and adolescents in industrialized countries.
- The natural history of this disorder is not yet completely understood, but an analysis of the available data show an elevated risk of developing progressive liver damage, up to cirrhosis and hepatocellular carcinoma, and other metabolic co-morbidities.
- NAFLD is now as considered the hepatic feature of metabolic syndrome, even if it is not actually included in the diagnostic evaluation of metabolic syndrome.
- Loss of weight and physical activity represent until now the cornerstones of treatment, but they are very difficult to achieve and to maintain. Several pharmacotherapeutic approaches including the use of insulin sensitizers, omega-3 fatty acids and vitamin E have been extensively studied in randomized trials, but with disappointing results.
- New treatments based on pathogenetic mechanisms leading to NAFLD are under evaluation to establish the effective pharmacological therapy of this disorder.

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of liver pathology characterized by the accumulation of triglycerides in the hepatocytes, which ranges from simple steatosis (accumulation of fats >5% of hepatocytes) to progressive damage, with differing degrees of inflammation, ballooning, and fibrosis, which may progress to advanced liver diseases and to cirrhosis [1].

NAFLD is an increasingly recognized worldwide cause of liver disease and is now the most common form of chronic liver disease in adults and children, related to the obesity epidemic and the metabolic syndrome (MetS) observed in the last decades.

The term "NAFLD" was coined by Ludwing and coworkers at the Mayo Clinic in 1980, referring to a subset of patients with histological evidence suggestive of alcoholic hepatitis but no history of alcohol abuse. The majority of these patients were obese females, and the most frequently associated co-morbidities were dyslipidemia and type 2 diabetes mellitus (T2DM). Shortly afterward, three cases of pediatric non-alcoholic steatohepatitis (NASH) were reported by Moran *et al.* and in the subsequent three decades, important progress has been made in the understanding of pathogenetic mechanisms, clinical implications, and therapeutic options for adults and children.

Hepatic steatosis is a common and non-specific finding, described in various liver diseases, not only in NAFLD (Table 15.1). Therefore, a careful diagnostic work-up is mandatory, in order to exclude other important and treatable causes of hepatic steatosis, such as Wilson disease, celiac disease, genetic and metabolic disorders, intoxications, drug toxicity, and viral hepatitis [1].

This chapter summarizes our understanding of the natural history, epidemiology, pathogenesis, diagnostic work-up, and treatment of pediatric NAFLD/NASH, with an overview of new therapeutic agents.

Epidemiology

The true prevalence of pediatric NAFLD is not known and the disease is probably underestimated. The difficulty in defining the exact prevalence is due to the different diagnostic tools used for diagnosis, and to heterogeneity of age, sex, and ethnicity of previous studies in children [1]. To date, certain

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Table 15.1 Causes of fatty liver disease in children.

Nutritional causes	Obesity
	Metabolic syndrome
	Protein energy malnutrition
	Anorexia nervosa
	Cachexia
	Total parental nutrition
Systemic disease	Polycystic ovary syndrome
	Obstructive sleep apnea
	Inflammatory bowel disease
	Celiac disease
	Nephrotic syndrome
	Thyroid disorders
	Type 1 diabetes syndrome
	Hepatitis C
	Hypothalamopituitary disorders or surgery
Drugs	Glucocorticoids
	Methotrexate
	Valproic acid
	Tamoxifen
	Tetracycline
	Nifedipine
	L-aspariginase
	Zidovudine
	Amiodarone
	Estrogens
	Phosphor
	Aspirin
	Paracetamol
	Vitamin A
Intoxication	Alcohol
	Ecstasy
	Cocaine
	Organic solvent
	Organic phosphates
	Carbon tetrachloride
Genetic metabolic disease	Alström syndrome
	Bardet–Biedl syndrome
	Lipodystrophy
	Turner syndrome
	Dorfman–Chanarin syndrome
Differential diagnosis	Tyrosinemia
	Galactosemia
	Glycogenosis
	Fructosemia
	Homocystinuria
	Organic acidosis
	Abeta/hypobetalipoproteinemia
	Defects of bile acid synthesis
	Hemochromatosis
	α1-Antitrypsin deficiency
	Shwachman syndrome
	Wilson disease
	Nieman–Pick disease type C
	Cystic fibrosis
	Congenital disorders of glycosylation

diagnosis of NAFLD/NASH requires liver biopsy, which is not feasible for population-based studies because of its invasiveness and high cost. Therefore, the majority of epidemiological studies on pediatric NAFLD are based on indirect non-invasive diagnostic tests, such as hepatic imaging (ultrasound, magnetic resonance imaging) and liver enzyme levels. Serum alanine aminotransferase (ALT) activity is the main screening tool used for indirect diagnosis of NAFLD, despite its lack of sensitivity as a percentage of children with biopsy-proven NAFLD may have normal ALT values. An Italian study to define liver involvement in a group of obese children reported the presence of fatty liver at ultrasound examination in about 50% of children, but hypertransaminasemia was described only in 25% of enrolled patients. Studies conducted on children in Europe and the USA estimated a 10% prevalence of NAFLD in the general pediatric population, with a higher percentage in obese children or patients with metabolic comorbidities (about 70%). NAFLD is now considered as the hepatic aspect of metabolic syndrome; patients with insulin resistance/T2DM, central obesity, and dyslipidemia have a higher incidence of NAFLD and show more severe forms of liver involvement (NASH) [2].

Clinical series of pediatric NAFLD have uniformly demonstrated that it is more common in boys than girls and there is a higher prevalence in Hispanic and Asian children than in white and black children. It is possible that this gender-based difference in the development of fatty liver is related to the influence of sex hormones. Racial/ethnic differences may be related to genetic, environmental, or sociocultural factors as well as differences in body composition. Moreover, NAFLD is more frequent in adolescents, probably because of the effect of sex hormones and insulin resistance (IR) in puberty or because of the unhealthy behavioral habits of adolescents (sedentary lifestyle and consumption of junk foods).

Pathogenesis

The pathogenesis of NAFLD is complex (Figure 15.1). In 1998 Day and James proposed the "two-hit" models, a theory in which a "first hit" induces fat accumulation in the hepatocytes, a prerequisite for the "second hit", caused by oxidative stress, which induces progression of liver damage. The complex interplay between genetic, epigenetic, environmental, and metabolic factors which has emerged in the last few years has meant that this theory has been revised to a "multi-hit" hypothesis, in which several factors are involved in NAFLD development and progression. Fat accumulation in the hepatocytes and IR are the essential events in the pathogenesis of NAFLD making the liver more prone to secondary factors that lead to NASH. The altered insulin signaling in the liver and the overload of fatty acids in the hepatocytes cause a disarray of lipid metabolism characterized by the overactivation of de novo lypogenesis transcriptional factors, causing more fatty acids and glucose products to be shunted into these lypogenetic pathways. Excess of fatty acids produces mitochondrial and peroxisomal oxidation, causing an elevated production of reactive oxygen species (ROS). In normal condition, the intracellular antioxidants and pro-oxidants systems are in dynamic equilibrium; the disruption of this balance leads to the alterated redox status of the cells,

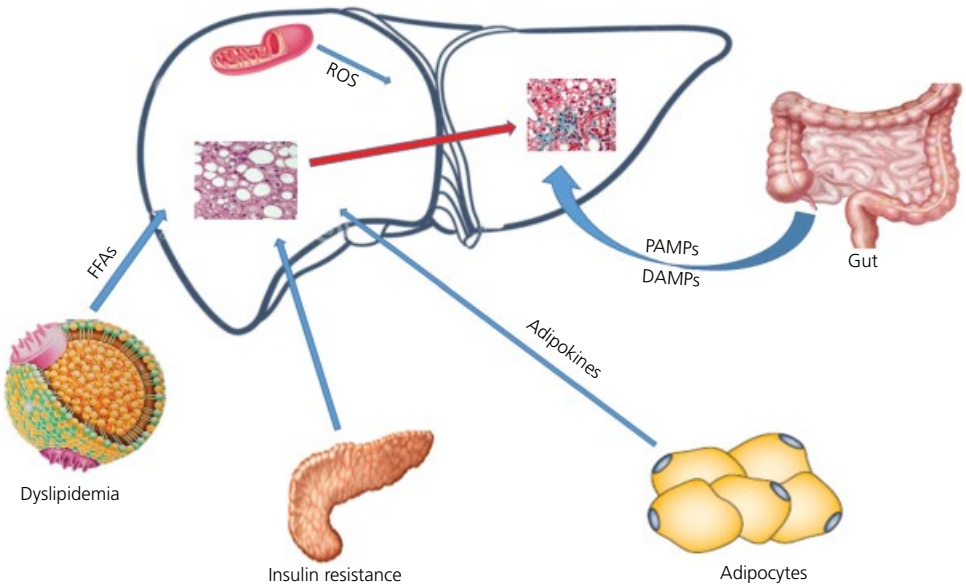


Figure 15.1 Schematic representations of non-alcoholic fatty liver disease (NAFLD) pathogenesis. FFA, free fatty acid; PAMP/DAMP, pathogen- and damage-associated molecular pattern molecules; ROS, reactive oxygen species.

triggering apoptosis and hepatic fibrogenesis and collagen production. Moreover, the exuberant fat accumulation in the liver induces a mechanism of lipotoxicity, characterized by local necroinflammatory response with subsequent damage of cell membrane and DNA [1, 3].

Key players in the progression of liver damage from simple steatosis to NASH are adipocytokines, produced by ectopic and hypertrophic adipose tissue and by inflammatory cells present in the adipose tissue in insulin-resistant conditions. Recent data have demonstrated that adipose tissue should be considered an endocrine organ, producing hormones that exert central and peripheral effects, influencing glucose and lipid metabolism and the immune response. Numerous studies conducted in human and animal models of NAFLD have shown that these adipocytokines, including adiponectin, leptin, resistin, tumor necrosis factor α (TNF- α) and interleukin (IL), IL-6 and IL-1, interfere with insulin signaling and modify hepatic FFA metabolism.

In normal condition, adiponectin binds to hepatic receptors, increasing hepatic insulin sensitivity and exerting anti-inflammatory effects. Several studies reported decreased serum levels of adiponectin in patients with fatty liver disease and NASH, with levels of adiponectin inversely related to necroinflammatory activity and grade of fibrosis, although associations between adipokine levels and the severity of hepatic steatosis and fibrosis have not been established.

Leptin is a peptide hormone secreted by adypocytes of white adipose tissue (WAT), which regulates food intake, body composition, and energy homeostasis. Levels of leptin are enhanced by proinflammatory cytokines, perpetuating the loop of chronic inflammation in obesity. Leptin levels are increased in NASH patients and are related to the grade of hepatic steatosis, while conflicting results exist about the possible relation between leptin levels and hepatic fibrosis.

Cytokines, principally proinflammatory cytokines, such as TNF-α, IL-6, and IL-1, are involved in the liver damage in NAFLD/NASH. Produced by inflammatory cells present in adipose tissue, these molecules enter the liver portal vein and exert their effects on hepatocytes by activation of inflammatory pathways and by recruitment and activation of Kupffer cells and transformation of hepatic stellate cells (HSC) into myofibroblasts. The levels of TNF-α and IL-6 are elevated in the liver and in the blood of patients with NASH, and the inhibition of these cytokines has been shown to improve NAFLD and represent potential treatments [3].

The so-called gut–liver axis is another crucial feature which may be relevant in the pathogenesis of NAFLD and chronic liver disease. Recent evidence reported that poor diet and slow intestinal transit, which is frequent in obese patients, may induce small intestinal bacterial overgrowth (SIBO), increasing the release of endotoxins, mainly of gut-derived lipopolysaccharides (LPS) and TNF-α. These inflammatory mediators easily cross the intestinal barrier that is more permeable in patients with NAFLD, increasing the severity of hepatic steatosis and promoting the progression of liver damage.

In addition, recent studies have shown that high-fat/high-fructose diets may influence gut microbiota, inducing a dysbiosis and the release of both pathogen- and damage-associated molecular pattern molecules (PAMPs or DAMPs). These molecules cross the more permeable intestinal epithelium and act as a trigger of liver damage, inducing necroinflammation and fibrosis. In NAFLD patients, disruption of tight junctions (TJ) has been demonstrated, which might explain the contribution of intestinal products (such as LPS) to the

progression of liver disease. These discoveries are relevant because of the possible therapeutic implications of prebiotics/probiotics and dietetic supplements [4].

Risk factors: role of gene and environment

It is now clear that NAFLD should be considered a multifactorial disorder in which genetic, epigenetic, and environmental factor closely interact, inducing disease development and progression.

Although environmental factors, such as sedentary lifestyle and hypercaloric diet leading to a progressive increase of body mass index (BMI) and visceral adiposity, play a central role in the pathogenesis of NAFLD, several recent studies have demonstrated the importance of genetic susceptibility in disease onset and natural history [3]. Many epidemiological, familial and twins studies have demonstrated a strong heredibility for NAFLD, as demonstrated also by the restricted number of confounding factors in childhood (duration of disease, presence of obesity, lifestyle, smoking, co-morbidities, and medications) [5].

Several studies have identified genetics determining factors of NAFLD through genome-wide association studies (GWAS). These genetic variants of the genes involved in lipid and glucoinsulinemic metabolism, in oxidative stress and the fibrogenetic process are associated with a high risk of disease development and progression. The polymorphism I148M of the patatin-like phospholipase domain-containing protein 3 (PNPLA3), involved in lipid metabolism, has been associated with severity of hepatic steatosis and hypertransaminasemia and progressive liver disease. Therefore, PNPLA3 genotype influences the severity of histological damage in NASH.

The manganese superoxide dismutase (SOD2), regulating SOD2 mitochondrial antioxidant activity, and the Kruppel-like factor 6 (KLF6), involved in the regulation of fibrogenetic process in HSCs, have polymorphisms which are associated with severity and progression of fibrosis in NASH [5].

Additional polymorphisms of genes implicated in insulin metabolism, such as the insulin receptor substrate 1 (IRS-1) appear to influence liver damage and progression of fibrosis in a number of studies including pediatric patients.

In a recent study, Nobili *et al.* evaluated the combined effect of a four polymorphisms genetic risk score in predicting NASH in NAFLD obese children with increased liver enzymes. This study suggests that a combined multi-single nucleotide polymorphism (SNP) analysis may be a useful tool in addition to other non-invasive diagnostic tests for predicting NASH in children and adolescents with fatty liver, facilitating NASH diagnosis and appropriate treatment before severe fibrosis and metabolic complications develop later in life [6].

Apart from the impact of the genetic background, hypercaloric diets (particularly those enriched in fat and fructose/sucrose) may act by encouraging the development of systemic IR accumulation of hepatic free fatty acid (FFA) or cause visceral fat deposition and consequent hepatic IR, leading to the development of fatty liver. According to the multiple-hit hypothesis, IR and FFA accumulation may predispose the fatty liver to secondary hits, including the imbalance of the production/release of hormones derived from adipose tissue (adipocytokines), oxidative stress, activation of specific nuclear receptors, and fibrogenesis.

Associated co-morbidities

Obesity

Childhood obesity represents a major public health problem, which is now considered global because it affects many low- and middle-income countries, particularly in urban settings. The global prevalence of overweight/obesity in children was estimated to be over 42 million in 2013. Obesity and its metabolic derangement are the principal risk factors for the development of NAFLD as reported in many epidemiological studies and there is a higher prevalence of NAFLD/NASH in overweight/obese patients compared to normal weight and age-matched pairs.

Recent reports suggest that waist circumference, in addition to BMI, is the best screening tool to identify the risk of NAFLD in overweight or obese children, because it reflects abdominal adiposity, which may be related to IR.

A sedentary lifestyle with an unhealthy diet facilitate the energy imbalance between caloric intake and consumption that leads to obesity. Particularly diets rich in sugar, especially fructose, and saturated fats increase not only the risk of obesity but also of fatty liver and metabolic syndrome. High fructose consumption induces de novo lipogenesis, leading to elevated triglyceride and cholesterol levels and dyslipidemia, with deleterious effects on ectopic fat deposition and glucose homeostasis, while in human studies, high-fructose diets caused increased intestinal permeability and translocation of endotoxin. Dietary fructose induces a dyslipidemic pattern, characterized by elevated levels of triglycerides, which correlates with the pathogenesis of NASH [1,3].

Insulin-resistance/type 2 diabetes mellitus

IR is a major risk factor in the development of NAFLD/NASH. Hepatic inflammation and lipid accumulation are believed to be the main drivers of hepatic IR in NAFLD. Several epidemiological studies conducted in adults and children have reported a higher prevalence of NAFLD in a subset of patients affected by alteration of glucoinsulinemic metabolism and that IR is more severe in individuals with NASH than in those with simple steatosis. Many studies have demonstrated that hypertransaminasemia and echographic

liver steatosis correlate positively with HOMA-IR (homeostatic model assessment of IR), a measure of IR, and negatively with measure of insulin sensitivity [7]. Patients with IR/T2DM may have clinical signs of IR, such as acanthosis nigricans (AN). Although the etiology is not known, it is likely that the increase in circulating serum insulin levels causes an increased stimulation of insulin and insulin-like growth factor 1 receptors, causing a progressive pigmentation of the skin and the development of papillomatous plaques. It has four grades (1–4) depending on the severity of IR. AN is found in 30–50% of children with NAFLD and IR.

Dyslipidemia

Atherogenic dyslipidemia, characterized by high levels of triglycerides and low levels of high-density lipoprotein (HDL) cholesterol is described in obese children and adolescents and in a percentage of pediatric patients with NAFLD. It is possible that this characteristic lipid profile is based on a polymorphism in adiponutrin/PNPLA3. The loss of function variant (PNPLA3 rs738409) predisposes a patient to steatosis by decreasing triglyceride hydrolysis in liver cells. There is a positive correlation between histological severity of NAFLD, assessed by NAFLD activity score and fibrosis score, and a higher atherogenic lipid pattern, suggesting that patients with NASH may be at higher risk of cardiovascular diseases compared to patients with simple steatosis [3].

Cardiovascular complications

NAFLD has emerged as a strong cardiovascular risk factor at an early age, independently from metabolic syndrome and other cardiovascular risk factors. Several studies conducted in children and adolescents demonstrated an interesting association between NAFLD and subclinical markers of atherosclerosis, such as increased carotid artery intima-media thickness and arterial stiffness. Moreover, NAFLD has also been associated with cardiac alterations, such as diastolic dysfunction and hypertrophy of the left ventricular structure. Subsequent studies demonstrated that the severity of liver injury, defined as NAFLD activity score and fibrosis score, correlates with increased atherogenic risk and cardiac changes, mainly with left ventricular hypertrophy. It is possible that this association is based on the known metabolic disarray in NAFLD, as IR and abnormal ectopic fat storage are associated with a proinflammatory chronic state [8].

Atherosclerosis in the pediatric setting may be a reversible phenomenon, if detected early and promptly treated, therefore early assessment of arterial damage and cardiac changes are mandatory to prevent future cardiovascular risk.

Several studies have demonstrated the association between altered omega-3 : omega-6 ratio and NAFLD, metabolic syndrome and cardiovascular risk. Omega-3 (N-3) fatty acids are essential, polyunsaturated fatty acids (PUFAs), which are found in large quantities naturally in fish oil, flaxseed, and some nuts. Omega-3 fatty acids derive from α-linolenic acid, and the two main component are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are both anti-inflammatory molecules. The other group of PUFAs are represented by N-6 fatty acids, which are found principally in grain; their main metabolite is arachidonic acid, which have proinflammatory and prothrombotic effects. These two class of PUFAs are competitively metabolized by the same pathways, with consequent anti- or proinflammatory effects. The optimal omega-3 : omega-6 ratio should be approximately 4 : 1, with a good balance between anti- and proinflammatory cascade, but in the modern diet, rich in omega-6 foods, this ratio is higher, about 15–16 : 1, increasing inflammation and oxidative stress and a potential risk factor of NAFLD.

Respiratory complications

Obstructive sleep apnea syndrome (OSAS) is characterized by episodes of chronic intermittent hypoxia and sleep fragmentation, which increase sympathetic activity and promote oxidative stress, proinflammatory cytokine production, endothelial dysfunction, and metabolic dysregulation, increasing cardiometabolic risk in adult populations. There is a possible association between OSAS and progression of liver damage in NAFLD and two randomized clinical trials suggested that treatment of OSAS might improve liver disease [9]. Interestingly, in 2014 Musso and coworkers described an association between NASH with advanced fibrosis and severity of OSAS, regardless of visceral fat, IR, and metabolic syndrome. These data are relevant to the diagnostic work-up and treatment of children with NASH, who, in fact, should be routinely screened for OSAS. Conversely, children with OSAS and fatty liver on ultrasound should be carefully evaluated in order to define liver involvement and the presence of NASH and advanced fibrosis.

Metabolic syndrome

Metabolic syndrome and NAFLD in children and adolescents are increasingly common with the outbreak of obesity. Although NAFLD is not traditionally considered for a diagnosis of metabolic syndrome, it is widely considered the "hepatic component" of metabolic syndrome. These disorders have a common pathogenetic origin, in which IR and oxidative stress play a major role. It has been reported that obese/overweight children with NAFLD had an odds ratio of 2.65 for having metabolic syndrome respect to obese/overweight children without NAFLD. Recently, an Italian study reported that including ultrasound evidence of fatty liver between the diagnostic criteria of metabolic syndrome, the prevalence of metabolic syndrome increased from 14 to 20% among white prepubertal obese children referred to a tertiary pediatric care unit [7].

Clinical features

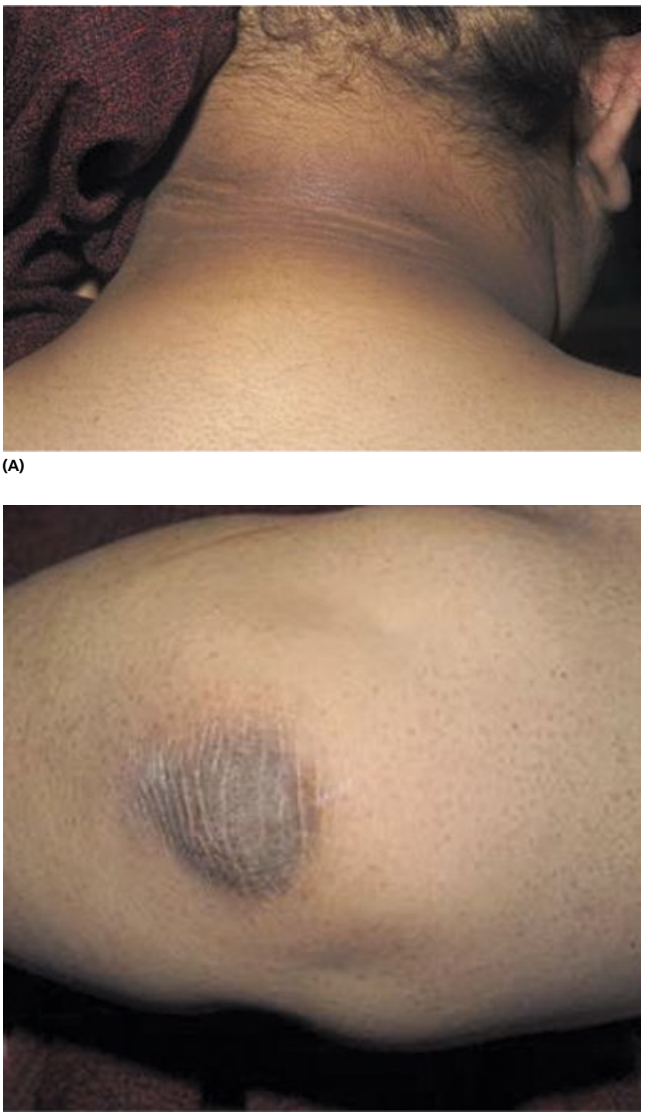
NAFLD is generally considered a "silent killer" because it is often asymptomatic and the presentation is insidious. The most common clinical presentations of NAFLD/NASH are:

- Incidental finding of elevated serum hepatic transaminases or hepatomegaly.
- Accidental discovery during abdominal ultrasound carried out for other clinical reasons or as diagnostic study of hypertransaminasemia or hepatomegaly.
- Vague right upper quadrant discomfort or fatigue (42–59% of cases) although most children are asymptomatic or may present with:
  - hepatomegaly in 50% of cases, with lower edge of liver palpable 1–2 cm below the right costal margin, in the absence of other signs of chronic hepatitis
  - overweight (BMI ≥85th percentile) or obese (BMI ≥95th percentile), with prevalent abdominal distribution of adipose tissue (visceral fat).
- Stigmata of metabolic syndrome with IR, AN, hypertension, dyslipidemia, cardiac and respiratory complications, and elevated waist circumference [3] (Figure 15.2).
- Hypothalamic dysfunction or surgery. Children with congenital or postsurgical hypothalamic dysfunction, for example, after resection of a craniopharyngioma, may be hyperphagic and obese. Recent reports suggest that they are at risk for NAFLD with rapid development of cirrhosis. NAFLD has been found in patients with Prader–Willi syndrome (PWS).

Metabolic disease

Fatty liver closely resembling NAFLD is associated with a number of metabolic or genetic diseases, which include the following.

- Alström syndrome is a rare autosomal recessive disorder which is similar to Bardet–Biedl syndrome. It is characterized by pigmentary retinopathy with infantile cone–rod dystrophy, obesity, sensorineural deafness, dilated cardiomyopathy, diabetes mellitus with IR, and normal intelligence. The genetic basis of Alström syndrome is mutations in the gene *ALMS1*. There are numerous reports of associated hepatic dysfunction with Alström syndrome: the liver disease includes a spectrum from mild steatosis, portal inflammation, and moderate fibrosis to hepatic steatosis with cirrhosis.
- Bardet–Biedl syndrome is characterized by progressive loss of visual acuity due to retinal dystrophy, central obesity, renal dysgenesis leading to progressive renal insufficiency, and male hypogonadism. Polydactyly or other abnormalities of the extremities are variable features, and mental retardation appears to occur in only a few patients. T2DM may develop in these patients because of defective insulin receptor function. Bardet–Biedl syndrome is genetically heterogeneous with at least six different loci associated with the phenotype. Cirrhosis has been reported in one patient previously.



(A)

(B)

Figure 15.2 (A,B) Acanthosis nigricans is a bluish-black pigmentation of the skin that is characteristic of insulin resistance from any cause. This young girl has grade 4 acanthosis nigricans in association with obesity, polycystic ovary syndrome, and non-alcoholic fatty liver disease.

- Polycystic ovary syndrome (PCOS) is a multisystem endocrine disorder of adolescent and young adult women characterized mainly by disorders of ovulation with menstrual disorders, features of androgen excess including hirsutism and acne, and structurally abnormal ovaries. Central obesity occurs in half the patients. AN is frequently present (see Figure 15.2). IR appears to be due to insulin receptor dysfunction or to postreceptor mechanisms. Hyperinsulinemia intensifies the adverse effects of androgen excess. Hypertriglyceridemia is often present. Recent reports confirm that NAFLD occurs in PCOS. Modest weight loss (5–10% overall) or metformin improves ovarian function and diminishes other features of androgen excess.

- Turner syndrome. Girls with Turner syndrome (XO) are often obese. Abnormal liver biochemistry has been attributed to hormonal treatment, including administration of growth hormone and estrogen, as in two girls, 13 and 14 years old, who had steatosis and fibrosis on liver biopsy.
- Lipodystrophy/lipoatrophy syndromes are primary disorders of insulin action, and hyperinsulinemia is associated with relative IR. They are genetically heterogeneous. *AGPAT2* is mutated in congenital generalized lipodystrophy. The gene product, 1-acylglycerol-3-phosphate-*O*-acyltransferase, is involved in the synthesis of triacylglycerol and glycerophospholipids. In a different form of congenital generalized lipodystrophy, mutations are found in *BSCL2*, whose gene product is a novel human protein called seipin, a protein of unknown function, although homologous to G protein. Some autosomal familial partial lipodystrophies are associated with mutations in LMNA, which encodes lamin A/C, a nuclear envelope protein. Other autosomal familial partial lipodystrophies are associated with mutations in *PPARG*, which encodes the peroxisome proliferator activated receptor γ. Patients with lipodystrophy/lipoatrophy syndromes have complete or partial lack of adipose tissue, elevated insulin, and low leptin levels. The most severely affected patients develop diabetes mellitus. NAFLD has been detected in patients with congenital forms of lipodystrophy, including one patient who later underwent liver transplantation. The severity of the hepatic steatosis is proportional to the extent of extrahepatic fat loss.

Clinical evaluation

The evaluation of children with suspected fatty liver disease includes:

- A careful assessment of feeding habits, lifestyle, and consumption of drugs.
- Identification of the presence of metabolic syndrome, obesity, and other co-morbidities in first-degree relatives.
- Anthropometric parameters, BMI and waist circumference, marker of visceral adiposity.
- Clinical signs of co-morbidities, such as AN, hirsutism, and striae rubra.
- Blood pressure should be compared with tables of percentiles for height, sex, and age.
- Hepatomegaly or splenomegaly, palmar erythema/spider nevi are suggestive of advanced liver disease.
- Exclusion of all possible causes of chronic steatotic liver disease and alternative diagnoses (Table 15.1) in children 3–8 years and especially in children <3 years old in whom genetic or metabolic diagnoses should be considered [10].

Diagnostic work-up

The early detection of fatty liver is important because it may identify those children with potential progressive forms of liver disease. A screening program for NAFLD has been introduced to identify subjects at risk, in particular those presenting with features of the metabolic syndrome. Recently, the Hepatology Committee of European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) have published diagnostic guidelines in order to guide physicians in the diagnosis of pediatric NAFLD [10]. In Figure 15.3, a flow chart with diagnostic tools used for diagnosis of NAFLD is shown.

Laboratory markers

In the laboratory assessment of pediatric NAFLD/NASH, baseline routine tests should include:

- Hepatic aminotransferases levels (AST and ALT), γ-glutamyltranspeptidase (GGT), albumin, international normalized ratio (INR), serum protein. Hypertransaminasemia with a mild elevation of liver enzymes (1.5–2 times the upper limit of normal) is a common finding in children with NAFLD, although levels of aminotransferases may fluctuate and be normal even with advanced liver damage, such as fibrosis and cirrhosis. Serum ALT and AST activity or ratio is a widely accepted test for NAFLD, but is not sensitive.
- Measurement of IR, including fasting insulin and glucose. Fasting insulin level >130 pmol/L is suggestive of IR.
- HOMA-IR has been validated for use in children. Fasting insulin (mU/L) × fasting glucose (μmol/L)/22.5.
- Hemoglobin A1c for diabetes mellitus.
- Thyroid function tests.
- Fasting (or 9 a.m.) cortisol.
- Lipid profile – cholesterol and triglycerides.
- Uric acid concentration.
- Exclusion of other liver diseases with antitransglutaminase antibodies, serum ceruloplasmin, and 24-h basal urinary copper excretion, α1-antitripsin levels, serum immunoglobulins, and autoantibodies. Other specific tests as suggested by history and examination are sweat test, serum lactate, amino acid and organic acids, plasma FFAs, acyl carnitine profile and urinary steroid metabolites, creatinine kinase (CK), ferritin, and lactate (Table 15.2) [10].
- A careful assessment of metabolic features with basal lipid and glucose and insulin profiles. In selected cases, oral glucose tolerance test may be needed to define IR with measurement of HOMA-IR and insulin sensitivity index (ISI).
- Screening of co-morbidities such as OSAS and cardiovascular abnormalities.
- Indirect non-invasive markers of liver fibrosis although none are specific or sensitive enough to diagnose NASH. Hyaluronic acid (HA) has been shown to predict liver fibrosis in chronic liver disease and in children with

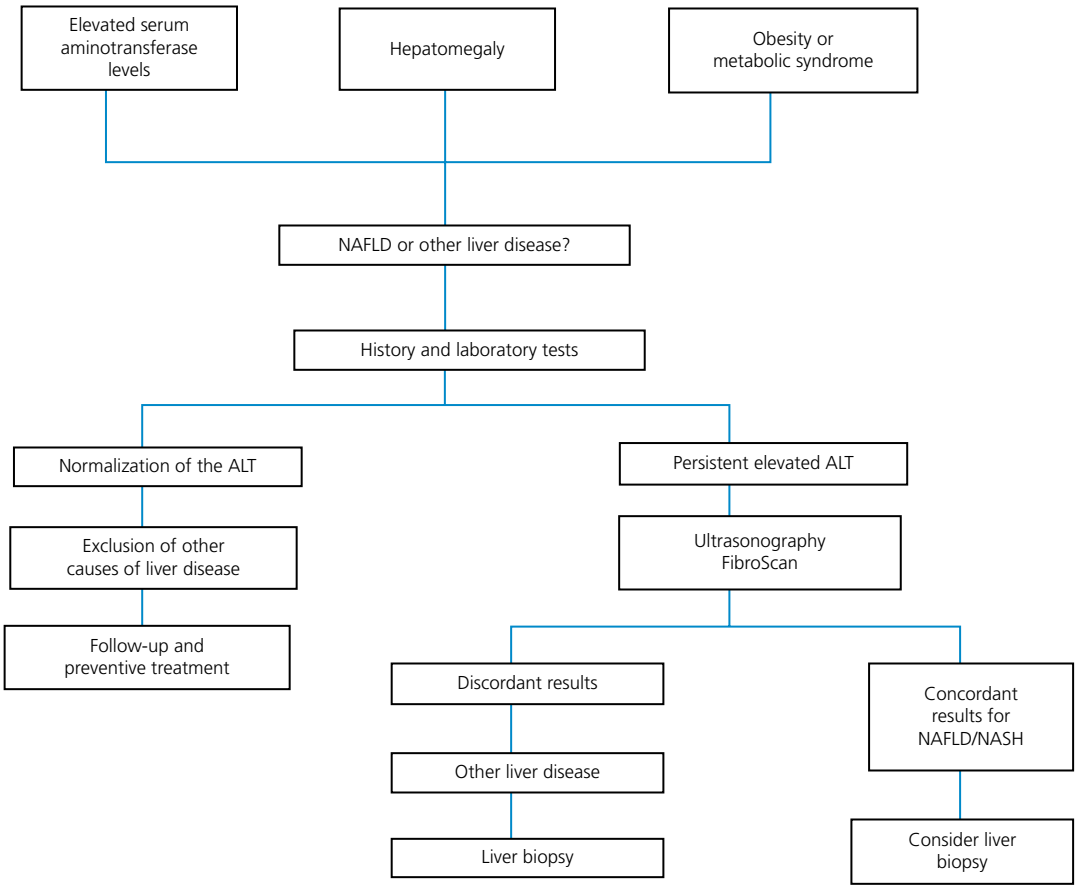


Figure 15.3 Management algorithm for children affected by non-alcoholic fatty liver disease (NAFLD). The discovery of increased circulating levels of alanine aminotransferase (ALT), the presence of hepatomegaly and/or the presence of obesity or one or more features of metabolic syndrome could mask the presence of NAFLD. Therefore, liver function tests and careful personal history are required to exclude chronic liver disease; if ALT levels normalize by 6 months and the primary evaluation for chronic hepatopathies are negative, the child should be maintained under observation and preventive treatment (that is, adequate diet and exercise and eventually treatment of co-morbidities). Conversely, if ALT elevation persists for at least 6 months, the child should begin the diagnostic program required to perform diagnosis of NAFLD. Firstly, imaging by non-invasive methods could be used (that is, ultrasonography and transient elastography). In cases of concordant evidence of NAFLD, the child can be managed with drug therapy and lifestyle and diet changes. In cases of discordant evidence, liver biopsy is required to confirm diagnosis of NAFLD before starting appropriate treatments or to consider other diagnosis.

NASH. More recent work suggests cytokeratin 18 (CK18) fragment levels, which are increased in NASH, may be more sensitive. In addition, cathepsin D, a lysosomal protease, is a novel marker of liver inflammation and may correlate with severity of liver inflammation and NAS score in children with NAFLD [3].

Imaging techniques

Liver ultrasound is the most used diagnostic tool to detect fatty liver, as it is safe, cheap, and widely available. It provides an accurate evaluation of the liver, identifying the presence and degree of hepatic steatosis based on standardized parameters which are hepatorenal echo contrast, liver echogenicity, visualization of intrahepatic vessels, and visualization of the liver parenchyma and the diaphragm (Figure 15.4). Several studies have firmly demonstrated that ultrasound scanning is highly sensitive and specific for the detection of NAFLD, and that an ultrasonographic score of steatosis is an accurate estimation of the grade of steatosis detected on liver biopsy. Unfortunately, the main limitation of liver ultrasound is the inability in distinguish between NAFLD and NASH because it does not differentiate liver fibrosis; moreover, the diagnostic sensitivity of ultrasonography decreases when fat liver content is <30% and when BMI is >40 kg/m^2.

The recent development of transient elastography (FibroScan), which is a technique based on the evaluation of tissue elasticity through ultrasound, is a promising non-invasive technique for the detection of advanced fibrosis caused by chronic hepatitis and NASH, although abdominal obesity may affect its utility in patients with NASH. Large studies are required to define normal values and the accuracy of transient elastography in children.

Computed tomography (CT) scan is more sensitive and specific than ultrasound in determining liver steatosis, but

Table 15.2 Laboratory tests in children with suspected non-alcoholic fatty liver disease (NAFLD).

Baseline routine tests	Blood counts, electrolytes, urea, uric acid, coagulation, INR, ALT, AST, GGT, TSH, FT4
Lipid profile	Cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, lipoproteins
Glucoinsulinemic pattern	Fasting glucose and insulin, OGTT, glycosylated hemoglobin, ISI, HOMA-IR
Exclusion other hepatopathies	Iron and ferritin
	Serum lactate
	Ceruloplasmin levels, 24-h urinary copper
	α1-Antitrypsin levels
	Antibodies transglutaminase IgA and total IgA
	Urinary steroid metabolites
	ANA, ASMA, LKM1, LC-1
	Viral serology
Metabolic screening, if indicated	Amino and organic acids
	Acyl carnitine profile
	Plasma free fatty acids

ALT, alanine aminotransferase; AST, aspartate aminotransferase; FT4, free thyroxine; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment insulin resistance; INR, international normalized ratio; ISI, insulin sensitivity index; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test; TSH, thyroid-stimulating hormone.

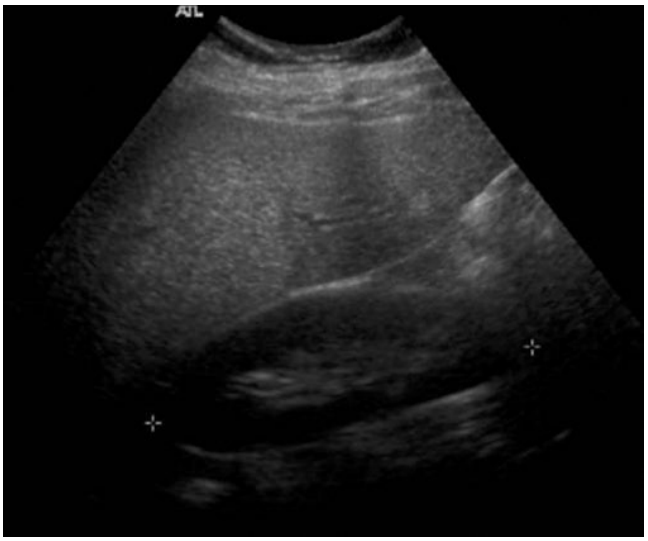


Figure 15.4 Sonogram of the liver in non-alcoholic fatty liver disease (NAFLD) demonstrates hyperreflectivity, suggestive of fatty infiltration. It should be noted how bright the liver is in comparison with the kidney. This is not specific for NAFLD, as similar appearances may be found in cystic fibrosis. Serial liver ultrasound examinations may demonstrate progression (or regression) of the disease.

the unjustified radiation exposure, the high costs and the masking effect of iron overload mean that CT is not recommended.

Magnetic resonance imaging (MRI) is now considered the best imaging technique to define qualitative and quantitative fat, because it provides a measurement of the hepatic content of triglycerides which is valid and reproducible. The principal limitations to its use are the cost and by the need for sedation, especially in younger children. Moreover, neither MRI nor CT accurately stage the disease and do not distinguish between hepatic steatosis and NASH or hepatic fibrosis. However, new imaging techniques with magnetic resonance spectroscopy may solve this problem [10].

Liver biopsy

Liver biopsy remains the gold standard for the diagnosis of NAFLD because it is the only way to distinguish between NAFLD and NASH, to determine the severity of liver damage and the degree of fibrosis and may also exclude other chronic liver diseases. The principal limitation of liver biopsy is that it is invasive and has potentially life-threatening complications in adults and children, so it is not suitable as a screening procedure and may not be available in all centers. Additionally, sampling error may lead to misdiagnosis.

Liver biopsy can be performed safely in both obese and non-obese children with low complication rates (see Chapter 5) in children. Recently, the Hepatology Committee of ESPGHAN has proposed indications for liver biopsy in chronic liver diseases in children [11] in which the indications for liver biopsy in NASH are outlined.

Liver biopsy should be performed in any child with suspected NAFLD/NASH as follows:

- In those who have signs/evidence of chronic liver disease, e.g., splenomegaly, abnormal diagnostic investigations (e.g. positive autoantibodies), strong family history, etc.
- To reach a diagnosis in patients with persistently abnormal LFTs (e.g., more than twice the upper limit of normal for >1 year) in the absence of evidence of chronic liver disease or other risk factors.
- To exclude other diseases,
- Liver biopsy must be performed prior to pharmacological or surgical treatment.
- Liver biopsy should be performed in the context of clinical research trials.

Histopathology

The main histological characteristics in NAFLD/NASH are macrovescicular fatty changes of hepatocytes, ballooning degeneration, a mixed lobular inflammation, and fibrosis (Figure 15.5) [12]. Other histological findings in NASH may include: acidophilic bodies, which result from hepatocyte apoptosis; mega-mitochondria; and vacuolated, glycogen-filled nuclei, which are also be seen in Wilson disease or in diabetic liver disease.

The histological features of pediatric NASH differ from those of adult NASH [12]. The National Institutes of Health NASH Clinical Research Network (NASH CRN) defines three subphenotypes of pediatric NASH:

- Type 1 NASH, characterized by steatosis with ballooning degeneration and/or peri-sinusoidal fibrosis, without portal involvement.

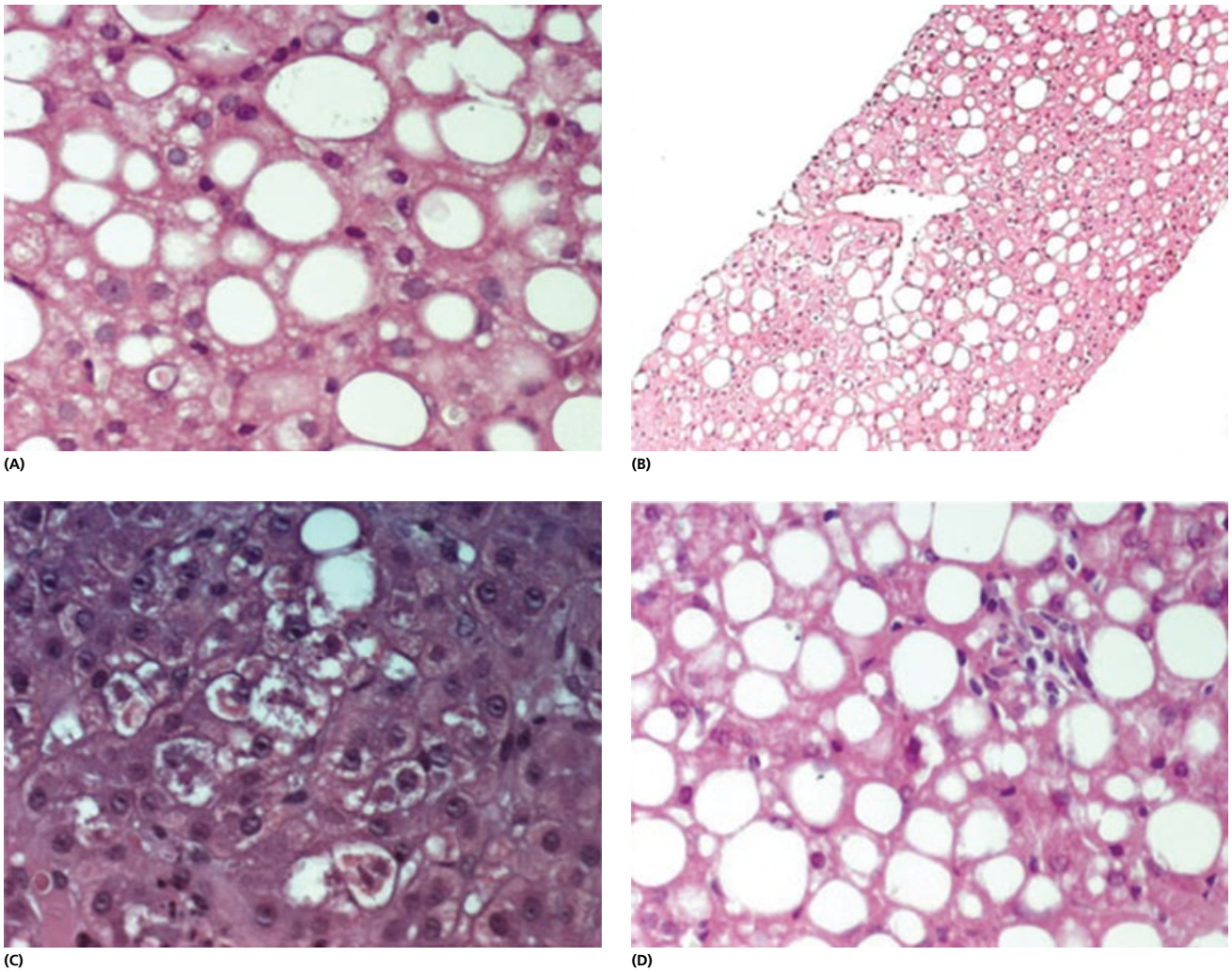


Figure 15.5 Major histological features of pediatric non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH). Steatosis is evident in (A) (×40 magnification) and (B) (×10 magnification); ballooning and lipogranulomas are present in (C) and (D), respectively (×40 magnification).

- Type 2 NASH, characterized by steatosis with portal inflammation and/or fibrosis, in the absence of ballooning degeneration or peri-sinusoidal involvement.
- NASH overlap type, in which characteristics from both types1 and 2 were present [13].

Steatosis

Steatosis is the main histological hallmark of NASH in which the fat droplets accumulate in >5% of hepatocytes. Two types of hepatic steatosis are described: macrovesicular, in which hepatocytes are distended by a single large fat droplet that displaces the nucleus peripherally; and microvesicular, in which the nucleus remains in the center of the cell surrounded by tiny fat drops. Steatosis in NAFLD/NASH is principally macrovesicular, but a mixed macro/microvesicular pattern is common. The pattern of distribution of fat in the liver in children typically starts in the periportal zone (acinar zone 1) or shows azonal distribution in contrast to adults in whom the steatosis is mainly localized in zone 3. The severity of steatosis is determined by the extent of parenchymal involvement. Semi-quantitative methods based on the percent of surface area involved are the most useful means of grading the steatosis [12].

Ballooning

Ballooning represents degeneration of the hepatocytes, which lose their typical polygonal shape, becoming swollen and round. It is due to intracellular fluid accumulation secondary to microtubule dysfunction and impaired protein secretion. The cytoplasm of ballooned cells becomes clear and vacuolated with inside characteristic lesions, called Mallory–Denk bodies (perinuclear clumps of amorphous

eosinophilic material). Ballooning is generally seen in zone 3 of the acinus and confers an increased risk of disease progression [12].

Inflammation

Lobular inflammation is the third component of pediatric NASH, constituted by a mix of lymphocytes and histiocytes, frequently associated with small numbers of polymorphonuclear leukocytes. Portal chronic inflammation is not required for the diagnosis of NASH even if present in liver biopsies in varying degrees [12,13].

Fibrosis

Fibrosis in pediatric NASH manifests in a pericellular "chicken wire" pattern, which distinguishes it from other causes of steatohepatitis. This pattern results from the deposition of collagen fibers in the perivenular, peri-sinusoidal spaces of Disse in acinar zone 3, usually in association with the other lesions of steatohepatitis. Special histochemical stains for collagen, such as trichrome stain (Masson, Van Gieson) or picrosirius red stain, are required for the detection of fibrosis in the initial stage. The progression of portal–periportal fibrosis results in septal linkage with vascular structures, which remodel the hepatic architecture and can lead to cirrhosis. HSCs, the principal collagen-producing cells in the liver, are considered responsible for the development of fibrosis in NASH, even though the exact pathogenetic mechanism is still unclear.

Scoring systems

NAFLD/NASH may be evaluated by using scoring systems, more commonly in adults than children. Two common tools are the Brunt score and the NASH-CNR (Italian Research Council) system. The Brunt score was designed to define the histological response to treatment and provides a semi-quantitative definition assessment of macrovacuolar steatosis, ballooning, and lobular and portal inflammation (mild or grade 1; moderate or grade 2; severe or grade 3) [12].

The NASH-CRN system provides a final score for grading the disease (the NAS score), that derives from the sum of different scores given to three items (steatosis, lobular inflammation, and ballooning degeneration). A NAS score ≤2 corresponds to "not NASH," while a score ≥5 corresponds to "definite NASH." NAS scores of 3 or 4 are considered borderline for a diagnosis of NASH, and these cases may benefit from assessment of the entire biopsy specimen, using other features of NASH histology [13]. Alkhouri *et al.* have proposed a new grading score for pediatric NAFLD to be used in clinical trials: the Pediatric NAFLD Histological Score (PNHS), which takes into account the presence of portal inflammation and other histological features [14].

Natural history

The natural history of pediatric NAFLD remains unknown, owing to the limited longitudinal data available. Recent observations suggest that children with NAFLD may be at increased risk for both hepatic and non-hepatic morbidity and mortality.

The prognosis is likely to be related to the severity of baseline liver histology. Advanced fibrosis is present at the time of diagnosis in 5–10% of children with NAFLD. Although data on the progression to cirrhosis are extremely limited, many reports have described progression to cirrhosis in pediatric NAFLD, with a few reports of adolescents undergoing liver transplantation for end-stage liver disease caused by NASH in the US [15]. As with any form of chronic liver disease, children with NAFLD may develop hepatocellular carcinoma in adulthood, and there is a recent report of hepatocellular carcinoma in an obese 7-year-old boy with NAFLD.

Treatment

The treatment of pediatric NASH is a challenge and in view of the co-morbidities, identifying an effective treatment is a public health necessity. Lifestyle intervention, with hypocaloric diet and regular physical exercise, is the first therapeutic approach, but its results are disappointing. Unfortunately, none of the drugs investigated in children are satisfactory and novel targets or pharmacological associations are being evaluated.

The final endpoint of treatment of NASH is to reverse the histological features of necroinflammation and fibrosis, in order to improve the patient's quality of life reducing long-term morbidity and mortality related to metabolic consequences of fatty liver (i.e., cardiovascular diseases, T2DM) [16]. To date, definite guidelines for treatment are lacking and the available therapy is directed against the diseases associated with fatty liver (i.e., obesity, IR, dyslipidemia).

Non-pharmacological approach: diet and lifestyle

The cornerstone of treatment of NAFLD is gradual weight loss, using a combination of diet and exercise. Convincing results in terms of normalization of serum aminotransferases levels, improvement of ultrasonic steatosis, and amelioration of liver histological damage have been demonstrated in both adults and children. Weight loss improves hepatic and extrahepatic insulin sensitivity by reducing the delivery of FFAs and by increasing peripheral glucose utilization. Moreover, weight reduction inhibits the production of reactive oxygen species (ROS) and adipocytokines, mediators of tissue inflammation [1,3,16].

There is no good evidence to support any particular dietetic scheme. Previously, a reduced calorie, balanced diet associated with regular and moderate aerobic exercise has been recommended. However, it may be that diets designed to reduce hyperinsulinemia are more effective than a simple hypocaloric diets because of the crucial role of hyperinsulinemia in the pathogenesis of NAFLD. Therefore, low-glycemic index diets, based on limited consumption of simple sugars, refined grains, and potatoes in favour of wholegrains, legumes, vegetables, and fruits might be more effective. Low-fructose diets are based on recent evidence demonstrating the critical role of high-fructose consumption in the pathogenesis and progression of NAFLD, with NAFLD patients consuming nearly two to three times more than healthy controls. Reduced dietary intake of saturated/trans-fat, increased intake of polyunsaturated fat (omega-3), and increased fiber intake have been proposed as dietary alternatives.

A program combining diet and regular physical exercise is more effective than dietetic intervention alone, because of the beneficial effect of physical activity on hyperinsulinemia.

Recently, to counter the difficult compliance to lifestyle intervention, family-based behavioral treatments (FBF) have been proposed, which actively involve both parents and children in nutritional education programs and behavioral changes, with better results [16].

It is not known what percentage of excess weight should be lost to obtain a significant improvement in liver disease and over what timeframe, but gradual weight loss is recommended because extreme diets may cause metabolic damage.

Unfortunately, despite all efforts, achieving and maintaining weight loss in children by lifestyle intervention is difficult, with <10% success rate 2 years after the onset of intervention.

Pharmacological approaches

The aim of treatment is the complete recovery of liver damage, with normalization of liver enzymes and histological lesions. Insulin-sensitizing agents, antioxidants, and cytoprotective drugs have all been tested in pediatric setting, but none have been effective. Only a few randomized controlled clinical trials (RCTs) are ongoing in the pediatric population and they are limited by small sample size, the heterogeneity of diagnostic tools used to detect NASH, and different outcome measures.

Insulin-sensitizing agents

Metformin, a biguanide, is the principal insulin sensitizer evaluated in children. Its action is mediated by the activation of the 5′ adenosine monophosphate (AMP)-activated protein kinase (AMPK) pathway, which increase lipid and glucose catabolism. In the last 10 years, contrasting data about the efficacy of metformin have been published. Initially, significant improvement in hepatic transaminase levels and steatosis, measured by magnetic resonance, were reported after 24 weeks of treatment with metformin in 10 children with biopsy-proven NAFLD. Since then, a number of studies, including the Treatment of NAFLD in Children (TONIC) study demonstrated that metformin was no more effective than lifestyle intervention in ameliorating levels of aminotransferases, steatosis, and liver histology [17].

Thiazolidinediones are agonists of the peroxisomal proliferator activated receptor gamma (PPAR-γ). In adult patients these drugs were effective in reducing liver enzymes and ultrasonic steatosis, with anti-inflammatory and antiapoptotic effects. These drugs have not been studied in childhood because of cardiotoxicity in adults.

Antioxidants

The main antioxidant agent tested in children has been vitamin E (α-tocoferol), a fat-soluble vitamin, with a good safety profile. Although in the first small studies vitamin E seemed to be effective in improving transaminases levels, subsequent well-designed RCTs in adults and children demonstrated no differences between vitamin E and lifestyle intervention. Similar results were reported by the TONIC trial, which evaluated the effect of 96 weeks of therapy with vitamin E (400 IU twice daily), insulin sensitizer metformin (500 mg twice daily), or placebo in 173 children affected by biopsy-proven NAFLD. In this study, vitamin E was no better than placebo in attaining a sustained decrease of ALT levels, and there was only a limited effect on hepatocellular ballooning and NAFLD activity score [17].

Cytoprotective agents

Ursodeoxycholic acid (UDCA), a tertiary hydrophilic bile acid, is widely used in liver disease, especially cholestasis for its choleretic and hepatoprotective properties. It had no effect on biochemical, ultrasonic, and histological features of NAFLD/NASH, even at higher dosage (32 mg/kg/day) in adults and children [16].

Omega-3 fatty acids

Omega-3 fatty acids are essential, PUFAs, which are found in large quantities naturally in fish oil, flaxseed, and some nuts. Several studies have demonstrated the association between altered omega-3 : omega-6 ratio and NAFLD, metabolic syndrome, and cardiovascular risk. Therefore supplementation of omega-3 to normalize the omega-3 : omega-6 ratio, has been evaluated in adults and children with NAFLD [18]. These studies described amelioration in hepatic steatosis, IR, and dyslipidemia, with a significant improvement in serum inflammation and oxidative stress markers. In a pediatric RCT significant improvement of histological damage, with reduction in steatosis, ballooning, and lobular inflammation, was observed after 18 months of treatment with omega-3; but there was no effect on liver fibrosis [19].

Probiotics

The theory of gut–liver axis suggests that gut microbiota and impaired intestinal barrier integrity play an important role in the development of NAFLD [4]. In this context, probiotics, as manipulators of intestinal bacterial microbiota, have been used in the treatment of fatty liver disease.

The probiotic food supplement VSL#3, a mixture of eight probiotic strains (Streptococcus salivarius subspp. thermophilus, Bifidobacterium (B. breve, B. infantis, B. longum), Lactobacillus acidophilus, L. plantarum, L. casei, and L. delbrueckii subspp. bulgaricus), has been tested in both animal and human studies, showing a beneficial effect on reducing inflammation and permeability of the intestinal barrier, and improving hepatic steatosis and aminotransferases levels [16].

New therapeutic strategies

The obesity epidemic, the lack of guidelines for therapy, and the absence of effective treatment leads researchers to search for new molecules for the treatment of NASH.

The renin–angiotensin system is involved in the recruitment of inflammatory cells and hepatic fibrogenesis by activation of HSCs. Previous data in adults have shown amelioration of aminotransferases levels and histological features in a small group of adults treated with losartan, which is now being studied in an RCTs in children with biopsy proven NAFLD (clinical trials identifier: NCT01913470) [16].

Current clinical trials to test angiotensin-converting enzyme inhibitors (ACE-I) and angiotensin receptor blockers (ARBs), cysteamine, vitamin D and choline (RCTs registered on ClinicalTrials.gov on May 2015) are ongoing [16].

The cysteamine is an antioxidant and anti-inflammatory amino thiol, derived by Coenzyme A. Following positive data in a pilot study, an ongoing RCTs using cysteamine bitartrate in the treatment of pediatic NASH with a histological primary endpoint is ongoing (NCT01529268).

Vitamin D has a central role in the metabolic disarray secondary to obesity. Some studies reported an association between low vitamin D levels and the severity of NASH and fibrosis in children and adults with NAFLD, and so an RCT evaluating the combination of vitamin D supplementation (800 IU) with DHA in the treatment of pediatric NASH with a histological endpoint is underway (NCT02098317) [16].

Several studies demonstrated an association between low-choline diet and liver damage; therefore, studies are in progress to evaluate the efficacy of choline supplementation, vitamin E, and DHA in NAFLD/NASH based on a histological endpoint (NCT01934777) [16].

Surgical approaches

Bariatric surgery

Bariatric surgery is increasingly used in the treatment of morbid obesity in adult patients and resolves NAFLD/NASH in about 75% of treated patients and is now used in the treatment of morbid obesity in adolescents. The Hepatology Committee of ESPGHAN produced a position statement about bariatric surgery in severe obese adolescents [20] in which they concluded that, although there are insufficient data to recommend widespread use of weight loss intervention in severe obese adolescents without co-morbidities, bariatric surgery should be considered a valuable therapeutic option in selected patients with BMI >40 kg/m^2 and severe co-morbidities (including NASH with advanced fibrosis) or with BMI >50 kg/m^2 and mild co-morbidities [20].

Temporary devices

Temporary non-pharmaceutical and non-surgical treatment of morbid obesity is based on minimally invasive intragastric balloons, such as BioEnterics Intragastric Balloon® (BIB) and Obalon®. These balloons have a variable volume and are designed to remain in the gastric cavity for a period of 3–6 months, inducing an amelioration of BMI and related co-morbidities with a very low complication rate. The first pediatric study on the use of Obalon in an adolescent with morbid obesity reported positive effects of an intragastric balloon on BMI and obesity-related co-morbidities, with reductions also of hepatic aminotransferase levels.

Conclusion

NAFLD/NASH is due to disordered insulin action, with hyperinsulinemia and relative IR. It is an important liver disease among children as the prevalence of childhood obesity continues to increase. Most children are either asymptomatic, or present with vague abdominal pain. The prognosis is uncertain, but progression to cirrhosis has been reported. Weight loss through dietary redesign with a low glycemic index diet and a regimen of regular exercise is the current mainstay for treatment. Primary prevention of NAFLD should be a priority for pediatric patients.

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CHAPTER 16

Hepatobiliary Disease in Cystic Fibrosis

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Key points

- Focal biliary cirrhosis is the most clinically relevant cystic fibrosis (CF)-associated hepatic problem, as extension of the initially focal fibrogenic process may lead to multilobular biliary cirrhosis, portal hypertension and related complications in around 10% of patients.
- CF liver disease (CFLD) develops before puberty, is usually subclinical, and has normal biochemical liver function tests until disease is advanced.
- Several methods such as transient elastography and acoustic radiation force impulse elastography, may help identify patients at risk for CFLD and document disease progression.
- Oral bile acid therapy, aimed at reducing bile viscosity and improving biliary secretion and bile acid composition, is the only available therapeutic approach for CF-associated liver disease, but there are no long-term data on the efficacy of UDCA in the prevention of liver disease.
- Once clinical signs of cirrhosis or portal hypertension develop, it is important to screen for varices and prevent bleeding, as many CF patients with severe portal hypertension may have stable liver function for years. Extreme care should be taken not to underestimate the degree of portal hypertension even if there is little evidence clinically or on imaging, as portal hypertension related to obliterative portal veinopathy without cirrhosis may occur.
- Early liver transplantation is recommended for children with deteriorating nutritional status and lung function as there is evidence that liver transplantation may prevent further decline.

Cystic fibrosis (CF) is the most common life-limiting autosomal recessive disease of the Caucasian population, with an incidence of approximately 1 in every 3000 live births worldwide; it is a multiorgan disease affecting more frequently the lungs, pancreas, sweat glands, and in males the wolffian ducts. Lung disease is the primary cause of morbidity and mortality and results from the combined and sustained effect of chronic infection from different respiratory pathogens and inflammation leading to progressive damage and eventually to respiratory failure.

Other clinical manifestations include exocrine pancreatic insufficiency with maldigestion and malabsorption, intestinal obstruction in the neonatal period (meconium ileus) or later in life (distal intestinal obstructive syndrome), and hepatobiliary disease. An increasing proportion of patients develops diabetes mellitus in the second decade of life and virtually all men with CF are infertile due to atresia or complete absence of the vas deferens.

In 1989 the discovery of the gene responsible for CF led to recognition of the key role of the encoded protein, the cystic fibrosis transmembrane regulator (CFTR), in maintaining fluid balance across epithelial cells. CFTR, a large protein of 1480 amino acids belongs to the adenosine triphosphate (ATP)-binding cassette family and functions mainly as a low conductance cyclic adenosine monophosphate (cAMP)-dependent chloride channel at the apical membrane of epithelial cell where it promotes transmembrane efflux of chloride ions [1]. The CF secretory defect determines inability to maintain the luminal hydration of ducts, leading to physicochemical abnormalities of secretions and duct obstruction.

The phenotypic expression of CF disease is, however, extremely heterogeneous in terms of the severity and type of organs involved. The major clinical manifestations of CF, their pathogenetic mechanisms, and relative frequencies are listed in Table 16.1.

Diseases of the Liver and Biliary System in Children, Fourth Edition. Edited by Deirdre A. Kelly.

Table 16.1 Major clinical manifestations in cystic fibrosis.

Organ involved	Functional/structural abnormalities	Clinical manifestation	Frequency
Sweat glands	Salt hyperconcentration	High sweat chloride concentrations	95–98%
		Salt loss syndrome	Undefined
Pancreas	Ducts obstruction with fibrocystic transformation	Pancreatic insufficiency	85%
	Inflammatory response in the surviving pancreatic tissue	Chronic/recurrent pancreatitis (if pancreatic sufficiency)	3–4%
	Islet cell strangulation	Diabetes mellitus	10–25%
Intestine	Thickened intestinal content	Meconium ileus	10–20%
		Distal intestinal obstruction	10%
		Rectal prolapse	1–2%
Liver and biliary tract	Thickened ductular secretions	Focal biliary cirrhosis	20–30%
		Multilobular biliary cirrhosis	10%
		Portal hypertension	2–5%
	Nutritional deficiencies	Liver steatosis	23–67%
		Micro-gallbladder	30%
	Lithogenic bile	Cholelithiasis	15%
Nose and paranasal	Mucus stagnation/infection	Sinusitis	90%
		Mucocele	16%
		Nasal polyps	15–26%
Lung	Defective mucus clearance, obstruction, infection, and inflammation	Chronic respiratory infections	97%
		Pneumothorax	5–8%
		Massive hemoptysis	5%
		Allergic bronchopulmonary aspergillosis	10%
Reproductive system: vas deferens	Atresia azoospermia	Male sterility	98%
uterine cervix	Cervical mucus abnormalities	Decreased female fertility	Undefined

CF is characterized by a striking genetic heterogeneity, with almost 2000 different CFTR mutations identified to date (www.cftr2.org; last accessed June 2016). Mutations have been grouped in six classes according to their functional outcome on CFTR protein [1]. Class I, II, and III mutations are defined as severe and result in a complete loss of chloride channel function at the apical membrane of epithelial cells through different molecular mechanisms (lack of production, defect in protein trafficking or in chloride channel regulation). In contrast, class IV, V, and VI mutations are classified as mild mutations, are associated with altered conductance properties, reduced synthesis or defective stability of normal CFTR, and to some residual CFTR membrane activity. This classification system based on functional classes of mutations allows the evaluation of genotype/phenotype correlations despite the wide genetic heterogeneity. A good association has been established exclusively for the exocrine pancreas, patients with pancreatic insufficiency being generally homozygous or compound heterozygous for class I, II, and III mutations. For other manifestations of CF, additional genetic factors (termed modifiers genes),and/or extrinsic factors (environmental, therapeutic, iatrogenic) are probably important in determining disease heterogeneity.

On clinical grounds, the term "classic CF" refers to the more severe form of the disease, with multiorgan involvement and pancreatic insufficiency, and is associated with the presence of two severe mutations. Non-classic forms of CF occur in around 10% of cases, are associated with mild mutations, and are characterized by residual pancreatic function and often single-organ disease (chronic bronchitis, sinusitis with nasal polyposis, pancreatitis, male infertility due to obstructive azoospermia).

When first described in 1938, the disease was almost invariably fatal during early childhood and for many years the basic defect has remained unknown. To date, median survival is over 40 years, but premature death due to respiratory failure is still a major problem. Contributors to improved survival over the past two decades include centralized care in dedicated CF clinics and advances treatment strategies [2], that so far have targeted the downstream effects of CFTR protein dysfunction to control symptoms. Further improvement in the outcome of the disease is expected due to development of a novel class of drugs (modulators of the CFTR protein) aimed at correcting the underlying basic defect of CF by enhancing synthesis or function of the protein [3].

The recent introduction of ivacaftor (a CFTR potentiator) and lumacaftor (a CFTR corrector) represents a major milestone and a stimulus for achieving mutation-targeted personalized medicine that may ultimately involve 90% of CF patient [3].

Table 16.2 Hepatobiliary manifestations in cystic fibrosis.

Type of lesion	Clinical manifestation	Frequency
Specific alterations ascribable to the underlying CFTR defect	Focal biliary cirrhosis	20–30%
	Multilobular biliary cirrhosis	10%
	Portal hypertension	2–5%
	Neonatal cholestasis	Rare
	Sclerosing cholangitis	Rare
	Micro-gallbladder	30%
	Cholelithiasis	15%
Lesions of iatrogenic origin	Hepatic steatosis	23–67%
	Drug hepatotoxicity	Undefined
Lesions reflecting the effects of a disease process that occurs outside the liver	Hepatic congestion	Rare
	Common bile duct stenosis	Rare

Spectrum of hepatobiliary manifestation of cystic fibrosis

There is increasing awareness of the wide spectrum of hepatic problems related to CF, which include specific alterations ascribable to the underlying CFTR defect as well as lesions of iatrogenic origin or reflecting the effects of extrahepatic disease (Table 16.2).

Liver disease (cholestasis/focal biliary cirrhosis/multilobular cirrhosis)

Focal biliary cirrhosis is the classic and pathognomonic hepatic lesion of CF and is considered a direct consequence of the basic defect that results from biliary obstruction and progressive periportal fibrosis. This is the most clinically relevant CF-associated hepatic problem, since extension of the initially focal fibrogenic process may lead to multilobular biliary cirrhosis, portal hypertension, and related complications. Unlike pulmonary and pancreatic diseases that affect the majority of CF patients, liver disease develops in no more than one-third of patients.

Pathogenesis

The pathogenesis is still not understood. CF-associated liver disease represents the only inherited liver disease resulting from impaired secretory function of the biliary epithelium. It is classified among genetic cholangiopathies, since it affects CFTR, one of the multiple cholangiocyte flux molecules that function as channels, exchangers, and transporters at the cholangiocyte plasma membrane. In the hepatobiliary system, CFTR expression is restricted to the apical membrane of cholangiocytes and gallbladder epithelial cells; at this level, CFTR regulates the fluid and electrolyte content of bile and participates in the choleretic effect of secretin [4].

Cholangiocyte injury, with irregular shapes, reduced microvilli, necrosis, and periductular collagen deposition, is a consistent finding even in CF patients without clinical evidence of liver disease. This suggests that damage to the bile duct epithelium is likely to represent the primary event in the development of periportal fibrosis.

In physiological conditions, the cAMP-stimulated Cl^- secretion through low-conductance Cl^- channels imposes a negative luminal potential and an osmotic gradient that triggers the passive secretion of Na^+ and water. The change in apical Cl^- gradient facilitates HCO_3^- extrusion via Cl^-/HCO_3^- exchange, providing the biliary alkalization required for the digestive function and for the solubility of organic components of bile (Figure 16.1).

Lack of CFTR has a greater impact on cell function than could be predicted by its role as a cAMP-stimulated Cl^- channel. CFTR plays an important role in the regulation of other membrane transport proteins, including Na^+ channels (EnaC), K^+ channels, outward rectifying Cl^- channels (ORCC) and Cl^-/HCO_3^- exchangers (AE2). There is also evidence that CFTR regulates cellular secretion of ATP, intracellular vesicle acidification, and processing and trafficking of certain proteins, including mucin secretion.

Ductal cholestasis due to reduced CFTR-related fluid and electrolyte transport by cholangiocytes is considered the central step in the pathogenetic sequence of CF-associated liver disease that has been shown to occur in a long-living CFTR knockout murine mice model.

In addition, a study in primary culture of human cholangiocytes has clearly documented that cAMP-stimulated Cl^- and HCO_3^- transport are both impaired in CF. The resulting reduction in bile fluidity and alkalinity would lead to plugging of intrahepatic bile ducts by inspissated secretions. Quantitative and qualitative abnormality in mucin secretion may also contribute to abnormal bile viscosity in CF patients: secretion of chondroitin sulfate was shown to be markedly increased in CF biliary epithelium in vitro, and its accumulation may well explain bile duct plugging by eosinophilic material, which is one of the early histological changes found in infants and children with CF. This process begins focally in the liver, possibly because of interductal connections that allow adequate bile drainage from some areas and is followed by portal fibrosis, bridging, and, eventually, cirrhosis. The retention of endogenous hydrophobic bile acids may be responsible for cell membrane injury and progressive liver fibrosis. Oxidative injury to the liver cell membrane may occur through increased free radical production favored by decreased lipid-soluble antioxidant activity. Injured cholangiocytes may release proinflammatory cytokines and growth factors that would recruit and activate hepatic stellate cells for collagen synthesis (Figure 16.2).

The progression from cholestasis to focal biliary cirrhosis and multilobular cirrhosis is a slow process that should be viewed as a continuum and may represent the anatomical expression of longstanding impairment in ductular bile flow. This may increase susceptibility of the biliary epithelium to damage by cytotoxic compounds excreted into bile and to aggression by microbial pathogens.

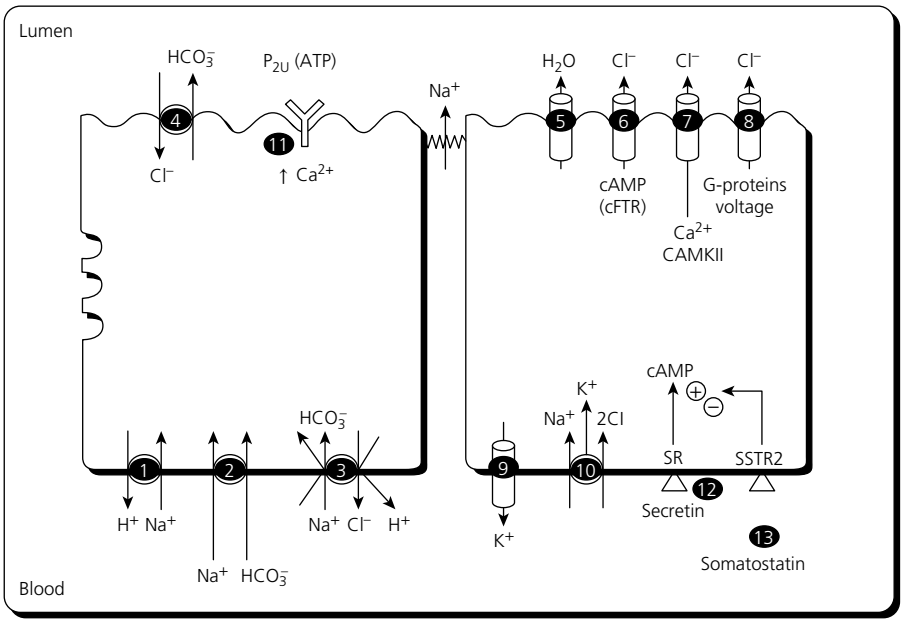


Figure 16.1 Model of cholangiocyte bile formation. Bile formation requires the coordinated function of two epithelial cell types that form a functional bile secretory unit. Hepatocytes secrete the major biliary osmolytes and constituents, such as bile acids, lipids, glutathione, organic cations, and anions, xenobiotics, proteins, and electrolytes. Cholangiocytes (represented in the figure) are located downstream, and are responsible for the rapid regulation of bile volume, fluidity, and alkalinity in response to a complex network of hormones, such as secretin (12) and somatostatin (13), and paracrine mediators, such as ATP (11). Their interplay results in the net secretion or absorption of osmolites, mainly Cl^- and HCO_3^-. Studies in isolated cholangiocyte preparations have recently elucidated the basic mechanisms involved in constitutive and stimulated Cl^- and HCO_3^- transport in the biliary epithelium. Basolateral Na^+/H^+ exchanger (1) and Na^+/HCO_3^- symporter (2) (Na^+-dependent Cl^-/HCO_3^- exchanger in humans (3)) mediate cellular HCO_3^- uptake. Bicarbonate is then secreted into the biliary lumen by a Cl^-/HCO_3^- exchanger (4) located at the apical membrane. Water channels (5) located at the apical and lateral membrane facilitate H_2O movements flowing across the established osmotic gradients. Basolateral Cl^- uptake involves a bumetanide-sensitive $Na^+/K^+/2Cl^-$ co-transporter (10), while apical Cl^- efflux is mainly mediated by a cAMP-activated Cl^- channel with electrophysiological features that are similar to CFTR (6). In addition to CFTR, a number of Cl^--conductive pathways, including a Ca^{2+}/CAMK II-activated Cl^- channel (7), and a G-protein-regulated, voltage-dependent Cl^- channel (8), are located at the apical cell membrane of cholangiocytes. Basolateral K^+ channels (9) modulate the membrane potential difference. (From Colombo *et al.* [22]. Reproduced with permission of Thieme.)

Increased fecal bile acid loss, a common finding in CF, suggests a defect in the ileal bile acid reabsorption, the cause of which remains elusive. Recently, the investigation of biliary dysfunction in cftr –/– and F508del mice without liver disease demonstrated disruption of the enterohepatic circulation of bile acids while cycling of bile acids to the liver was maintained, at least partly, by a cholecystohepatic shunt [5]. In CF, this mechanism may, with pancreatic defects, contribute to fat malabsorption but may also restrict the amount of toxic secondary bile acids entering the liver.

Another area of investigation relates to the role of alterations in gut microbiota and intestinal inflammation reported in CF. Experimental studies in the CF mouse have generated a hypothesis whereby small intestinal bacterial overgrowth (SIBO), impaired intestinal motility, and aberrant gut microbiota (i.e., gut dysbiosis) in CF induce inflammation with increased intestinal permeability and endotoxinemia. This activates the innate immune system in the biliary epithelium, promoting chronic biliary disease. A link between gut dysbiosis, intestinal inflammation, and cirrhosis was recently reported in CF patients [6].

In summary, current evidence suggests that liver disease in CF is related to the basic defect at the hepatobiliary level. However, it remains to be explained why only one-third of CF patients develop liver disease and why liver disease shows a great degree of variability in terms of severity. It should be noted that, in addition to CFTR, a number of Cl^--conductive pathways, including a Ca^{2+}-activated Cl^- channel and a G-protein-regulated voltage-dependent Cl^- channel, are located at the apical cell membrane of the cholangiocytes and may partly compensate the CF secretory defect in the liver (see Figure 16.1). Indeed, in human CF cholangiocytes, alternative Ca^{2+}-activated Cl^- channels were shown to be able to support HCO_3^- secretion.

Hepatic steatosis

Steatosis can be detected in a substantial proportion (23–67%) of CF patients of any age. Its pathogenesis is still unknown, but does not seem to be directly related to the CF basic defect. Massive steatosis, once frequently observed in newly diagnosed patients with pancreatic insufficiency and severe malnutrition, is now infrequent due to earlier diagnosis and better nutritional care. Mild steatosis is more

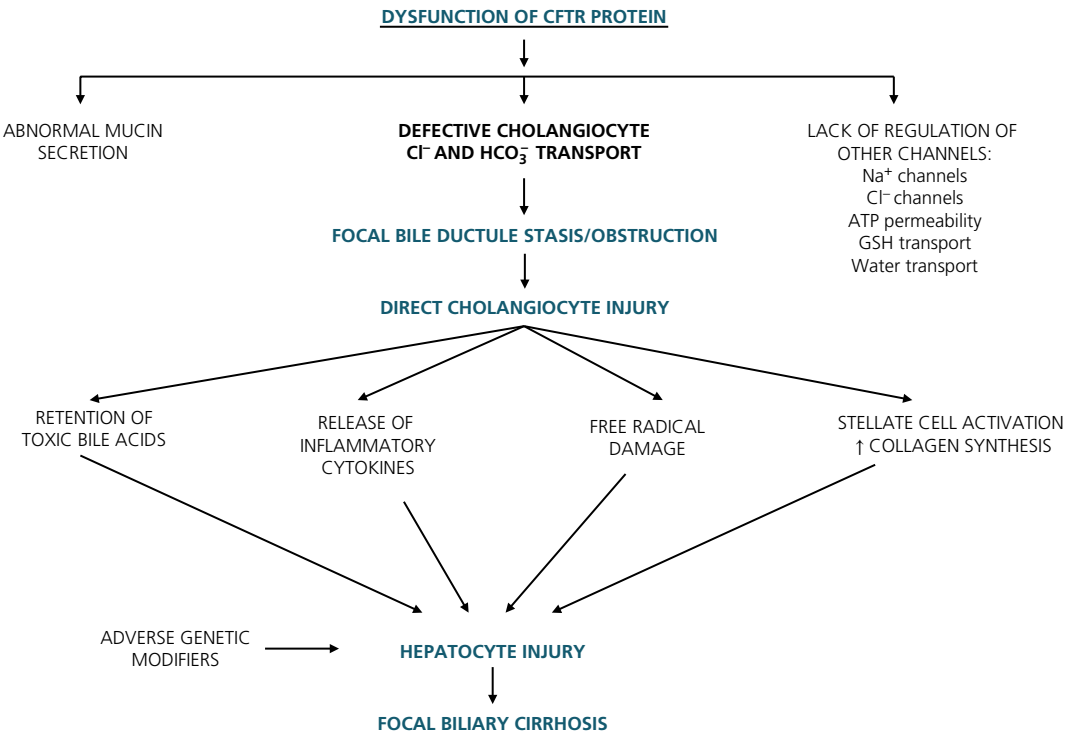


Figure 16.2 Proposed pathogenetic model for CF-associated liver disease. ATP, adenosine triphosphate; GSH, growth-stimulating hormone.

common and has been associated with selective nutritional deficiencies including essential fatty acids, carnitine, choline, trace elements, and minerals, and altered phospholipid metabolism in CF.

Finally, steatosis may be also a consequence of diabetes or long-term antibiotic therapy. As increasingly recognized in non-CF adult populations, steatosis may be the hepatic consequence of the metabolic syndrome and insulin resistance, which is a common event in CF patients due to chronic infection and inflammation. Steatosis may result from the effect of circulating cytokines on hepatic fatty acid oxidation or mitochondrial function in CF patients chronically colonized by respiratory pathogens. Although steatosis has been considered a benign condition in CF, without a proven relationship to the development of cirrhosis, recent data on the progression of non-alcoholic steatohepatitis to cirrhosis in adults may change this view.

Non-cirrhotic portal hypertension

Obliterative portal venopathy (OPV) has been recently recognized in a subset of CF patients with portal hypertension without cirrhosis, i.e., non-cirrhotic portal hypertension (NCPH) [7]. The cause of this portal branch venopathy remains obscure. It could be due to spillover of inflammatory infiltrate of the bile ducts, to microthrombosis from platelet activation, or to endothelial injury related to the CFTR defect. The prevalence of OPV in CF patients remains unknown.

Prevalence of liver disease

There are marked differences in the reported prevalence of CF-associated liver disease, which may be explained by differences in the diagnostic criteria and in the populations studied. The highest figures have been provided by autopsy studies, which documented a progressive increase in the prevalence of liver disease with age, from 10% in infants to more than 70% in adults. However, autopsy data in CF may be affected by significant bias since liver disease may have contributed to death or may have been the reason for postmortem evaluation. On the other hand, histological data from liver biopsy during life have not provided reliable epidemiological information.

Prevalence figures obtained by retrospective analysis in clinical settings range between 4.2% and 17%, but cross-sectional studies using biochemical and ultrasonographic assessment of liver disease have noted higher prevalence figures (from 18% to 37%); increasing through childhood with a peak in mid-adolescence without further increase.

Prospective long-term follow-up of different cohorts of CF patients carefully monitored for hepatic status demonstrated a cumulative incidence of liver disease ranging between 27% and 35%, without incident cases after the age of 18 years [8, 9].

Overall these data suggest that the mechanism and risk factors for liver damage present in early childhood for those CF patients who develop liver disease.

Risk factors for liver disease in CF

Identification of CF patients at risk of developing liver disease is important as therapeutic intervention is likely to be more effective in patients with early liver disease. Factors significantly associated with the development of CF liver disease (CFLD) include severe genotype, male sex, history of meconium ileus, and age at diagnosis of CF.

The development of liver disease appears to be restricted to patients with severe genotypes (i.e., carrying class I, II, or III mutations on both alleles), but no specific *CFTR* mutation has been associated with the presence and severity of liver disease, suggesting a multifactorial pathogenesis. Although familial clustering of liver disease has been reported, the poor concordance of liver disease in sibling pairs excludes a major role for environmental factors. On the other hand, discordance for liver expression in CF siblings has suggested that genetic factors inherited independently from the *CFTR* gene could modulate the clinical expression and severity of liver disease in CF.

There is some evidence that polymorphisms in genes that upregulate inflammation, fibrosis, or oxidative stress may increase susceptibility for its development. The role of modifier genes such as *SERPINA-1* whereby the heterozygous Z-allele mutation of α1-antitrypsin is overrepresented in children with CFLD and portal hypertension compared with those without CFLD, has been reported [10]. The identification of genetic modifiers for liver disease is a research priority, as it may allow early identification of patients at risk who might benefit from prophylactic strategies.

The preponderance of male subjects among CF patients with liver disease has been consistently reported, suggesting the possible role of endocrine factors in the development of this complication.

A few studies have reported a significantly higher incidence of liver disease in CF patients with a positive history of meconium ileus. This association, linking inspissated gut content and biliary secretions, was first described by a necropsy study, but was not consistently found in cross-sectional studies involving different CF patient populations. In patients with a positive history of meconium ileus, additional risk factors for the development of liver disease include abdominal surgery with extensive small bowel resection, poor nutrition in early life, and prolonged total parenteral nutrition.

Finally, age at diagnosis of CF was suggested to be an important risk factor for the development of liver disease. The finding that a delay in diagnosis (with a poor nutritional status) may predispose children with CF to liver disease, supports the newborn screening programs aimed at earlier diagnosis of CF.

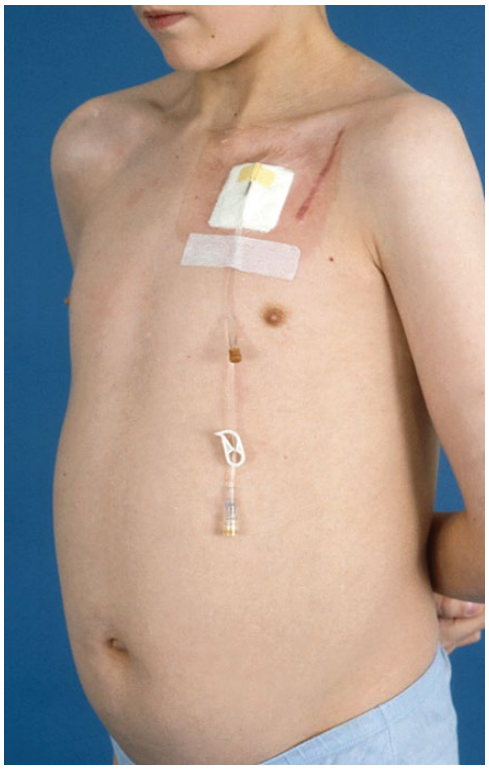


Figure 16.3 The commonest presentation of cystic fibrosis is with malnutrition, abdominal distension, and hepatosplenomegaly secondary to cirrhosis and portal hypertension.

Clinical features

Liver disease associated with CF usually develops before puberty, is often asymptomatic, and is slowly progressive. Signs of chronic liver disease such as jaundice, palmar erythema, and spider nevi are rarely present and are limited to patients with end-stage liver disease. The most common presentation is the occasional finding of hepatomegaly on routine physical examination (Figure 16.3) that may be associated with abnormalities in liver biochemistry but the spectrum includes neonatal disease and gallbladder disease.

Neonatal cholestasis

Neonatal cholestasis, caused by the obstruction of extrahepatic bile ducts by viscous biliary secretions, may be the presenting symptom of CF (Table 16.2) and may mimic biliary atresia. Infants with meconium ileus are at greater risk, particularly those with another risk of cholestasis, i.e., total parenteral nutrition or abdominal surgery.

Although CF infants may require a much longer period to clear jaundice than infants with other medical disorders presenting with neonatal jaundice, cholestasis generally resolves spontaneously over the first months of life, perhaps because of resolution of physiological cholestasis and maturation of biliary secretion. Serum cholesterol concentrations

are generally normal. In addition, these infants do not seem to be at higher risk of developing cirrhosis.

Hepatomegaly and portal hypertension

A more usual presentation in older children is with the following:

- Intermittent elevation of hepatic transaminases, a raised alkaline phosphatase, and γ-glutamyl transpeptidase.
- Hepatomegaly due to steatosis, fibrosis, or right ventricular failure in severely ill children.
- Splenomegaly and hypersplenism.
- Variceal hemorrhage from portal hypertension.
- Malnutrition.
- Clubbing.
- Diabetes mellitus.

The development of splenomegaly, palmar erythema, and telangiectasia suggests cirrhosis. Multilobular biliary cirrhosis is considered to develop sequentially from focal biliary cirrhosis in around 10% of patients. The progression of liver disease from early asymptomatic stage to liver failure remains unpredictable, and may take years to occur; however, in a minority of patients, often in the pediatric age group, liver disease may be the dominant manifestation of CF and its progression may be unusually rapid, suggesting the role of adverse genetic modifiers.

In contrast, the hemodynamic consequences of cirrhosis are often prominent, favoring early development of portal hypertension and related complications. Once cirrhosis is established, the risk of gastrointestinal bleeding from esophageal or gastric varices is high. Splenomegaly is often asymptomatic, but hypersplenism may develop, with thrombocytopenia and leukopenia. Massive splenic enlargement may cause abdominal discomfort or pain and deterioration of pulmonary function due to diaphragmatic splinting; ascites, encephalopathy, fatigue, and coagulopathy occur later with decompensated cirrhosis and are an indication for transplantation.

Portal hypertension may precede the onset of cirrhosis in some patients with NCPH related to obliterative portal venopathy [7]. Extreme care should be taken not to underestimate the degree of portal hypertension, even if there is little evidence clinically or on imaging.

Progression of liver disease

Esophageal varices develop in a high percentage of CF patients with cirrhosis; in addition, variceal bleeding may be the only clinical consequence of liver disease, as hepatic function remains well preserved for a long time. Prolonged survival after variceal bleeding has been reported in CF patients (median survival of 8.4 years), compared to a 1-year survival of 34% in other cirrhotic patients [11]. This supports the observation that some CF patients may develop NCPH [7].

Prospective studies using histological [8] and clinical [9] findings demonstrate a slow progression of liver disease in CF patients (Table 16.3). More recent evidence suggests that the impact of liver disease on the outcome of CF is negligible compared with the general CF population until end-stage liver failure develops. There is no significant increase in the rate of respiratory failure, the need for oxygen therapy, the frequency of hospitalization, or mortality in CF patients with liver disease, and children with CF-associated liver disease may have a better pulmonary prognosis than those without liver disease.

Table 16.3 Incidence of liver disease and its complications in cystic fibrosis.

	Incidence: no. per 100 patient-years (95% CI)
Liver disease:	
Overall	1.8 (1.3–2.4)
First 10 years of life	2.5 (1.8–3.3)
Cirrhosis, liver disease patients	4.5 (2.3–7.8)
Portal hypertension, cirrhotic patients	28.8 (15.4–49.3)
Liver decompensation, cirrhotic patients	0.4 (0–2.0)
Death, any cause, or liver transplantation:	
Liver disease patients	0.4 (0.1–1.2)
Cirrhotic patients	1.6 (0.3–4.7)

Impact of end-stage liver disease on cystic fibrosis

In contrast, the impact of advanced liver disease on pulmonary function and nutritional status of CF patients is now evident. CF patients are at risk of developing complications of portal hypertension, malnutrition and wasting, hepatic osteodystrophy, and deterioration of pulmonary status. Malnutrition is a common complication of CF, but may be exacerbated by the development of liver disease with an increase in fat malabsorption and protein wasting (Figure 16.3) (see Chapter 21).

The pathogenesis of malnutrition is multifactorial and involves the increase in resting energy expenditure, abnormalities in nutrient intake (due to anorexia and, in patients with encephalopathy, to protein restriction), malabsorption (due to the combined effect of cholestasis and pancreatic insufficiency), and abnormal metabolism of nutrients (Box 16.1). Many CF patients develop decompensated liver disease as adolescents, when glucose intolerance or diabetes are more likely to develop. In addition, advanced liver disease may induce insulin resistance, increasing the risk of developing CF-related diabetes.

End-stage liver disease may also induce significant changes in body composition, including osteoporosis and reduced fat and lean body mass, that will be reversed by liver transplantation, due to restoration of hepatic function and bile flow.

With regard to pulmonary status, cirrhosis and portal hypertension negatively affect respiratory function due to organomegaly, ascites-induced diaphragmatic splinting, and

Box 16.1 Causes of malnutrition in cystic fibrosis patients with liver disease.

- Reduced caloric intake:
  - anorexia
  - protein restriction (if encephalopathy)
- Malabsorption of lipid-soluble nutrients/vitamins:
  - reduced bile flow
  - pancreatic insufficiency
- Abnormal metabolism of nutrients:
  - carbohydrates: glucose intolerance/diabetes, reduced hepatic glycogen, insulin resistance
  - protein: hypercatabolic state, increased tyrosine and phenylalanine, decreased branched amino acids
  - lipids: essential fatty acid deficiency
  - vitamin D: reduced 25-hydroxylation
  - vitamin A: defective release from liver stores
- Increased resting energy expenditure

intrapulmonary shunting. This leads to recurrent respiratory infections from multiresistant bacteria, frequent hospital admissions, and significant deterioration of quality of life.

Hepatic congestion

Hepatic congestion from right-side heart failure occurs in some older patients with advanced CF.

Gallbladder abnormalities

CFTR is expressed in the gallbladder epithelium [4], which may explain the high frequency of gallbladder abnormalities observed in CF patients, but symptomatic biliary disease is uncommon. Symptoms and signs may include:

- Asymptomatic gallstones on ultrasound, which are found in 20–30% of patients (Figure 16.4).
- Micro-gallbladder on ultrasound, which is present in 10–40% of patients.
- Non-visualized, non-functioning gallbladder on hepatobiliary scintigraphy, which is found in over 50% of patients and does not need any therapeutic intervention.
- Submucosal cysts, septate gallbladders, and adenomyomas have been also described.

Cholelithiasis is an occasional finding in CF patients, with symptoms occurring in less than 4% of cases and prevalence increasing with age (from 8–12% in late childhood to more than 20% in adults). This complication has decreased over the last decade due to better correction of pancreatic insufficiency and improved nutritional status of CF patients. The pathogenesis of cholelithiasis was considered to be related to the production of lithogenic bile as a result of bile acid malabsorption, but this is unlikely as the main component of CF gallstones is calcium bilirubinate, not cholesterol. This might explain why ursodeoxycholic acid (UDCA) therapy is not effective in dissolving radiolucent gallstones in CF patients. Other problems include nidus formation from bile stasis and abnormalities in the biliary mucus; intrahepatic stones, and motor abnormalities of the extrahepatic biliary tree (dyskinesia, often associated with gallbladder distension and biliary pain).

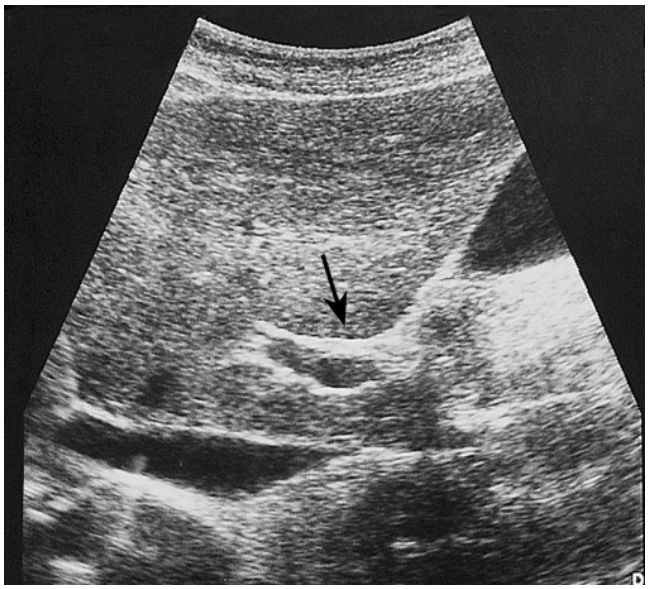


Figure 16.4 Ultrasound examination often demonstrates a coarse liver texture and a micro-gallbladder with gallstones (arrow).

Sclerosing cholangitis

Sclerosing cholangitis, with strictures and beading of the larger intrahepatic bile ducts, was initially reported in adult CF patients, sometimes in association with inflammatory bowel disease. Using magnetic resonance cholangiography (MRCP), typical cholangiographic abnormalities of the intrahepatic bile ducts have been reported in the majority of adult patients with CF-associated liver disease and in a significant proportion of CF patients without clinically apparent liver disease, suggesting that they may be related to the underlying CFTR defect at the hepatobiliary level. Interestingly, an increased prevalence of *CFTR* mutations has been reported in patients with primary sclerosing cholangitis, suggesting that patients with inflammatory bowel disease who are heterozygous carriers of *CFTR* mutations may be at increased risk of developing sclerosing cholangitis (Figure 16.5).

Common bile duct stenosis

Stenosis of the intrapancreatic portion of the common bile duct due to pancreatic fibrosis or common bile duct stenosis are rare complications that cause complete or partial biliary obstruction. Clinical features include jaundice, right upper quadrant pain (related to inflammation proximal to the stenosis or to a pressure effect producing gallbladder distension), and steatorrhea. Treatment includes interventional radiology or surgical decompression.

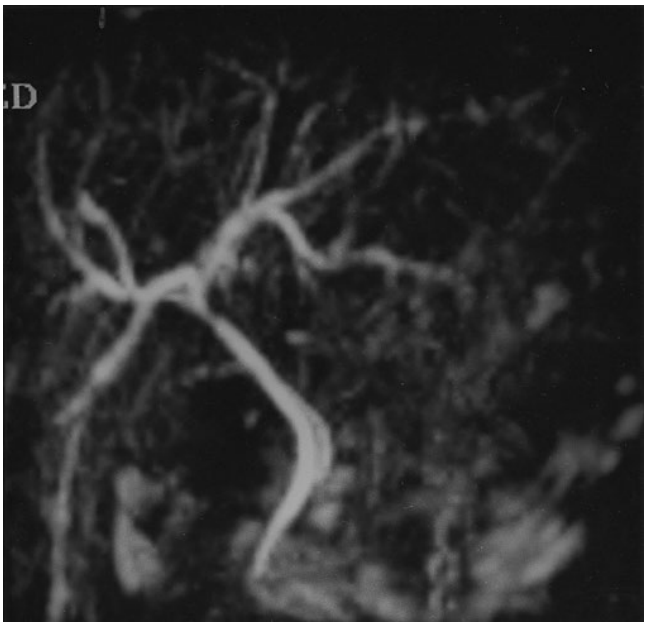


Figure 16.5 Magnetic resonance cholangiopancreatography is less invasive than endoscopic retrograde cholangiography and produces excellent imaging demonstrating variable dilation of the bile ducts.

Diagnosis of cystic fibrosis-associated liver disease

Evidence of liver disease in CF patients is usually subclinical, with normal biochemical liver function tests until disease is advanced, therefore it is often underdiagnosed. Early detection is difficult but essential, as early lesions may be reversible. There are no reliable tests for identification of individuals with CF who are at high risk for the development of cirrhosis. The diagnosis of CF-associated liver disease is based on clinical examination and a combination of biochemical tests and imaging techniques (Figure 16.6).

Physical examination

Regular clinical examination is essential for the detection and evaluation of liver disease in CF. The liver may be firm and nodular, often extending more than 3 cm below the right costal margin and its enlargement may be limited to the right or, more often, to the left lobe that protrudes centrally. Since the liver is often pushed down as a result of pulmonary disease, it is important to measure the liver span at the right mid-clavicular line through percussion and palpation. Splenomegaly is the first sign of portal hypertension and requires close monitoring at every follow-up visit. Attention should be paid to the presence of peripheral signs of chronic liver disease, including spider nevi, palmar erythema, jaundice, edema, distension of abdominal wall veins, and eversion of the umbilicus (see Figure 16.3).

Liver biochemistry

Biochemical abnormalities are frequently mild or intermittently present and have shown low sensitivity and no correlation with histological findings. Not infrequently, CF patients with multilobular biliary cirrhosis have completely normal liver biochemistry [12]. Non-specific biochemical abnormalities have been documented in more than 50% of infants with CF, with complete normalization in most cases by 2–3 years of age and no impact on future development of liver disease [8]. Common findings include:

- Intermittent rises in plasma transaminases (aspartate and alanine aminotransferase) in 30% of patients.
- Increased serum levels of alkaline phosphatase that is difficult to evaluate in growing children.
- Increased serum levels of γ-glutamyl transpeptidase in those with more serious liver disease.

Occasional biochemical abnormalities occur as a result of drug treatment, infection, or malnutrition. Therefore it is important to exclude other causes of acute or chronic elevation of hepatic aminotransferases (infectious and autoimmune hepatitis, metabolic disorders, drug hepatotoxicity). Isolated elevation of serum transaminase levels, with normal concentrations of enzyme related to cholestasis suggests the presence of steatosis, and requires correction of nutritional deficiencies, if present.

Ultrasonography

Ultrasonography of the hepatobiliary system is the most suitable initial method of investigation. Ultrasound technology has improved in recent years and reliably distinguishes normal parenchyma, steatosis, fibrosis, cirrhosis, portal hypertension, and ductal abnormalities. Abnormal echogenicity may precede clinical and biochemical manifestations of liver disease, suggesting that routine ultrasonography may be a valuable marker of early liver disease in CF [13]. Doppler ultrasound can evaluate the flow patterns of hepatic vasculature: decreased portal venous flow velocities or reversal of flow (hepatofugal) in the portal vein are indicative of portal hypertension. Thrombosis of the portal or splenic veins as a cause of splenomegaly will also be visualized.

Attempts to standardize the method of assessment and scoring systems to overcome inter- and intraobserver variability have been developed, based on coarseness of liver parenchyma, nodularity of the liver edge, and increased periportal echogenicity, but their use in clinical practice is limited.

Computed tomography

Computed tomography may be helpful in confirming the presence of multilobular biliary cirrhosis and portal hypertension, but it lacks both specificity and sensitivity in diagnosing focal biliary cirrhosis.

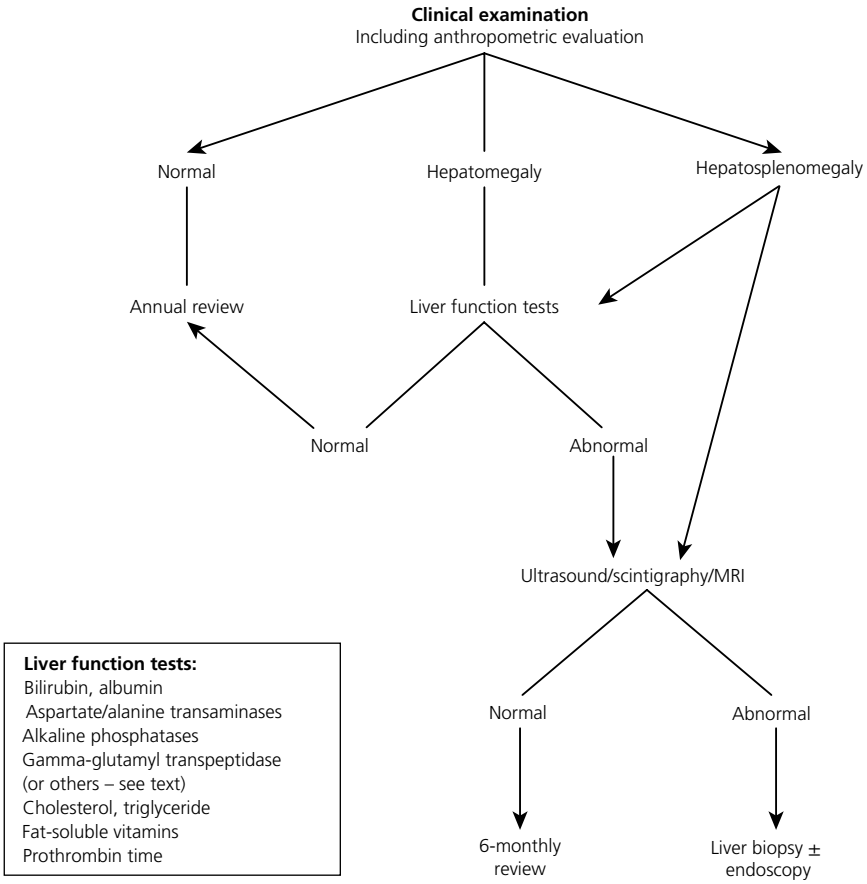


Figure 16.6 Algorithm of routine investigations for liver disease in cystic fibrosis.

Hepatobiliary scintigraphy

Hepatobiliary scintigraphy with third-generation iminodiacetic acid derivative tracers (that concentrate well in the biliary tree) has been used to screen for biliary tract disease in CF patients in the research setting. Scintigraphy may document the impairment of biliary drainage, the dilation of intra- and extrahepatic bile ducts and delayed biliary excretion, and the intestinal appearance of the tracer. Scintigraphy has been employed to document time-related progression of liver disease, to monitor the response to treatment with UDCA (Figure 16.7), and, in association with percutaneous transhepatic cholangiography, to screen for the presence of true stenosis of the common bile duct.

Cholangiography

Percutaneous transhepatic cholangiography and endoscopic retrograde cholangiography (ERCP) are invasive procedures, but may be useful for the investigation and therapy of patients with symptoms and signs of sclerosing cholangitis, distal stenosis of the common bile duct, or choledocholithiasis.

Magnetic resonance cholangiography

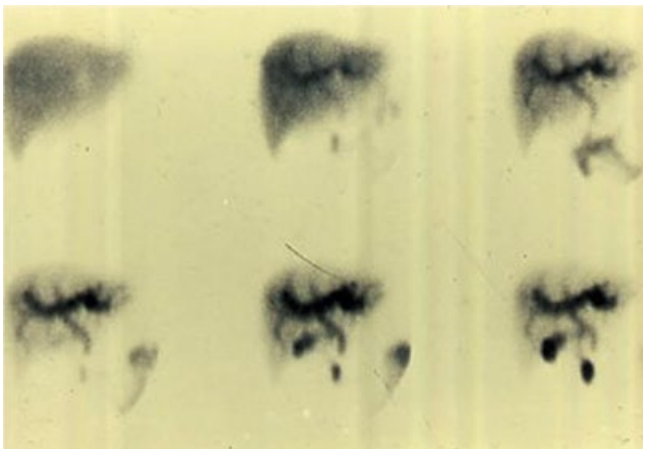
Magnetic resonance cholangiography imaging of the biliary tree is less invasive than ERCP and produces excellent imaging, demonstrating variable dilation of the bile ducts. MRCP has revealed cholangitic lesions in patients with or without clinically apparent liver disease, suggesting that it may be employed for early detection of intrahepatic biliary tract involvement (see Figure 16.5).

1H-magnetic resonance spectroscopy (1H-MRS) is a non-invasive technique for quantifying hepatic fat content. 1H-MRS measures the resonance signals derived from protons in triglycerides, which are quantified to assess the severity of steatosis. 1H-MRS has not been performed in CF patients so far.

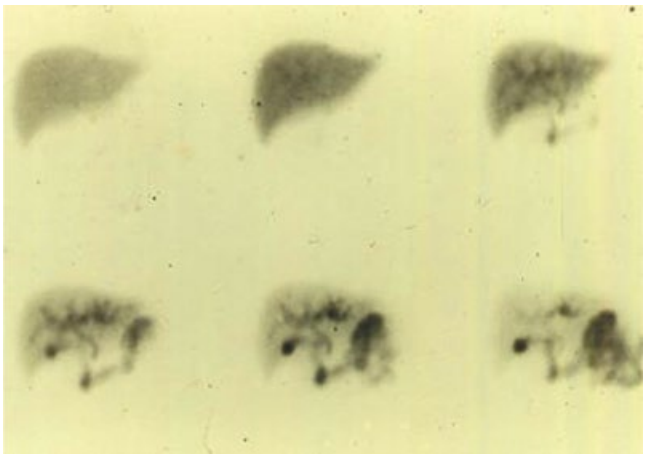
Liver pathology

Histological assessment, which represents the gold standard in the diagnostic work-up of many chronic liver diseases, is controversial in CF patients because of the potential risk of sampling error due to patchy distribution of lesions and underrepresentation of the extent of the disease. Nevertheless, studies have demonstrated that there is a good correlation between wedge biopsies and needle biopsies. Furthermore, using a dual-pass needle core liver biopsy increased the sensitivity of the detection of hepatic fibrosis by 22% compared with a single-pass liver biopsy [12].

The histological hallmark of CF-related liver disease is the deposition of inspissated bile (appearing as eosinophilic material with variable degrees of periodic acid–Schiff-positive reaction) in dilated cholangioles. There is focal periportal



(A)



(B)

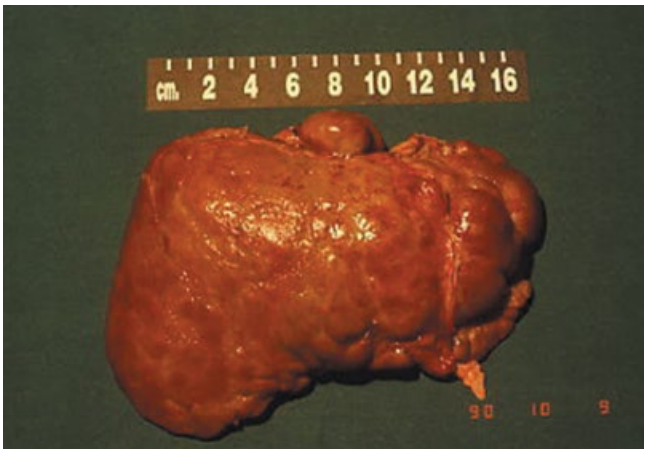
Figure 16.7 Hepatobiliary scintigraphy before (A) and after (B) UDCA therapy demonstrating improved biliary flow. (From Colombo *et al.* [23]. Reproduced with permission of John Wiley & Sons.)

obstructive disease with bile duct proliferation and cholangitis, a variable combination of inspissation, inflammation around the portal tracts, fatty infiltration (hepatocyte vacuolization with micro- and macrodroplet steatosis), and fibrosis (starting around the portal tracts and then extending intralobularly). Fibrosis and fatty infiltration are the most common features, whereas the presence of inspissated bile in cholangioles is infrequent. Particular attention should be paid to portal vein branches (obliteration, absence of portal veins) suggesting obliterative portal venopathy [7].

Liver biopsy may provide important information on the type of predominant lesion (steatosis or focal biliary cirrhosis), the extent of portal fibrosis, the rate of progression of liver disease, and the response to treatment with UDCA (Figure 16.8).

Non-invasive assessment of hepatic fibrosis

Several methods have shown promise for the detection of clinically silent fibrosis and are being evaluated among the CF population.



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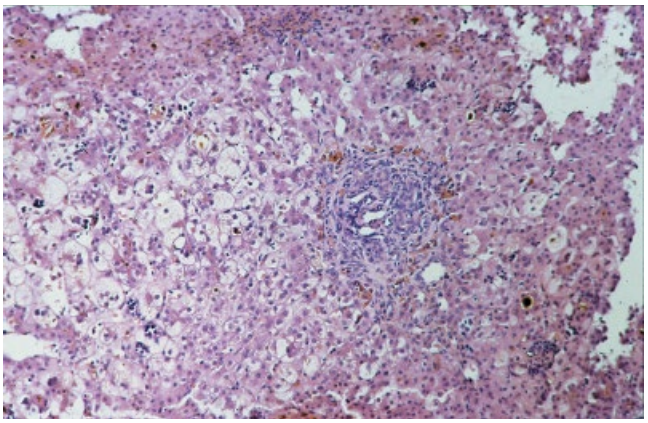

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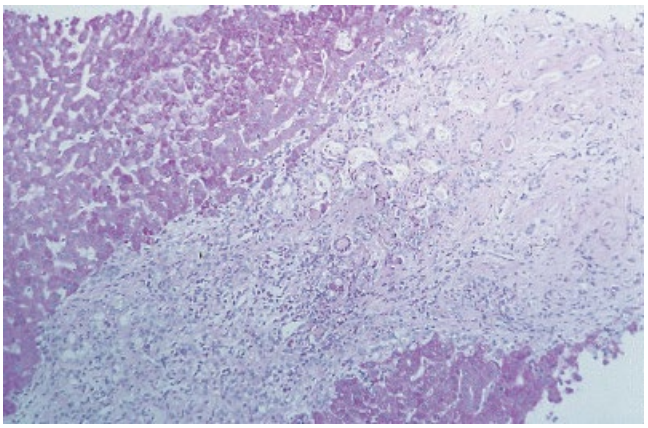

(C)

Figure 16.8 Multilobular biliary cirrhosis in a 9-year-old boy with cystic fibrosis (A). Histology may also demonstrate steatosis and chemical cholangitis (B), or focal biliary fibrosis (C) with portal inflammation.

Biomarkers

Identification of serum markers of liver fibrosis would be useful for the early detection of patients with CFLD. A recent study suggests that serum-based miRNA analyses could be used as diagnostic biomarkers with potential to predict early hepatic fibrosis [14].

Elastography

Transient elastography (TE; Fibroscan®, Echosens, Paris, France), acoustic radiation force impulse (ARFI; Siemens AG, Erlangen, Germany) and magnetic resonance elastography (MRE) are now used to assess the degree of fibrosis in various liver diseases and might identify patients at risk for CFLD and document disease progression.

TE is a reliable method for the diagnosis of significant fibrosis and cirrhosis but does not differentiate between normal and mild fibrosis. In addition, liver stiffness may be influenced by hepatic steatosis [15].

ARFI imaging combines conventional ultrasonography with the measurement of ultrasound-guided liver stiffness and shear wave velocities. A potential advantage is that ARFI imaging may not be influenced by hepatic steatosis. In a recent prospective study evaluating TE and ARFI simultaneously in 55 adult CF patients, both TE and ARFI did not significantly discriminate between non-cirrhotic CFLD and CF without liver disease but only discriminated between CF patients with liver cirrhosis and earlier stages of CFLD [16].

MRE is a new technology to detect earlier stages of liver fibrosis, but has not been evaluated in the CF population.

Hemodynamic measurements of hepatic venous pressure gradient

Hepatic venography should be performed in patients with portal hypertension without evidence of cirrhosis to measure the hepatic venous pressure gradient (HVPG), especially if transplantation is being considered. Intrahepatic presinusoidal (non-cirrhotic) portal hypertension is indicated if the HVPG is normal (≤5 mmHg) or only slightly increased (5–10 mmHg) [7]. In view of the good hepatic synthetic function, management of patients with CF who have NCPH should probably seek the alleviation of this portal hypertension by shunting procedures rather than referring these patients for liver transplantation.

Hepatic follow-up of cystic fibrosis patients

Regular monitoring of hepatic status with liver biochemistry and ultrasound scanning is essential in all patients with CF and should be included in their routine annual schedule [13].

In patients with cirrhosis, α-fetoprotein levels should be measured annually to monitor the possible development of hepatocellular carcinoma; an increased risk of biliary tract cancer has been recently reported in CF patients.

Upper gastrointestinal endoscopy is most useful in detecting the presence of esophageal varices and portal hypertensive gastropathy, and should be performed at least annually in patients with portal hypertension. Although safe and well tolerated, the sensitivity of esophageal capsule endoscopy for the evaluation of esophageal or small bowel varices is being investigated in the pediatric population.

Treatment options for cystic fibrosis-associated liver disease

Because of the decreasing mortality from extrahepatic causes, treatment of liver disease in CF patients is a relevant clinical issue. At present, management of CF-associated liver disease depends on clinical manifestations: oral bile acid therapy is started early in the course of the disease when the patient is asymptomatic, whereas end-stage liver disease often requires a complex and multidisciplinary approach, including a variety of surgical interventions.

Bile acid therapy

Oral bile acid therapy, aimed at reducing bile viscosity and improving biliary secretion and bile acid composition, is the only available therapeutic approach for CF-associated liver disease. Liver disease in CF is probably the result of bile duct obstruction related to the CFTR defect in cholangiocytes, and toxic retention leading to peribiliary fibrosis. Thus UDCA, a hydrophilic and choleretic bile acid, has been widely used in CF patients and has been shown to improve liver functions tests, biliary drainage, early hepatic ultrasonographic changes, and liver histology.

Other mechanisms of action may be involved, including direct cytoprotection of biological membranes, a protective effect against apoptosis induced by endogenous hydrophobic bile acids and the stimulation of bile secretion by hepatocytes and bile duct epithelial cells. The beneficial effects of UDCA in CF-associated liver disease may be also related to the stimulation of Cl^- secretion through Ca^{2+}-dependent Cl^- conductance and to concomitant reduction in mucin secretion. This may lead to improvement in biliary drainage, which has been documented at hepatobiliary scintigraphy (see Figure 16.7).

A dose–response study in CF patients has shown that the degree of biliary enrichment with UDCA and the biochemical response are better with a dose of 20 mg/kg/day, which is higher than that used in other cholestatic diseases (10 mg/kg/day) [17]. Although no significant side effects have been reported related to the long-term use of this drug, concerns were raised after patients with primary sclerosing cholangitis experienced a high rate of major adverse events in a randomized, double-blind, placebo-controlled trial of high doses of UDCA (28–30 mg/kg/day). It was thought that the adverse events were due to the possible biotransformation of UDCA in toxic bile acids. Analysis of the serum bile acid composition in CF patients on long-term UDCA treatment did not show significantly greater concentrations of potentially toxic bile acids in CF patients [18].

Unfortunately, due to the long natural history of CF-associated liver disease, there are limited data on the effectiveness of UDCA on long-term outcomes including death or need for liver transplantation. A recent Cochrane review concluded that there is insufficient evidence to justify its routine use in cystic fibrosis [19]. Asymptomatic patients with early-stage liver disease are more likely to benefit from UDCA administration, but there are no long-term data on the efficacy of UDCA in the prevention of liver disease. The potential therapeutic use of bile acid analogs and nuclear factors requires evaluation.

Nutritional support

The recognition that malnutrition is an important complication of CF means that most children with CF are prescribed high-energy diets with pancreatic enzyme supplements. The development of liver disease may further exacerbate malnutrition by increasing fat malabsorption and protein loss (see Chapter 21).

The following dietary management is recommended:

- An increase in energy intake of 150% the estimated average requirement, which may be achieved by adding carbohydrate supplements such as glucose polymers and increasing the percentage of fat.
- Increasing the proportion of fat to 40–50% of the energy content or a diet with special attention to increase supplementation of polyunsaturated fatty acids.
- Providing protein supplements to ensure an intake of 3 g/kg/day.
- Ensuring that sufficient pancreatic enzymes are prescribed to allow optimal absorption of long-chain triglycerides and essential fatty acids. In infants these supplements may be added directly to the formula, although use of a modular feed may allow careful adjustment of the food constituents. In older children supplementation of regular food with vegetable oils is preferable but can be supplemented by high-energy carbohydrate and protein drinks. In children in whom anorexia is a problem, enteral nasogastric or gastrostomy feeding may be required to ensure adequate caloric intake. Gastrostomy feeding is not recommended in children with advanced liver disease, varices, or portal gastropathy because of the risk of gastric hemorrhage.
- Fat-soluble vitamin supplementation

It is justified to prescribe high oral doses of vitamin A (5000–15,000 IU daily), vitamin E (α-tocopherol 100–500 mg daily), and vitamin D (α-calcidiol 50 ng/kg to a maximum of 1 μg). Vitamin K is sometimes required (1–10 mg daily). In addition to their nutritional importance, supplementation with vitamins, which are also antioxidants, such as α-tocopherol, ß-carotene, and vitamin C, might reduce lipid peroxidation and tissue damage. Supplementation with vitamin A should be carefully monitored with plasma levels to prevent toxicity.

Treatment of portal hypertension and end-stage of liver disease

The management of CF patients with advanced liver disease, severe portal hypertension, and hypersplenism is similar to that for other patients (see Chapter 21). There are few data regarding prophylactic therapy for portal hypertension before the first episode of variceal bleeding; the efficacy of α-receptor blockade has not been evaluated in CF because of the adverse effects of α-blockers on airway reactivity. Although adult data demonstrate the efficacy of endoscopic variceal ligation compared with β-blockers for primary prophylaxis of variceal bleeding, there are no data in children with CF. It is important to treat the complications of portal hypertension effectively [13], as many CF patients with severe portal hypertension and hypersplenism may remain stable for years and long-term survival has been reported after variceal bleeding [11].

Variceal bleeding may require sclerotherapy or variceal ligation during the acute episode. Vasopressin or octreotide may be used to control bleeding, but may cause systemic hypertension and splanchnic ischemia. Although endoscopic treatment is successful in most cases, in some patients gastric variceal bleeding or portal hypertensive gastropathy develop and may require additional therapeutic interventions (see Chapters 21 and 27).

There are no prospective studies to assess the indications, optimal timing, and actual benefits of different therapeutic interventions – including esophageal band ligation, transjugular intrahepatic portosystemic shunt (TIPSS), and surgical portosystemic shunts in CF. Esophageal band ligation is a preferable alternative to sclerotherapy and should be repeated until varices are eradicated, which has implications for children with CF who require repeated anesthetic and the necessary antibiotic prophylaxis. Neither procedure addresses the clinical complications related to hypersplenism. Alternatively, TIPSS has been employed for portal decompression in patients with recurrent bleeding, both as a long-term therapy for portal hypertension or as a bridge for liver transplantation. Elective surgical portosystemic shunts represent a more definitive treatment option for refractory bleeding in patients with preserved liver function and without severe pulmonary disease, allowing prolonged postoperative survival. Potential complications include development or worsening of hepatic encephalopathy, shunt thrombosis, and occlusion and may make transplantation more hazardous.

Splenectomy has been performed, alone or in association with a splenorenal shunt, in CF patients with hypersplenism showing an accelerated decline in lung function and/or variceal bleeding. These procedures are presently not recommended as transplantation may be a more effective strategy.

Liver transplantation

Liver transplantation is an effective therapeutic option for CF patients with end-stage liver disease, but selection criteria and timing have not been clearly established. This is made more complex because liver failure is a late event, therefore biochemical parameters and classification systems used to monitor severe liver disease – such as the Pugh and Child score, model for end-stage liver disease (MELD), and pediatric end-stage liver disease (PELD) model– are less suitable for CF-associated liver disease in which the complications of portal hypertension may occur with isolated hepatic fibrosis and good hepatic synthetic function.

Opinions vary between those who feel transplantation should be performed early to prevent progression of pulmonary disease, and those who feel transplantation is only indicated if there is clear evidence of liver failure. A poll among European CF and transplant centers on current practice and outcome for liver transplant in CF patients in Europe revealed that in the majority of cases the decision to transplant was based on the complications of portal hypertension and often transplantation was performed before the development of end-stage liver disease.

The agreed indications for liver transplantation in CF liver disease are:

- Progressive hepatic dysfunction (falling albumin <30 g/L; increasing coagulopathy, not corrected by vitamin K).
- Development of ascites and jaundice.
- Intractable variceal bleeding that is not controlled by conventional means.
- Deteriorating quality of life related to liver disease.
- Deteriorating lung function.

In summary, although the ideal candidates for liver transplantation are CF patients who have clear evidence of hepatocellular failure rather than CF patients with severe portal hypertension, adequate consideration should be given to the severity of other organ involvement as an indication for transplantation. In order to address this issue, exceptions to the MELD and PELD scores have been proposed for CF patients with severe pulmonary disease to give them additional priority and improve their access to the few suitable donors of both liver and lung.

The transplant assessment should evaluate pulmonary and cardiac function in order to establish whether liver transplantation alone is required or a combined heart/lung/liver transplant is more appropriate. With increasing experience, early liver transplantation is recommended for those children with deteriorating nutritional status and lung function as there is evidence that liver transplantation may prevent further decline [20].

Preoperative management includes adequate treatment of lung disease such as vigorous physiotherapy, control of infection, and mucus-dissolving agents. It is essential to plan appropriate postoperative antibiotic therapy by ensuring that regular sputum cultures are performed to identify the antibiotic sensitivity of colonized organisms. Preoperative information about pancreatic endocrine function is important because the immunosuppressive drugs used post transplantation, such as steroids and tacrolimus, have a diabetogenic effect (see Chapter 31).

Results for isolated liver transplant are satisfactory, with acceptable waiting times and survival. Survival after liver transplantation is similar to that in other groups of children (see Chapter 31). The 1-year survival rate after transplantation in CF patients is approximately 90%, with beneficial effects on lung function, nutritional status, body composition, and quality of life in most. A 5-year survival rate of 85% has been reported, late mortality being generally related to progression of pulmonary disease [20]. However, further grafts may be required and renal impairment is a frequent complication.

Survival data for combined liver/lung transplantation are also comparable to those observed in other groups of patients receiving lung transplants, with reported 1- and 5-year actuarial survival rates of 85% and 64% [20].

Future treatment options

With better knowledge of mechanisms involved in the pathogenesis of CF-associated liver disease, there is potential for several therapeutic strategies.

Somatic gene therapy aimed at replacing the defective gene in the biliary epithelium has been shown to be feasible in the experimental animal and may be curative. However, cholangiocytes are less accessible to targeted drug and gene delivery than the airways epithelium and the clinical application of this approach has been poorly explored.

Strategies aimed at stimulating fluid secretion by cholangiocytes may be easier to achieve.

Pharmacological correction of the CF ion transport defect by targeting the mutant *CFTR* gene (with correctors and/or potentiators) is presently an area of intensive investigation, and may prove to be an effective therapeutic approach for CF-associated liver disease in the near future [21].

Activation of alternative chloride channels that may compensate for CFTR dysfunction may also correct the defective anion secretion in intrahepatic ducts. Extracellular ATP is known to be a potent Cl^- secretagogue and can activate the Ca^{2+}-dependent Cl^- channel in different cell types, including cholangiocytes.

Finally, docosahexaenoic acid (DHA) supplementation has been recently shown to induce a significant amelioration of the severity of liver disease in cftr –/– mice, with a striking reduction in the degree of periportal inflammation. The beneficial effect of DHA that may be linked to its ability to inhibit cytokines and/or eicosanoid metabolism and to release endogenous inhibitors of inflammation, must be confirmed in CF patients.

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SECTION 7

Acute Liver Disease

CHAPTER 17

Non-Viral Infectious Liver Disease

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Key points

- The liver is commonly involved in severe systemic bacterial infections resulting in hepatitis or infective cholangitis.
- The infecting organism and resultant pathogenesis depends on host immunity and the presence of existing liver disease.
- Chronic bacterial infection of the liver (with *Mycobacterium*) result in granulomatous lesions that require prolonged treatment.
- Pyogenic liver abscesses are more common in immunocompromised patients and carry a high mortality rate if left untreated.
- Parasites and worms can infect the liver resulting in cyst or abscess formation.
- In immunocompromised patients and those post-transplant systemic fungal infection (commonly *Candida*) can involve the liver and requires prompt recognition and treatment.

Systemic acute bacterial infection

Bacterial sepsis

Pediatric sepsis is a clinical syndrome caused by a dysregulation of the immune system in response to infection. It has a wide clinical spectrum, which can involve liver inflammation and multiorgan failure. Sepsis is a serious problem worldwide accounting for the majority of deaths (>60%) in children under 5 years [1]. Table 17.1 outlines the common bacterial causes of sepsis by age group.

Clinical features and diagnosis

In addition to other features of sepsis, children may have liver involvement manifesting as jaundice and elevation of serum transaminases. This is diagnosed as a raised bilirubin (>4 mg/dL) (not applicable for neonates) and alanine aminotransferase (ALT) above twice the normal age-appropriate range. There may be several factors that contribute to liver injury in sepsis the most common of which include hypoxic hepatitis, resulting in hepatocellular toxicity and sepsis-associated cholestasis [3] (Figure 17.1).

Treatment is primarily supportive including effective antibiotic therapy, specific treatment for hypotension, anemia, coagulopathy, bleeding and shock, and supportive care in an intensive care unit. As children with liver involvement will be more susceptible to hepatotoxic drugs it is important to identify and stop these if possible.

Children who are immunocompromised post-transplantation or who have end-stage liver disease are also susceptible to overwhelming bacterial infection, with any number of organisms. *Streptococcus* and *Staphylococcus* are important organisms. A study in Spain showed that extended β-lactamase producing Enterobacteriaceae, *Pseudomonas*, meticillin-resistant *Staphylococcus aureus* (MRSA), and *Enterococcus* were important organisms in cirrhotic patients [4]. Mortality was significantly increased when infections were caused by multiresistant organisms. *Clostridium* is also important, particularly in the context of necrotizing enterocolitis (NEC) in neonates and in immunosuppressed children. Jaundice and hepatic involvement are common and can include gas in the portal system and abscesses [5]. In this case surgical debridement is important in addition to antibiotic treatment. In immunocompromised individuals overwhelming infection leading to liver failure can also be caused by a number of other organisms including viral infections (cytomegalovirus, adenovirus) and fungi. Coinfection with a number of different pathogens is common.

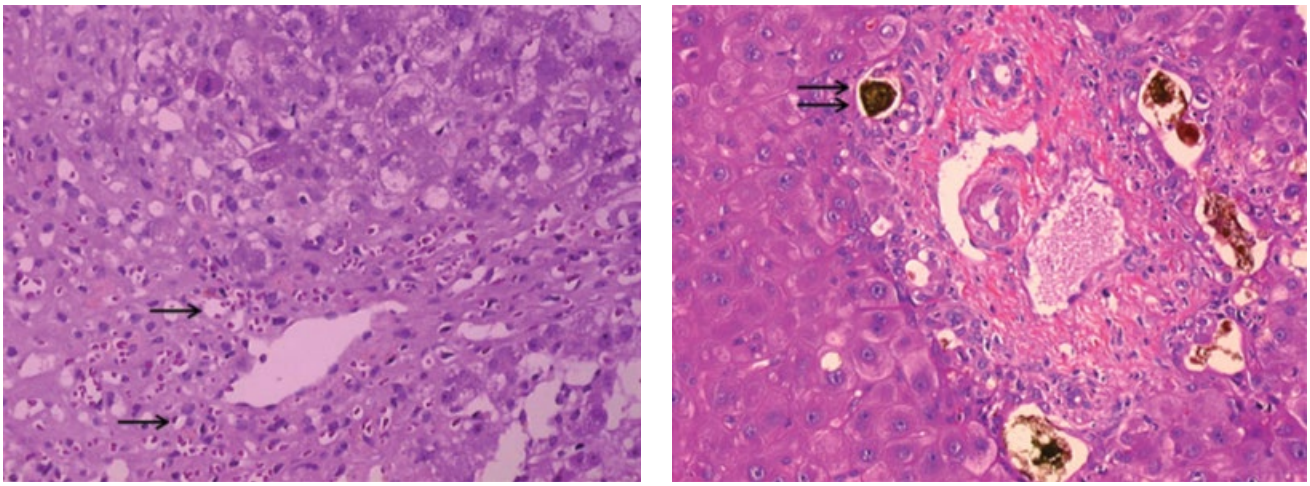
Prognosis

Without treatment, mortality for severe sepsis involving multiorgan failure is over 80% but with treatment is around 10% overall [2]. This is likely to be worse in immunocompromised patients with resistant bacteria and/or coinfection. Prompt recognition and targeted treatment improves survival and can reduce associated organ damage.

Diseases of the Liver and Biliary System in Children, Fourth Edition. Edited by Deirdre A. Kelly.

Table 17.1 Common bacterial causes of sepsis by age group. (Adapted from Plunkett and Tong 2015 [2].)

Age	Gram positive	Gram negative
Early neonatal sepsis (first 72 h of life)	Group B streptococci *Staphylococcus aureus* Coagulase-negative staphylococci Enterococci *Listeria monocytogenes*	*Escherichia coli* *Haemophilus influenzae*
Late neonatal sepsis (72 h to 28 days)	Coagulase-negative staphylococci (associated with vascular devices in neonates) Any of the above organisms	–
Infants and young children	*Streptococcus pneumoniae* *Staphylococcus aureus* Group A streptococci	*Neisseria*
Hospital acquired	Dependent on local microbiology But consider coagulase-negative staphylococci in catheter-associated sepsis Meticillin-resistant *Staphylococcus aureus*	*Pseudomonas aeruginosa* *Klebsiella* *Escherichia coli* *Acinetobacter* spp.
Immunocompromised	As above Also consider *Salmonella* and other encapsulated organism	–



(A) (B)

Figure 17.1 Hypoxic hepatitis (A) showing perivenular hepatocyte necrosis with cell loss, acidophil bodies, and moderate congestion; and (B) with inspissated bile in dilated periportal bile ductules (also called cholangitis lenta). (From Lescot *et al.* 2012 [3]. Reproduced with permission of Wolters Kluwer Health.)

Listeria monocytogenes

Infections with *Listeria* are rare and almost all cases of hepatic involvement with *Listeria* occur in the presence of immunosuppression or in neonates. An hepatic illness with fever, raised transaminases, and jaundice may occur, diagnosis being confirmed by isolating *Listeria* organisms from blood cultures. Treatment with antibiotics (ampicillin intravenously for at least 14 days) is usually effective. Hepatic abscess formation due to *L. monocytogenes* infection has also been described.

Salmonella

Salmonella typhi (typhoid fever) is a common infection worldwide (see Chapter 34). Serious hepatic involvement is uncommon but can involve cholecystitis, fulminant hepatitis, and hepatic abscess, mediated by endotoxin release from the bacteria.

Clinical assessment is important looking for fever, right upper quadrant pain, and tender hepatomegaly. There are raised transaminases but the bilirubin is usually not very high. Abdominal ultrasound may reveal sludge in the biliary tree or abscess formation.

Diagnosis is usually dependent on cultures growing *Salmonella typhi* but polymerase chain reaction (PCR) may be helpful in the diagnosis.

Treatment is with effective antimicrobials such as ceftriaxone and ciprofloxacin, but this depends on local sensitivities.

Prognosis is good with early diagnosis and effective treatment although underlying liver disease is an independent risk factor for mortality [6].

Other bacterial infections

Children who are immunocompromised are also at risk of liver disease from a number of other infecting bacteria.

Yersinia enterocolitica is a Gram-negative bacillus that causes a range of illness from acute enteritis to serious systemic infection and is associated with contaminated food consumption. It presents with abdominal pain and diarrhea and can rarely have hepatic involvement, which manifests as multiple abscesses and ascites [7]. *Yersinia* is an iron-loving bacteria and therefore children with iron overload syndrome such as hemachromatosis may be at increased risk. There is a high mortality in immunosuppressed children with liver involvement. Treatment is dependent on microbiology but is usually susceptible to co-trimoxazole or cephalosporins.

Actinomycosis is an infection with *Actinomyces* spp. and is uncommon but important invasive organisms particularly in immunocompromised hosts. Liver and biliary tract involvement can occur with local spread from abdominal sites. It may be slowly but continually progressive in any body site and can mimic malignancy. The liver may have multiple small or large abscesses which may require surgical resection [8]. Treatment is with penicillin-based antibiotics and is likely to be prolonged.

Legionella pneumophila has also been associated with a hepatic illness in the immunosuppressed host. Characteristically jaundice is minimal and there is hepatic steatosis and necrosis on biopsy. Treatment of the underlying infection is the mainstay of treatment.

Chronic/granulomatous bacterial infection

Mycobacteria

Bacille Calmette–Guérin

Bacille Calmette–Guérin (BCG) is an attenuated strain of *Mycobacterium bovis*, which is used as a vaccine against tuberculosis and in adults as intravesical treatment of bladder cancer. In the immunocompetent host local effects (regional lymphadenitis and local vaccine abscess) are common. In the pediatric population, disseminated disease is usually associated with immunodeficiency which is either primary (severe combined immunodeficiency, interferon-γ pathway defects) or secondary (human immunodeficiency virus (HIV) infection, immunosuppression). Systemic BCG infection affects most solid organs including the liver. It can affect the liver in several forms, either as a cold abscess, granulomatous disease, or hepatitis. There may also be an inflammatory component to liver damage.

Clinical features

In the context of an immunodeficient patient, its symptoms may be masked. The presence of fevers, weight loss, hepatomegaly, generalized lymphadenopathy, and rashes may all be signs of the underlying immunodeficiency, but clinical suspicion is raised if there is confirmation of BCG vaccination in the recent past history.

Diagnosis

In immunocompromised patients, the diagnosis is usually one that requires exclusion of other causes of hepatitis. Multiple infections (viral and fungal) which themselves also cause hepatitis, can also occur. Radiologically ultrasound may reveal granulomas or heterogeneous liver echo texture. Histologically biopsy shows the presence of granulomatous hepatitis which may be non-caseating and not typical of what is seen in primary tuberculosis. Histochemical stains may be positive for alcohol acid–fast bacilli (AAFB). Rapid identification using broad PCR to identify tuberculosis complex is helpful (greater sensitivity if the sample is AAFB positive); BCG can then be identified by its absence of ESAT 6. Mycobacterial culture is the gold standard to confirming the diagnosis but this takes several weeks to yield a positive result.

Treatment

Mycobacterium bovis and the attenuated BCG are inherently resistant to pyrazinamide; treatment involves use of the other three first-line agents – ethambutol, isoniazid, and rifampicin. Certain strains also show some low-level resistance to isoniazid, as well as cycloserine and prothionamide. Treatment should be with a minimum of the three agents but in systemic illness, addition of second-line agents (fluoroquinolones, aminoglycosides, clarithromycin) is recommended to control disease [9]. Treatment courses are prolonged and usually for months. If immunodeficiency is confirmed and corrective therapy (stem cell transplant, antiretroviral treatment) is implemented there is secondary phenomenon of BCG immune reconstitution syndrome, which would exacerbate hepatitis and may require adjunctive use of steroids.

Prognosis

Infection and associated hepatitis can improve with antimycobacterial therapy. However, a few of the first-line agents can cause drug-induced hepatitis so close monitoring of biochemical markers is essential. Investigation for an immunodeficiency must be undertaken if a pediatric patient has confirmed BCG liver involvement. Despite treatment it carries a high morbidity and mortality up to 50% in some case series. True control of the disease and reduction of disease recurrence is achieved with treatment of the underlying immunodeficient condition.

Tuberculosis

Clinical features

Tuberculous hepatitis is a component of disseminated, extrapulmonary tuberculosis which occurs via *hematogenous* spread from the initial foci of infection – the lung. Tuberculosis can affect the liver in three ways: as distinct granulomas, tuberculous hepatitis, and hepatic abscess (Figure 17.2).

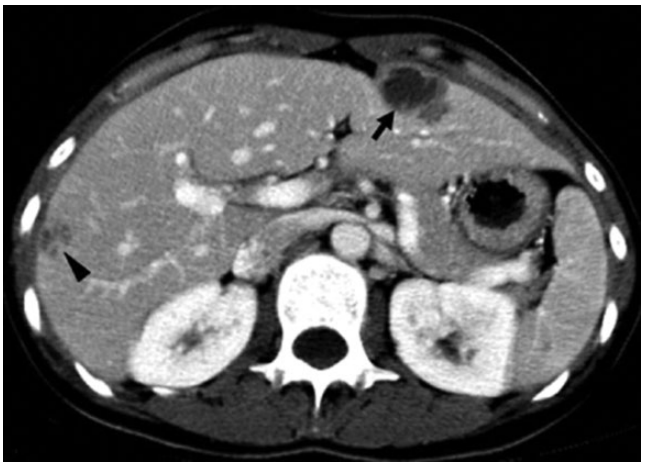


Figure 17.2 CT of miliary tuberculosis of the liver. Two abscess pockets are also indicated with arrows. (From Hwang *et al.* 2009 [22]. Reproduced by permission of The Korean Association for the Study of the Liver.)

Risk factors for dissemination include immunocompromise and chronic disease states as well previous underlying hepatic disease. Features of fevers, night sweats, cough, and weight loss are the most common features in tuberculosis. Jaundice is unusual, but abdominal pain can be a feature. The presence of hepatic enlargement and tenderness or presence of liver lesions on ultrasound must always be explored.

Diagnosis

Diagnosis is based on good clinical history; known tuberculous contacts, positive smear or presence of AAFB on biopsy or sputum samples. Interferon-gamma release assay are more often positive but false negative results can occur. The definitive diagnosis is made on culture, which take up to 3–10 weeks but a more rapid diagnosis can be made using tuberculosis complex PCR. On liver histology periportal granulomas, caseating and non-caseating, can be seen.

Treatment

Treatment consists of empiric use of the standard four drug regime (isoniazid, rifampicin, ethambutol, and pyrazinamide), whilst awaiting drug sensitivity testing from culture. Treatment is usually safe but close monitoring is required as both rifampicin and isoniazid in combinations as well as pyrazinamide can cause significant liver derangement. Pre-existing liver disease is risk factor for side effects.

Prognosis

The outcome is variable. If tuberculosis is fully sensitive and the patient is treatment adherent then hepatitis is recoverable but can carry significant morbidity and mortality. If left untreated fulminant liver failure can occur. With poor adherence or the presence of drug-resistant treatment then the outcomes are less favourable. Pre-existing co-morbidities also affect outcome for tuberculosis and not necessarily related to its hepatic complications.

Bartonella

Bartonella are usually opportunistic bacteria and are transmitted by vectors such as tics, flies, and mosquitos. *Bartonella henselae* is the organism responsible for cat-scratch disease.

Clinical features

Regional lymphadenopathy, fever, and mild systemic symptoms are the most common features of *Bartonella* infections (*B. henselae*) [9]. A small number of cases (1–2%) are also associated with hepatitis as a complication. The typical features with this include fever, malaise, right upper quadrant pain, and headaches [10]. The triad of clinical parameters (including the euphemistic scratch by a cat) may not be apparent. There are raised transaminases and raised bilirubin but biochemical parameters are usually non-specific as to a cause. Hepatitis can occur in as part of a multisystem disorder in the immunocompromised but is known to occur in immunocompetent hosts.

Diagnosis

Histologically hepatic bartonellosis is characterized by necrotizing granulomas, but these are not specific; there can be evidence of reticuloendothelial lesions (bacillary peliosis) [10]. *Bartonella*-specific histochemical stains on tissue can help in identification but they are not specific or sensitive. Direct culture of hepatic tissue or of blood rarely identifies the organism due to the fastidious nature of this Gram-negative organism in culture. Serology can be useful but the development of *Bartonella*-specific PCR has made it possible to rapidly identify the organism and tailor treatment.

Treatment

Bartonella has a broad susceptibility to fluoroquinolones, azithromycin, trimethoprim–sulfamethoxazole, doxycycline, gentamicin, and rifampicin [10]. As most of the agents are bacteriostatic, combination treatment is needed and a prolonged course of treatment of up to several months in disseminated disease and in the immunocompromised is recommended.

Prognosis

Infection usually improves if treated early. The use of steroids may be beneficial but could trigger endocarditis complications. Close monitoring of symptoms, and biochemical and clinicoradiological findings should guide treatment.

Brucellosis

Clinical features

This is a zoonotic illness caused by a Gram-negative coccobacilli. Children are infected by ingestion of unpasteurised milk or undercooked meat from infected animals. In children its onset may be acute or insidious. Symptoms of fever, malaise, lymphadenopathy, abdominal pain, and arthralgia are most common; neurological involvement

and endocarditis can occur. More rarely, hepatic involvement in the form of abscesses and hepatitis has been observed. The most common species is *B. melitensis* but *B. abortus* and *B. suis* can cause human disease. There may be elevation of liver enzymes but this is not specific.

Diagnosis

Definitive diagnosis is isolation by culture from blood, tissue, or bone marrow. Prolonged culture is needed. Serum agglutination tests are the gold standard. Paired acute and convalescent sera showing a fourfold rise in titers from samples taken 2 weeks apart will confirm the diagnosis. Liver histology may show the presence of non-caseating granulomas or portal/septal lymphocytic infiltrate [11].

Treatment

A prolonged course of combination antibiotic treatment is needed to prevent relapse. In children less than 8 years of age trimethoprim–sulfamethoxazole treatment is recommended along with rifampicin. Gentamicin is added for the first 2 weeks of treatment in severe infections. Treatment course should be for a minimum of 4–6 weeks but may be extended in more serious infections to 4–6 months. For older children doxycycline (or tetracycline) in combination with rifampicin should be used [9].

Prognosis

Most diagnoses are made during the chronic phase of illness, thus optimizing treatment using dual therapy and for prolonged duration reduces the rate of relapse. Severe disease or complications require longer courses from the outset to minimize complications.

Localized bacterial infection

Pyogenic liver abscess

Pyogenic liver abscesses are rare in healthy children but tend to be reported more often in children who are immunocompromised (e.g., chronic granulomatous disease, acute lymphoblastic leukemia), children with underlying diseases (biliary atresia, chronic inflammatory bowel disease, hemoglobinopathies), and in children from developing countries. A rich blood supply in the liver and an extensive reticuloendothelial system generally present an effective barrier against bacterial invasion. In one autopsy series, the incidence was 38 per 1000 in children under 15 years of age. Retrospective hospital series suggest a lower incidence of 3 per 100,000 [12].

Pathogenesis and etiology

Bacterial infection can become established in the liver through systemic *hematogenous* spread (80%), usually in immunocompromised patients. About 10–15% occur following portal vein inflammation and bacteremia, secondary to appendicitis/periappendiceal abscess or chronic inflammatory bowel disease. The incidence of liver abscess, following undiagnosed appendicitis and its complications, is much rarer than it was 50 years ago. A small number of cases follow extension of infection from contiguous structures (e.g., from biliary tract disease as an ascending cholangitis). Infants may develop hepatic abscesses, following omphalitis or umbilical vein catherization. This latter procedure may result in septic thrombophlebitis, focal necrosis of the liver, and liver abscess. A small number of hepatic abscesses are cryptogenic in origin and often present as a pyrexia of unknown origin. Most liver abscesses occur in the right lobe of the liver and the majority are solitary. Multiple liver abscesses constitute 20–25% of all cases.

Although *Staphylococcus aureus* is the leading cause of pyogenic liver abscess in most cases, the etiology is commonly polymicrobial with Gram-positive cocci (*Staphylococcus aureus*, streptococci spp.), enteric Gram-negative bacilli, and anaerobes.

Clinical presentation

Pyogenic bacterial abscesses often present with non-specific manifestations such as fever, nausea, vomiting, anorexia, malaise, and abdominal pain, which often is localized to the right upper quadrant. Hepatomegaly is present in more than half of the patients although jaundice in uncommon. A pyrexia of unknown origin and abdominal pain may indicate a diagnosis of liver abscess, especially in an immunocompromised patient. A single solitary abscess is often insidious in its presentation, while multiple abscesses usually present more acutely.

Diagnosis

Ultrasound of the liver is the imaging of first choice. Rounded or oval hypoechoic lesions may be identified on ultrasound (Figure 17.3). These lesions may have a heterogeneous texture.

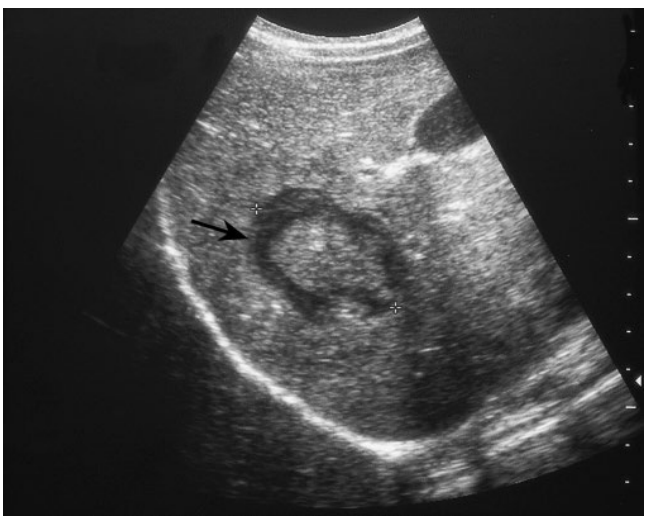


Figure 17.3 This hepatic ultrasound demonstrates an abscess in a patient with abdominal pain and hepatic tenderness.

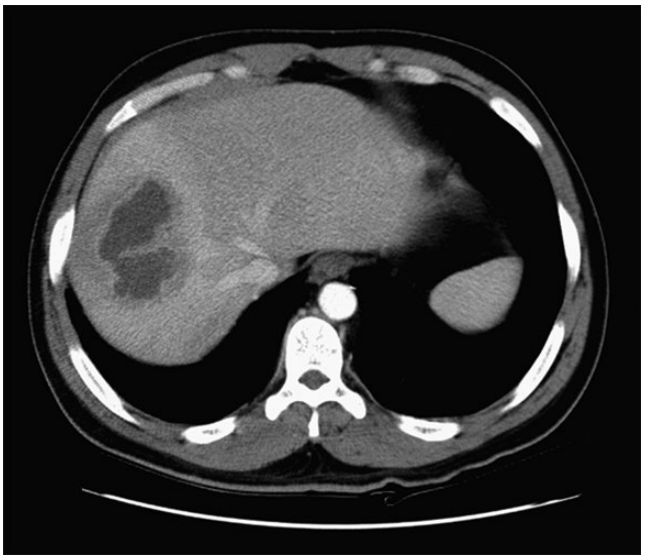


Figure 17.4 CT showing large fluid-filled liver abscess caused by *Staphylococcus aureus* in an adult presenting with fever and cough. (From Mueller 1998 [23]. Reproduced with permission of Thieme.)

CT scanning is more sensitive at picking up small abscesses. A classic picture is of a hypodense lesion with low attenuation areas and an enhancing rim (Figure 17.4). Liver abscesses on MRI appear as hypointense and hyperintense lesions in different sequences. Ultrasound is the best modality for following up resolution of abscess during treatment.

A microbiological diagnosis is made following culture of fluid/pus, obtained following needle aspiration or percutaneous drainage. Anemia, leukocytosis, and raised erythrocyte sedimentation rate (ESR) are also the usual accompaniments. Blood cultures are rarely positive and altered liver function is unusual. Serology tests (enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination) for amoebiasis should be included early on, when empiric therapy is commenced, although amoebic abscess is rare in children.

Treatment

In general, treatment involves the administration of antibiotic therapy and drainage of liver lesions. Empiric antibiotics are started immediately and this could be a combination of an antistaphylococcal agent, an aminoglycoside, and anaerobic agent (e.g., flucloxacillin, gentamicin, and metronidazole). Other acceptable combinations include: piptazobactam and gentamicin or clindamycin and gentamicin. Once the results of culture of aspirated material are known, definitive therapy can be instituted. Antibiotic treatment is for 4–6 weeks; multiple liver abscesses may require treatment for up to 12 weeks.

Abscesses, less than 4 cm in diameter, can be successfully treated with needle aspiration (or repeated aspiration), along with antibiotics. Abscesses greater than 4 cm in diameter need continuous drainage using CT- or ultrasound-guided percutaneous aspiration, and antibiotics. Open surgical drainage is reserved for those patients whose liver lesions do not resolve with percutaneous drainage or those with rupture of the abscess. Serial ultrasonography should be performed to document a reduction in the size of the abscess cavity prior to stopping antibiotic therapy.

Prognosis

Mortality from undrained and untreated lesions tend to be high, however with prompt diagnosis, facilitated by better imaging, improved antibiotics, and percutaneous drainage, mortality in most series has been less than 15%. The commonest complications are pleuropulmonary which include pleural effusion, empyema, and hepatopleural fistula. Other complications are ascites, intraperitoneal rupture, and peritonitis. The presence of jaundice, liver failure, acute abdomen, encephalopathy, large abscess volume, and hypoalbuminemia are indicators of poor prognosis. The aim of treatment is complete resolution although this may take a few months. Serial ultrasound can be useful in assessing the success at reducing the abscess size. There will be an eventual calcification of the lesion in most cases, with no long-term sequelae.

Infective cholangitis

(see Chapters 8, 11, 25, and 27)

Infection of the biliary tree in the immunocompetent usually signifies an underlying structural abnormality. Predisposing disorders include:

- Primary sclerosing cholangitis.
- Choledochal cyst.
- Biliary atresia (after portoenterostomy).
- Cholelithiasis.
- Caroli disease.
- Portal vein cavernoma with biliary obstruction.
- Biliary strictures.

Presentation may be with fever, jaundice, and biliary obstructive symptoms such as pale stools and dark urine, accompanied by tenderness in the right hypochondrium. Occasionally the only symptom may be fever. Blood cultures may yield bacterial pathogens, but are frequently negative, and treatment with broad-spectrum antibiotics is indicated such as:

- Ceftazidime: 30 mg/kg t.d.s. for 10 days.
- Amoxicillin: 20 mg/kg t.d.s. for 10 days.
- Ciprofloxacin: 4–7 mg/kg/dose 12 h i.v. for 10 days.

If there is persistent biliary obstruction and sepsis, external biliary drainage, or biliary dilation may be required.

Unusual organisms that may cause cholangitis include *Ascaris* infection and *Aspergillus*. *Cryptosporidium* and *Microsporidium* may cause cholangitis in those with immunodeficiency.

Spirochetes

Spirochetes are bacteria that belong to a distinct phylum and include the bacteria that cause Lyme disease and syphilis. Leptospirosis and *Treponema* are most commonly associated with liver disease.

Leptospirosis

Leptospira icterohaemorrhagicae is a spirochete that is carried in the kidneys of both wild and domestic animals, particularly the rat, with contamination of streams and rivers occurring through infected urine. Human exposure occurs in vets and farm workers, and by swimming in contaminated water. Transmission occurs via skin abrasions or mucous membranes, with person-to-person spread being rare.

Clinical features

Following an incubation period of 1–2 weeks, bacteremia is established and leads in the majority to a subclinical infection or a mild flu-like illness with fever and myalgia. Symptoms usually persist for 1 week, with their severity relating to the number of infecting organisms and the immune status of the host. In less than 10% of symptomatic cases, severe systemic disease, Weil disease, occurs. Symptoms are due to damage to the endothelium of the small vessels and to seeding of the leptospires in meninges, liver, or kidneys. They include:

- Fever, headache, myalgia.
- Extensive vasculitic rash and circulatory collapse.
- Renal failure.
- Myocarditis.
- Pneumonitis.
- Hepatitis: jaundice and hepatomegaly are characteristic, and transaminases are usually only mildly elevated. Fulminant liver failure may occur.

In Egypt, 16% of 392 patients with undiagnosed acute hepatitis had serological evidence of *Leptospira* IgM but local prevalence will vary geographically [13]. It is important to take a full history of possible exposure to infected water sources in children with suspected leptospirosis.

Diagnosis

Diagnosis is made by:

- Demonstrating leptospires by dark-ground microscopy in:
  - blood during the bacteremic phase
  - urine during phase of organ involvement.
- Detecting in serum specific IgM antibody or rising titer of IgG antibody.

Treatment

Treatment with penicillin G (200,000–250,000 U/kg/day i.v. in six divided doses for 1 week) may have a beneficial effect on the illness if given in the first 4–7 days. Tetracycline or erythromycin is also effective. Most patients recover without long-term sequelae.

Treponema

Treponema pallidum causes syphilis. The most common cause of syphilis in children is congenital, when there is transplacental transmission of *Treponema* to the fetus. In utero infections can result in stillbirth, prematurity, and a wide range of clinical manifestations. Two-thirds of babies with congenital syphilis are asymptomatic at birth but the most severe forms are already evident by this point. Syphilis is a particular problem in the developing world, although there have been increased numbers of cases reported in the UK in the last 10 years. Cases of acquired syphilis through sexual transmission are more rare in children.

Clinical features

The most common early features of congenital syphilis (within the first 2 years of life) include [14]:

- Hepatosplenomegaly (71%).
- Morbilliform rash (68%).
- Fever (42%).
- Neurosyphilis (23%).
- Rhinitis "syphilic snuffles" (14%).
- Generalized lymphadenopathy (14%).
- Ascites (9%).

There is an associated leukocytosis in 72% patients and hemolytic anemia in 60%. Syphilitic hepatitis leads to jaundice with elevated serum transaminases and alkaline phosphatase. Prothrombin time may also be prolonged. Histological examination of the liver reveals a hepatic picture with spirochetes demonstrated on silver stain. Syphilis can involve multiple organs including bones (which usually resolves within 6 months of age), skin (bullous pemphigus), central nervous system (neurosyphilis), eyes, heart, pancreas, gut, and kidney (Figure 17.5).

Diagnosis

There are direct and indirect methods of diagnosing syphilis. Methods for direct identification of *T. pallidum* in clinical specimens are:

- Dark-field microscopy of a clinical specimen high in bacterial load (but with few blood cells – such as nasal discharge) (Figure 17.6).
- Direct fluorescent antibody.
- PCR – does exist but not widely used.

Specific tests for *T. pallidum* antibody exist and are widely used (including the *T. pallidum* particle agglutination (TPPA) test). Antibody titers remain positive for life but do not correlate with disease activity.

Indirect tests include rapid plasma reagin (RPR) and the venereal disease research laboratory test (VDRL). These actually detect antibodies to cardiolipin which reflect disease activity. False positive/negative reactions can occur and cord blood should not be tested for this reason. Cardiolipin levels can be used to test for treatment success.

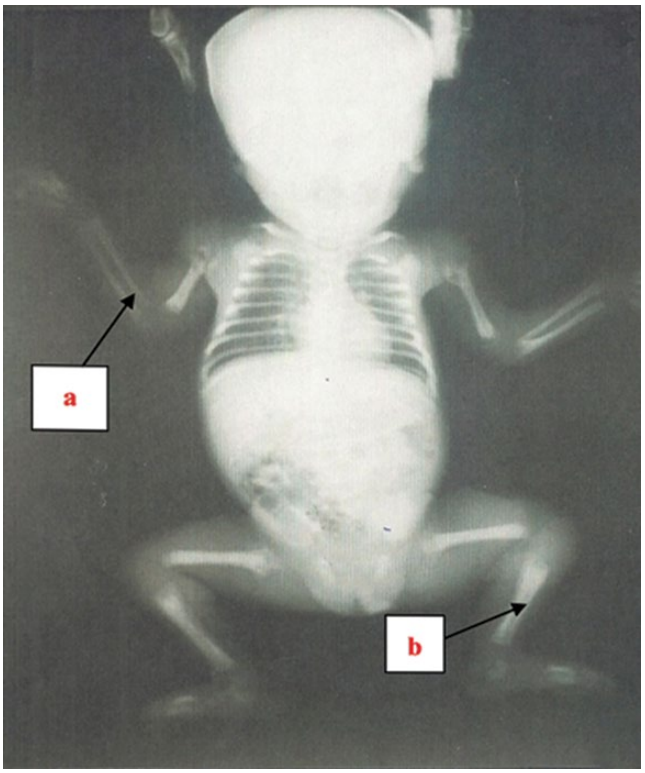


Figure 17.5 Anteroposterior radiograph of a preterm infant with congenital syphilis demonstrating osteoarticular lesions (a,b) and an enlarged liver. (From Megnier-Mbo *et al.* 2014 [24]. Reproduced with permission of Megnier-Mbo *et al.* and Scientific Research Publishing Inc.)

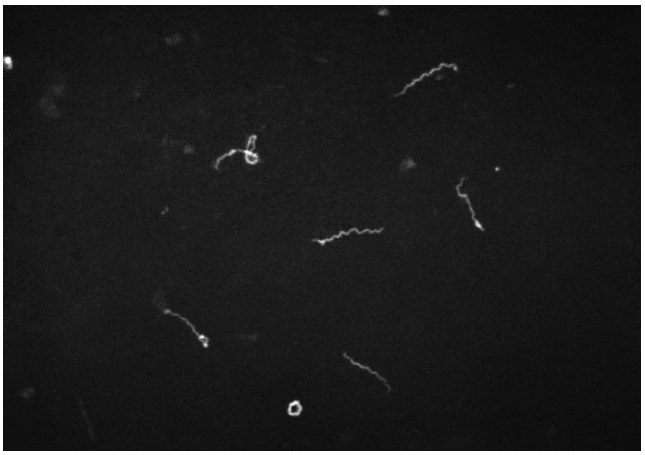


Figure 17.6 Dark-field microscopy reveals the presence of *Treponema pallidum*. Available at http://phil.cdc.gov/phil/details.asp?pid=10179 (last accessed July 2016). (Courtesy of Centers for Disease Control and Prevention.)

Treatment

Evaluation and treatment of the infant exposed to maternal syphilis but with no clinical features at birth depends on timing and adequacy of maternal treatment and maternal and infant non-direct treponemal serology. For infants with clinical congenital syphilis at birth, a positive dark-field or fluorescent antibody and a high antibody titer (more than 4× the maternal titer) should be given 10 days of either:

- Aqueous crystalline penicillin G, i.v.
  OR
- Procaine penicillin G, i.m.

A full 10 days is preferred even if the infant is on ampicillin for possible sepsis. Full blood count and cerebrospinal fluid should be collected. Investigation into other organ involvement such as the eye and hearing test should be done as clinically indicated.

Rickettsia

These are a genus of non-motile Gram-negative bacteria which are carried by many arthropods such as ticks, fleas, and lice. They can cause disease in humans worldwide depending on the region, e.g., Rocky Mountain spotted fever, African tick bite fever, and Q fever.

Q fever

Clinical features

Q fever is a zoonotic illness that is caused by an obligate intracellular Gram-negative organism, *Coxiella burnetii* which is spread by tick vectors. It is found worldwide (except New Zealand). In children the majority have mild and self-limiting features of fever, malaise, and non-specific gastrointestinal symptoms. There may be a rash in up to 50% of pediatric cases. Rarely it can present as an acute, severe illness of which hepatitis is one such manifestation. In such cases there will be raised liver enzymes (alkaline phosphatase as well the transaminases) with hyperbilirubinemia.

Diagnosis

It is difficult to diagnose in the initial stages, but a history suggestive of this infection can help to guide initial therapy. Culture is difficult due to the fastidious nature of the organism and must be done in highly specialized laboratories due to the risk it poses to personnel. Serological testing, using immunofluorescent assay on paired acute and convalescent serum, can help confirm the diagnosis; a four-fold rise in phase 2 IgG antibody titers or a single high titer could confirm the illness [15]. The use of PCR/nucleic acid amplification in blood or tissue can provide more rapid diagnosis if done early in the disease – it can be negative as the antibody titer rises. Histologically, Q fever hepatitis is characterized by fibrin ring granulomas (Figure 17.7) but this can also be seen in other infective causes of hepatitis [15].

Treatment

With severe illness the treatment in all age groups is doxycycline for a period of 14 days [9]. Mild disease in pediatric patients especially those less than 8 years of age, could be treated with trimethoprim–sulfamethoxazole. Chronic Q

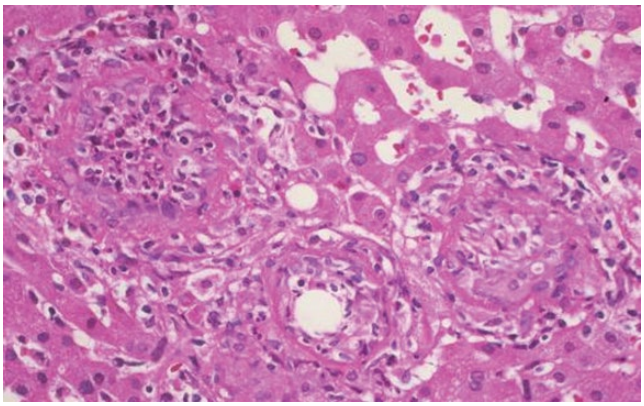


Figure 17.7 Liver biopsy showing classical fibrin ring granulomas in a patient with Q fever. These are typical but not specific for Q fever. (Courtesy of www.pathologypics.com/PictView.aspx?ID=545; last accessed July 2016.)

fever or those with associated endocarditis require combination therapy (doxycycline and hydroxchloroquine) for much longer. Other treatments requiring fluoroquinolones have also been used.

Prognosis

Treatment of the acute illness requires careful monitoring of serology and decrease in IgG titers. There is a risk of progression to chronic Q fever; frequent review and assessment is needed. Screening for endocarditis should be done in slow to respond cases and treatment options (combination treatment and duration) reviewed.

Parasites

Malaria is an important worldwide parasitic infection involving the liver. This is covered in Chapter 34.

Toxoplasmosis

Toxoplasma gondii is a protozoan parasite whose animal reservoir is the cat. Excretion of cysts by cats contaminates the soil and leads to infection in other animals, particularly sheep. Infection in humans occurs following ingestion of meat contaminated by *Toxoplasma* cysts, or due to prenatal acquisition (see Chapter 8).

The majority of acquired infections are asymptomatic and unrecognized. Symptomatic acute infection is manifest by fever, fatigue, and lymphadenopathy, resembling infectious mononucleosis, and may be accompanied by hepatitis. Lymph node biopsy may be characteristic and may permit isolation of the parasite.

Recovery can be prolonged over several weeks but is usually complete.

Treatment is only indicated in the immunocompetent with acquired infection if there is clinically overt visceral involvement or severe or persistent symptoms [16]. A good response may be seen with pyrimethamine and sulfadiazine. The optimal duration of therapy is not determined, but should be from 1 to 4 months, until symptoms resolve. Folinic acid (5–10 mg every 1–2 days) should be given to prevent significant bone marrow suppression occurring due to pyrimethamine.

Helminthic infections

Helminths, or parasitic worms, lead to a wide range of liver disease, the type and severity of which depends not only on the type of worm but also on the intensity of the infection and the host response. Simultaneous infection with more than one type of worm may occur. Children are particularly at risk, as infection may occur following close contact with infected animals, ingestion of infected soil, or contaminated food.

Echinococcus multilocularis (alveolar echinococcosis) and *E. granulosus* (hydatid disease)

The primary host for the adult *Echinococcus* tapeworm is the dog. Ova are excreted in feces, and although sheep are the usual intermediate host, people may become infected following close contact with infected dogs. In the UK, hydatid disease is most prevalent in Wales. Following ingestion of ova, the embryo develops and penetrates the stomach wall, reaching the liver via the portal venous circulation.

Hydatid disease

In hydatid disease, cysts develop most frequently within the lungs and liver. Hepatic cysts are usually slow growing and lead to asymptomatic hepatomegaly, but may become manifest due to secondary infection or because of their size. Aspiration of the cysts is hazardous due to the risk of dissemination and hypersensitivity to the daughter cysts contained within the fluid-filled parent cysts.

Serological diagnosis is highly sensitive. Treatment is by careful surgical excision if the cysts lead to symptoms, or with daily mebendazole for at least 3 months.

Alveolar echinococcosis

This occurs in people who have swallowed the eggs of the *Echinococcus* tapeworm (Figure 17.8). In alveolar echinococcosis there is liver involvement but this can spread to other organ systems. The cysts resemble a slow-growing tumor, with symptoms due to local pressure and parenchymal infiltration.

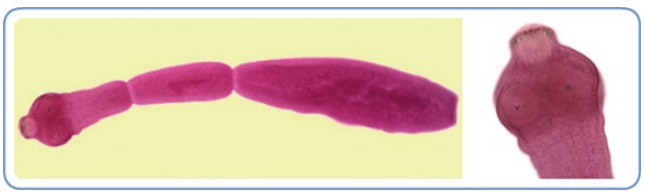


Figure 17.8 The tiny tapeworm *Echinococcus multilocularis* (1.4 mm) that is responsible for alveolar *Echinococcus* disease in humans. (Available at http://www.cdc.gov/parasites/echinococcosis/; last accessed July 2016. Courtesy of Centers for Disease Control and Prevention.)

As growth is slow, clinical symptoms may be delayed for decades, but presentation in childhood is reported and may be associated with immunodeficiency [17]. Metastatic spread to the lung and brain may occur.

Ultrasound reveals a typical solid, heterogeneous mass, which may resemble a malignant lesion, with a necrotic center. If complete surgical excision is not possible, mebendazole 40 mg/kg/day may arrest growth of the lesion.

Ascaris lumbricoides

Ascaris (intestinal round worm) has a worldwide distribution and often may cause no symptoms. Heavy infestation may lead to intestinal obstruction. Migration into the biliary tree, gallbladder, and liver may lead to obstructive jaundice and secondary pyogenic infection with cholangitis and abscess formation [18]. Diagnosis is made following recognition of *Ascaris* eggs or mature worms in infected feces. Treatment is with levamisole (single dose) mebendazole (twice daily for 3 days), or piperazine (single dose) and is effective in 90% of cases.

Toxocara canis and *T. catis*

Adult worms of *Toxocara* are found in the intestine of dogs (*T. canis*) or cats (*T. catis*). Infection in people follows ingestion of ova due to food contaminated by infected feces. Larvae develop in the small intestine, invade the portal circulation and lead to tissue damage with granulomata in the liver and other organs. Infection is characterized by fever, hepatosplenomegaly, and eosinophilia: "visceral larva migrans." Diagnosis is presumptive or by serology. Treatment is with thiabendazole 25 mg/kg/day for 5 days or diethylcarbamazine 6 mg/kg/day for 21days [19].

Schistosoma mansoni (Middle East and Africa) and *S. japonicum* (Far East)

The ova of these flukes infect snails, emerge in water as cercariae which then gain access to man as the intermediate host by penetrating the skin. Following invasion into the circulation, ova may embolize to the liver, become impacted in presinusoidal portal veins and give rise to a granulomatous hepatitis with progressive fibrosis and portal hypertension. Diagnosis is made by detection of the ova in stools or in rectal biopsy material. Treatment is a 1 day course of praziquantel, two doses given for *S. mansoni*, and three for *S. japonicum* [20].

Fasciola hepatica

This sheep liver fluke inhabits large bile ducts. The eggs, after excretion in feces, hatch in water and infect snails. Cercariae emerge from the snail and thus contaminate water and vegetation such as watercress. Human infection is common where watercress is eaten.

Following ingestion, *F. hepatica* may invade the biliary tree by migration through the gastrointestinal mucosa, peritoneal cavity, and hepatic parenchyma. This may be accompanied by fever, tender hepatic enlargement, anorexia, nausea and vomiting, and with allergic symptoms, urticarial, and eosinophilia. Severe infection may lead to biliary tract involvement including hyperplasia, necrosis, dilatation, and inflammation.

Diagnosis is by recognition of the ova in infected feces. Serological tests are also of value. Treatment is with bithionol 30–50 mg/kg given on alternate days for a total of 10–15 doses [20] as it is notoriously difficult to treat and failure with other anti-helminths such as praziquantel have occurred. Recovery is usually complete.

Fungi

The liver is often involved in deep fungal infections either through invasion across the gut wall in patients with impaired gastrointestinal mucosa, or through hematogenous spread causing local seeding in the liver. *Candida* and *Aspergillus* are mostly seen in immunocompromised children. In contrast histoplasmosis and *Cryptococcus* can be seen in both immunocompetent and immunocompromised.

Candida

Candidal infection of the liver and spleen usually occurs in immunocompromised infection, particularly neutropenic children who are receiving chemotherapy. Invasive *Candida* infection can also occur as a complication post-liver transplantation. *Candida* spp. accounts for more than half of all fungal infections post-transplantation, with *Candida albicans* being the most commonly isolated species [21]. Many centers will use prophylaxis against invasive *Candida* peritransplantation.

Clinical features

These are dependent on whether the route of spread is via the gut or hematogenous. Post-transplantation the route is thought to be via the gut, although this may then lead to systemic fungemia. Symptoms can often be insidious particularly in immunocompromised patients but include:

- Fever.
- Abdominal pain.
- Hepatomegaly.
- Splenomegaly.

There may be intra-abdominal abscesses (Figure 17.9), peritonitis, or episodes of recurrent cholangitis due to biliary strictures.

Invasive *Candida* is also common in neonates and should be considered in any neonate who remains febrile despite treatment with appropriate antibiotics.

Diagnosis

Alkaline phosphatase is usually raised but bilirubin and aminotransferases less so. Imaging is important with CT or MRI being the modality of choice. Ultrasound shows multiple

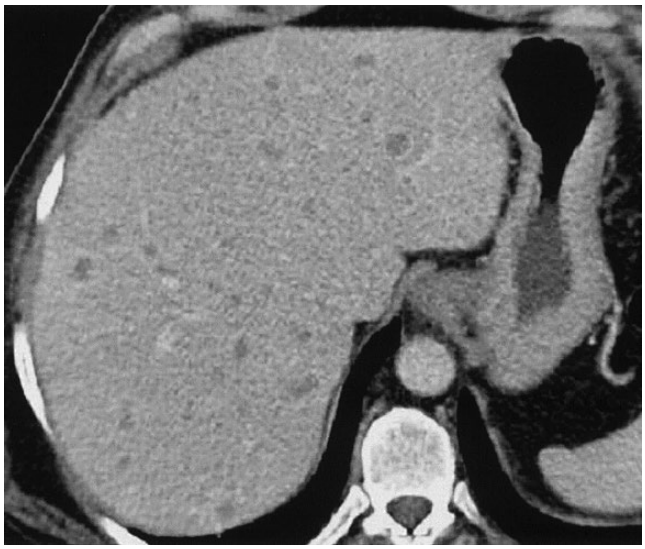


Figure 17.9 At contrast-enhanced CT, candidal microabscesses usually appear as multiple round, discrete areas of low attenuation. (From Berlow 1984 [25]. Reproduced with permission of Wolters Kluwer Health.)

hypoechoic small lesions which is not specific for *Candida* but highly suggestive if the clinical picture also fits. Blood cultures for funghi should be carried out but if there is no organism identified it may be required to obtain liver tissue for a histological and microbiological analysis, although this is difficult (and not appropriate in neonates). Histology will show hepatic necrotic lesions containing pseudohyphae. Lesions develop into granulomas. Lesions may also be culture positive or stain positive for funghi.

Treatment

This may be empiric if definitive diagnosis is very difficult. In neutropenic patients liposomal amphotericin B is the mainstay of treatment. Caspofungin can also be used. In non-neutropenic patients fluconazole (which is also used for prevention) can be used. Treatment may need to continue for several months until radiological resolution of lesions.

Prognosis

Invasive candidiasis carries a high mortality in immunocompromised patients (up to 60%) although improved diagnosis, recognition, and transplant techniques have reduced the incidence.

Aspergillus

Aspergillus infection of the liver is relatively uncommon. There are cases of intra-abdominal *Aspergillus* in children post-liver transplantation. These occur later than *Candida*, usually after the second month post-transplant and the mortality is extremely high. The patients most at risk are those with acute fulminant liver failure prior to transplantation. Patients undergoing transplantation should receive an antifungal that covers *Aspergillus*.

Histoplasma

Histoplasmosis is endemic in parts of the US and can be found worldwide. Many who become infected are asymptomatic, however disseminated disease can occur (more commonly, but not exclusively in immunocompromised patients). *Histoplasma* commonly infects the lung, however in disseminated disease hepatosplenomegaly can occur, particularly in infants. Laboratory findings include anemia, thrombocytopenia, and neutropenia. Jaundice, raised aminotransferases, and raised alkaline phosphatase can occur. Diagnosis is by *Histoplasma* serology and by finding organisms in the affected tissues. Liver histology may reveal granulomas. There is Kupffer cell infiltration, which contain fungus and periportal hepatocyte necrosis. Treatment is with amphotericin B or fluconazole [21].

Cryptococci

Cryptococcal infection is largely opportunistic in immunocompromised patients, although there are reports of disseminated *Cryptococcus* in children with no obvious immune dysfunction. Disseminated *Cryptococcus* can cause hepatitis progressing to long-term liver damage [21]. Histological examination of the liver reveals granulomatous nodules around fungal organisms. Extrahepatic obstruction and cholangitis can also occur. Diagnosis is by identifying the infecting organism using culture, microscopy, immunoassays, and potentially PCR. Treatment is with a broad-spectrum antifungal such as amphotericin B.

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CHAPTER 18

Acute Liver Failure

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Key points

- The causes of pediatric acute liver failure in childhood vary by age.
- Viral hepatitis, especially herpes simplex, is common in newborns, metabolic disease more common in infants and indeterminate hepatitis and paracetamol toxicity more common in teenagers.
- The etiology of pediatric acute liver failure is unknown in 30–50% of cases.
- Poorly regulated inflammatory responses may contribute and/or propagate ongoing hepatic injury in patients with both determined and undetermined etiologies.
- Care of these patients is supportive and should be delivered in the intensive care unit of a transplant center so that consideration for transplant can be anticipated.
- Prognostic models identify acidosis, advanced hepatic encephalopathy, and profound coagulopathy as predictors of poor outcome but are not accurate enough to supersede clinical judgment.

The broadest definition of acute liver failure (ALF) is hepatic necrosis resulting in loss of liver function within weeks or a few months of the onset of clinical liver disease. ALF is the current preferred name for such disease, although other terms – such as "fulminant hepatic failure" and "fulminant hepatitis" – have been used in the medical literature to describe this condition. The narrow definition of ALF that is currently accepted includes the onset of hepatic encephalopathy (HE) and coagulopathy (which defines failure of liver function) within 8 weeks of the onset of liver disease and the absence of pre-existing liver disease in any form. There are several problems with this definition in children. Firstly, some patients with acute hepatocellular disease develop encephalopathy later than 8 weeks into the course of the illness and are defined as having subacute hepatic failure, subacute hepatic necrosis, or late-onset hepatic failure. Secondly, ALF may be the first presentation of a previously unrecognized autoimmune or metabolic liver disease, e.g., Wilson disease or tyrosinemia type I. Thirdly, many cases of hepatic failure in neonates are secondary to an inborn metabolic error or an intrauterine insult, which would represent a pre-existing disease. In addition, encephalopathy may be difficult to detect in infants and small children and may be less severe than coagulopathy. Finally, some important pediatric disorders – such as Reye syndrome and inborn errors of metabolism mimicking Reye syndrome – produce a syndrome similar to ALF in which the encephalopathy is metabolic and secondary to liver failure.

A consensus of the members of the Pediatric Acute Liver Failure (PALF) study group, a multicenter and multinational consortium, resulted in a working definition for ALF that is the summation of clinical and biochemical parameters, as follows:

- The acute onset of liver disease with no known evidence of chronic liver disease.
- Biochemical and/or clinical evidence of severe liver dysfunction:
  - hepatic-based coagulopathy, with a prothrombin time (PT) ≥20 s or international normalized ratio (INR) ≥2.0, that is not corrected by parenteral vitamin K
  - and/or HE (must be present if the PT is 15.0–19.9 s or INR 1.5–1.9, but not if PT ≥20 s or INR ≥2.0)

In the UK, the unique difficulty in detecting encephalopathy in infants means that this is no longer a criterion for super-urgent listing for transplantation.

Etiology

The etiology of ALF is age dependent (Table 18.1). While acute viral hepatitis is the most common identifiable cause in all series, there is a distinct geographic impact on the frequency of

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Table 18.1 Causes of acute hepatic failure in children.

Etiology	Disease	Incidence
Neonates		
Infectious	Herpesviruses, echovirus, adenovirus, HBV	Frequent
Metabolic*	Galactosemia*, tyrosinemia*, neonatal hemochromatosis*, mitochondrial disease	Moderately frequent
Ischemia	Congenital heart disease, cardiac surgery, myocarditis, severe asphyxia	Rare
Older children		
Infectious	HAV, HBV, HEV, herpesviruses, sepsis*, other	Frequent
Drugs	Valproate, isoniazid, paracetamol, carbamazepine, halothane	Moderately frequent
Toxins	*Amanita phalloides*, carbon tetrachloride, phosphorus	Rare
Metabolic*	Hereditary fructose intolerance*, Wilson disease†	Rare
Autoimmune	Hepatitis	Rare
Ischemia	Congenital heart disease, cardiac surgery, myocarditis, severe asphyxia, Budd–Chiari syndrome	Rare
Other	Malignancy	Rare

HAV, hepatitis A virus; HBV, hepatitis B virus; HEV, hepatitis E virus.
* These diseases do not fulfill the definition of ALF.
† Rare under 3 years.

diagnosis, particularly with regard to the frequency with which hepatitis A and B infections are implicated. Table 18.2 details the causes of ALF in the first 418 children enrolled in the PALF study, representing the incidence of causation in 19 pediatric sites in the US, Canada, and the UK.

Infectious disease

Hepatitis virus infection

Early reports of ALF in children suggest that viral hepatitis accounts for the largest proportion of ALF in children of all age groups and infectious illness continues to be a prominent cause in developing countries. However, within the expanded PALF dataset which includes nearly 1000 cases, identified viral infection accounted for only 8% of the overall cases. Viral infection was a much more common cause of liver failure in newborns, 27% infants ≤28 days of age, with the majority being secondary to HSV. Also of note, HSV was identified in almost all age groups within the PALF dataset, with the oldest patient being 16 years [1].

Commonly recognized hepatotropic viruses were less common in PALF with five cases of hepatitis A virus (HAV), three cases of hepatitis B virus (HBV), one case of hepatitis C virus (HCV), and two cases of hepatitis E virus (HEV). The prevalence of HAV among patients of all ages with ALF in published series has varied from as low as 1.5% to as high as 31%. In areas of the world where HAV is endemic, this infection is a frequently diagnosed cause of ALF. Regional outbreaks of HAV causing ALF in unimmunized populations within developed areas have also been reported. In the US, HAV generally causes <5% of cases of ALF.

The prevalence of acute HBV infection in large series of ALF ranges from 25% to 75%, making it the commonest cause worldwide. It is uncommon to document HBV infection in children with ALF from Western Europe and the US, except in infants born to mothers who are positive for HBV and negative for hepatitis B e antigen (HB_eAg), while in endemic areas it plays a much greater role. The prognosis in HBV-related ALF is generally worse than with other etiologies, with spontaneous recovery occurring in fewer than 20% of cases [2]. Fortunately, universal hepatitis B vaccination in endemic areas of the world, such as Taiwan, has resulted in a significant decline in the mortality associated with ALF secondary to HBV. HCV is a very unusual cause for ALF.

HEV has rarely been associated with ALF in children, although hepatitis E infection is common in endemic areas and in returning travelers. Most experience with HEV comes from the Indian subcontinent, where 38% of PALF cases were due to HEV alone or in combination with HAV [3]. The case-fatality rate from ALF approaches 10% among pregnant women, with women in the third trimester particularly at risk. A higher incidence of HEV-associated PALF may be seen if the frequency of serologic testing for HEV increases.

Infection with viruses other than hepatitis viruses

The viruses in the herpes family are highly cytopathic and can cause severe hepatic necrosis, often in the absence of significant inflammation. HSV, varicella-zoster virus, cytomegalovirus, and Epstein–Barr virus (EBV) have been reported to cause ALF, especially in immunocompromised hosts, with EBV most frequently implicated. However, HSV is not as often evaluated as a cause of liver injury in immunocompetent patients beyond the neonatal period. A recent analysis within PALF, which included supplemental testing for viral pathogens, identified 5.6% of patients over the age of 2 years had evidence of new-onset HSV infection [1]. Yet, HSV infection was listed as the cause of liver failure in only two of 13 older subjects. This experience highlights the need to evaluate for herpes virus infection across the entire age spectrum.

As noted above, viral infection, including echovirus (principally type 11), adenovirus, and coxsackievirus, is an

Table 18.2 Final diagnosis in children with acute liver failure in the Pediatric Acute Liver Failure (PALF) study registry.

Diagnosis	Age group		
	<3 years (%)	>3 years (%)	Total (%)
	162 (39)	256 (6)	418
Paracetamol (n = 48)	2 (2)	46 (21)	48 (14)
Indeterminate (n = 169)	68 (54)	101 (46)	169 (49)
Autoimmune (n = 22)	6 (5)	16 (7)	22 (6)
Infectious (n = 20)	9 (7)	11 (5)	20 (6)
• Adenovirus (n = 2)	1 (1)	1 (0)	2 (1)
• Cytomegalovirus (n = 1)	1 (1)	0 (0)	1 (0)
• Epstein–Barr virus (n = 6)	1 (1)	5 (2)	6 (2)
• Enterovirus (n = 1)	1 (1)	0 (0)	1 (0)
• Hepatitis A (n = 3)	0 (0)	3 (1)	3 (1)
• Hepatitis C (n = 1)	0 (0)	1 (0)	1 (0)
• Herpes simples virus (n = 6)	5 (4)	1 (0)	6 (2)
Non-paracetamol drug-induced liver disease (n = 17)	1 (1)	16 (7)	17 (5)
• Mushroom (n = 2)	0 (0)	2 (1)	2 (1)
• Anesthetic (n = 1)	0 (0)	1 (0)	1 (0)
• Co-trimoxazole (Bactrim®) (n = 1)	0 (0)	1 (0)	1 (0)
• Pemoline (Cylert®) (n = 1)	0 (0)	1 (0)	1 (0)
• Cyclophosphamide (Cytoxan®)/phenytoin (Dilantin®) (n = 1)	0 (0)	1 (0)	1 (0)
• Phenytoin (Dilantin) (n = 1)	0 (0)	1 (0)	1 (0)
• Isoniazid (n = 2)	0 (0)	2 (1)	2 (1)
• Iron (n = 1)	0 (0)	1 (0)	1 (0)
• Methotrexate (n = 1)	0 (0)	1 (0)	1 (0)
• Minocycline (n = 1)	0 (0)	1 (0)	1 (0)
• Pravastatin (n = 1)	0 (0)	1 (0)	1 (0)
• Valproate (n = 3)	1 (1)	2 (1)	3 (1)
Metabolic (n = 36)	23 (18)	13 (6)	36 (10)
• α_1-Antitrypsin (n = 1)	1 (1)	0 (0)	1 (0)
• Fatty acid oxidation defect (n = 4)	4 (3)	0 (0)	4 (1)
• Galactosemia (n = 2)	2 (2)	0 (0)	2 (1)
• Fructose intolerance (n = 1)	1 (1)	0 (0)	1 (0)
• Mitochondrial disorder (n = 4)	2 (2)	2 (1)	4 (1)
• Niemann–Pick type C (n = 1)	1 (1)	0 (0)	1 (0)
• Respiratory chain defect (n = 7)	7 (6)	0 (0)	7 (2)
• Reye syndrome (n = 1)	0 (0)	1 (0)	1 (0)
• Tyrosinemia (n = 4)	4 (3)	0 (0)	4 (1)
• Urea cycle defect (n = 2)	1 (1)	1 (0)	2 (1)
• Wilson disease (n = 9)	0 (0)	9 (4)	9 (3)
Other (n = 20)	11 (9)	9 (4)	20 (6)
• Budd–Chiari (n = 2)	0 (0)	2 (1)	2 (1)
• Hemophagocytic syndrome (n = 4)	2 (2)	2 (1)	4 (1)
• Leukemia (n = 2)	1 (1)	1 (0)	2 (1)
• Gestational alloimmune liver disease (n = 6)	6 (5)	0 (0)	6 (2)

important cause of ALF in the neonate. These infections typically include multisystem involvement and are associated with high mortality [4]. Overwhelming viral infection is a contraindication to liver transplantation and thus those who survive must do so with supportive care alone. Infants that survive spontaneously do not develop chronic liver disease, although follow-up liver biopsy is rarely performed, physical evidence does not suggest cirrhosis.

Non-viral infectious hepatitis

Infectious agents other than viruses rarely lead to ALF. These include: congenital syphilis, leptospirosis, and, in endemic areas, *Coxiella burnetii* (Q fever), *Plasmodium falciparum*, and *Entamoeba histolytica*. Systemic sepsis may occasionally present as ALF.

Gestational alloimmune liver disease

Gestational alloimmune liver disease (GALD), formerly termed neonatal hemochromatosis results from an intrauterine alloimmune liver injury (see also Chapter 10). Maternal immunoglobulin G appears to activate fetal complement that leads to the formation of membrane attack complex resulting in liver cell injury [5]. The degree of liver injury can be so profound that death from liver failure can occur within the first few weeks of life. Therefore, liver failure associated with GALD is technically a terminal event of a chronic intrauterine liver

disease. However, the phenotype of the family's index case of GALD is one of ALF and thus deserves to be included in this section for clinical purposes.

Characteristic clinical features include refractory hypoglycemia, severe coagulopathy, hypoalbuminemia, elevated serum ferritin (>1000), and ascites. Strikingly, serum aminotransferase levels are normal or near normal and should alert the clinician to the possibility of this diagnosis. Extrahepatic iron deposition is a hallmark finding. Hemosiderin deposition in the minor salivary glands obtained by a buccal mucosal biopsy is often seen. Alternatively, magnetic resonance imaging (MRI) of the abdomen would suggest the diagnosis of GALD with the finding of reduced T2-weighted intensity of the liver and/or the pancreas relative to the spleen. Exchange transfusion and high-dose intravenous immunoglobulin is the preferred treatment for GALD.

Drug and toxin-related hepatic injury

After viral hepatitis, liver injury due to drugs and toxins is the most common etiology of ALF in children and adults. Box 18.1 lists the drugs and toxins that have been associated with ALF in children, grouped according to their mechanism of action. The three most common drugs implicated in children are paracetamol, isoniazid, and propylthiouracil. Twelve percent of cases in the PALF study were the result of paracetamol overdose. Paracetamol may also be responsible for some cases of ALF in which a cause is not readily identified. The role of fasting and alcohol consumption in potentiating paracetamol toxicity in patients exposed to high doses (60–100 mg/kg) remains controversial, but measurement of serum paracetamol protein adducts, a new biomarker of paracetamol hepatotoxicity in patients with ALF may help unravel this question [6]. Patients with hepatic injury secondary to paracetamol ingestion have a higher rate of recovery than patients with viral hepatitis and should be observed as long as possible before liver transplantation is considered. Drugs that cause steatosis (sodium valproate, amiodarone) may cause liver failure. The complex chemotherapy that is used to treat childhood cancer may occasionally result in hepatic failure.

Box 18.1 Drugs and toxins associated with acute liver failure.

Hepatotoxic agents*

- Paracetamol overdose
- Chlorinated hydrocarbons
- *Amanita* spp.
- Salicylate (overdose)
- $FeSO_4$ (overdose)
- 2-Nitropropane
- Yellow phosphorus
- Solvents

Drugs associated with idiosyncratic reactions

- Isoniazid
- Propylthiouracil
- Sodium valproate
- Halothane
- Amiodarone
- Non-steroidal anti-inflammatory agents
- Tetracycline
- Carbamazepine
- Lamotrigene

Recreational drugs associated with hepatic injury

- Cocaine
- Ecstasy

* Listed in approximate order of frequency as causes of acute liver failure in children. Mechanism of action according to Arundel and Lewis.

Autoimmune hepatitis

Autoimmune hepatitis is a common cause of liver failure in patients referred for liver transplantation. Children with both type I and type II autoimmune hepatitis have been reported with ALF, although it is more common in type II. In the PALF study, autoimmune hepatitis accounted for 7% of pediatric patients registered. Many of these patients will respond to medical therapy (corticosteroid and azathioprine), avoiding the need for transplantation. Liver biopsy shows signs of chronic hepatitis (portal fibrosis and interface hepatitis), in addition to severe lobular hepatitis. The majority of patients have titers of antinuclear, antismooth muscle, or anti-liver–kidney microsomal antibodies in serum and elevated immunoglobulin G (IgG).

Inherited and metabolic diseases

In the PALF study, metabolic disease – including α_1-antitrypsin deficiency and Wilson disease – accounted for 10% of pediatric patients registered. The metabolic disorders that present in the neonatal period or infancy with hepatic failure are galactosemia, hereditary fructose intolerance, and tyrosinemia type I. A recent report highlights the less frequent but recognized presentation of disorders of the urea cycle, especially ornithine transcarbamalase deficiency OTC as ALF. Inborn errors of bile acid synthesis can rarely present as ALF in infancy. Zellweger disease and Alpers disease cause cerebral degeneration and disordered hepatic function, and may present with ALF and be confused with primary hepatic failure if the neurological symptoms characteristic of these disorders are not obvious. Disorders of fatty acid oxidation and of oxidative phosphorylation produce episodes of recurrent hepatic dysfunction and coma that can be confused with Reye syndrome or severe hepatitis at any age. Wilson disease is most likely to produce ALF in the older child.

Other causes of acute hepatic failure in children are listed in Table 18.1.

Indeterminate hepatitis

Hepatitis of indeterminate cause is diagnosed when there is evidence of acute hepatitis in the absence of markers for hepatitis virus infection, the absence of clinical and/or serological evidence of systemic infection with other infectious agents, no exposure to drugs or toxins, and negative markers of autoimmune disease. It is the most important cause of ALF in children in Western developed countries, comprising 25–40% of PALF cases in series from Western Europe and the US [7,8]. In the PALF series, 444 of 863 non-paracetamol cases were attributed to acute hepatitis of indeterminate cause. It is

possible that some cases are classified as indeterminate cause because a complete diagnostic evaluation has not been performed. Review of the diagnostic tests performed on patients classified as indeterminate within the PALF network revealed that for a significant proportion of patients a full evaluation was not completed. Most important among these omissions might be evaluation for autoimmune-mediated liver injury, a potentially treatable disease. Work-up for metabolic diseases was also deemed inadequate in up to 55% of cases, even when expectations were tailored by patient age [9]. Thus, the true percentage of cases in which a diagnosis cannot established may not be as high as previously thought. However, an important subset of this patient group do have liver injury that eludes current diagnostic criteria.

It has also been proposed that acquired defects in immunoregulation, distinct from autoimmune hepatitis, and sometimes associated acute viral infections play a central role in initiating or propagating the liver injury. Evidence of immune activation, as manifested by high levels of serum soluble interleukin 2 receptor (sIL-2R) was identified in approximately half of the patients with an undetermined diagnosis in the PALF Network [10]. Patients with indeterminate PALF who had the highest sIL-2R levels were more likely to need for liver transplantation or have a risk of death within 3 weeks.

Serum from the PALF biorepository was assayed for a panel of 26 of inflammatory mediators to identify biomarker patterns that could predict the probability of spontaneous recovery. Although raw inflammatory mediator levels assessed over time did not predict outcomes, dynamic network analysis revealed distinct inflammatory networks that distinguished spontaneous survivors from those who died [11]. It is possible that some causes of PALF are the result of untethered inflammatory responses, initiated by a variety of lessor insults which propagate ongoing injury or cause direct liver injury at a stage when the primary insult is resolving.

The overall prognosis in indeterminate ALF is poor, with a rate of spontaneous recovery ranging from 5% to 40%, indicating the need for early referral to a liver transplant center. Ongoing research efforts are aimed at establishing better approaches to diagnostic evaluation and at exploring and manipulating immune mechanisms to dampen the inflammatory response and augment regeneration.

Pathology

The pathological features of ALF differ depending on the etiology. There are three basic lesions.

Hepatic necrosis

Severe hepatitis with loss of lobular architecture, secondary to extensive hepatocyte necrosis with collapse of the reticulin framework, characterizes the pathological lesion seen in either viral infection or an idiosyncratic drug reaction (Figure 18.1). In viral hepatitis, necrosis tends to be pan-acinar in distribution, while in toxic injury it is zonal (Figure 18.2). There may be diffuse necrosis of individual hepatocytes, or pericentral sublobular necrosis. Most ALF is associated with massive confluent necrosis. In many cases, it is difficult to identify any remaining viable hepatocytes. The reticulin framework of the lobule is collapsed, and the mass of the liver is small. A moderate acute inflammatory infiltrate may be evident, and this may be helpful in establishing the etiology. In HBV hepatitis, there is a minimal inflammatory infiltrate, while in EBV fulminant hepatitis, centrilobular necrosis with bridging and collapse may be obvious, with cholestasis and lymphoid "blast cells." In some cases, no evidence of regeneration can be found, while in others there is a proliferation of duct-like structures that probably results from attempts at regeneration. The degree and pattern of necrosis do not correlate with the development of encephalopathy or cerebral edema.

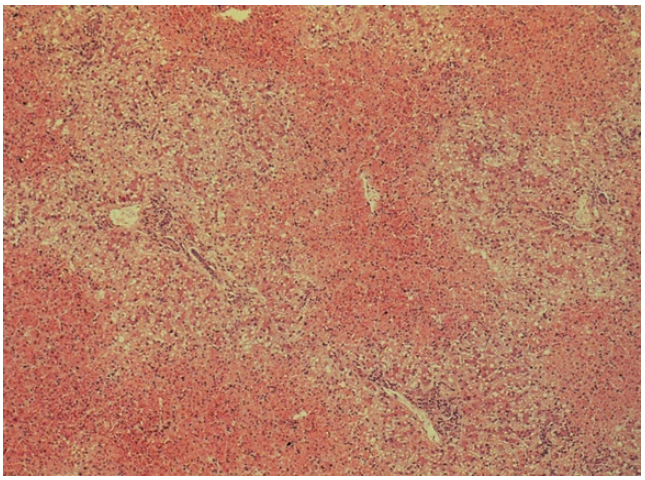


Figure 18.1 Liver histology is usually obtained postmortem or at transplantation. There is usually severe hepatic necrosis with reticulin collapse and biliary proliferation, irrespective of etiology.

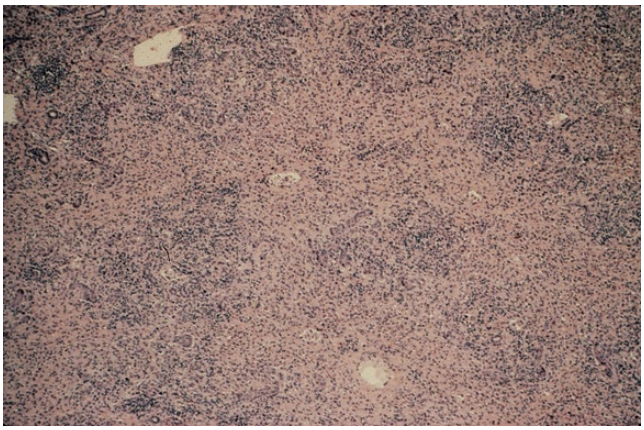


Figure 18.2 Acute liver failure secondary to viral hepatitis may show significant lobular inflammation, which may differentiate it from drug toxicity.

Hepatocellular degeneration

In ALF due to metabolic or toxic injuries in children, the prominent lesion is hepatocellular degeneration with diffuse fatty infiltration of hepatocytes. There is minimal hepatocyte necrosis or inflammatory infiltrate. In Reye syndrome and similar disorders, the intracellular fat is microvesicular and does not displace the nuclei. This lesion is seen in association with toxic injury (valproic acid, aspirin) and inborn errors of metabolism (disorders of fatty acid oxidation). Rarely, macrovesicular steatosis is seen with drug and toxic injury (hydrocarbon ingestion, amiodarone therapy). The absence of cell necrosis in association with failure of liver function implies organelle failure as the etiology. The hepatic mass is usually increased, and hepatomegaly is evident. Serum aminotransferase levels are usually elevated, but only to a mild to moderate degree (usually <400 IU/L), indicating that hepatocytes generally remain intact. Jaundice is minimal (serum bilirubin usually <200 μmol/L), which suggests that some organelle function remains intact and also that bilirubin production is probably not increased. Full histological recovery is the rule if the patient survives.

Patients with ALF due to hereditary fructose intolerance, acute-onset tyrosinemia type I, and – to a lesser degree – galactosemia have a lesion characterized by diffuse swelling of hepatocytes with condensation of organelles and cytoplasmic elements. Hepatocyte necrosis is spotty and usually not prominent. Macrovesicular fat with displacement of nuclei is seen in a variable proportion of hepatocytes, sometimes a majority. This lesion suggests organelle injury, probably resulting from chemical alteration of macromolecules that is severe enough to cause the death of some hepatocytes. Aminotransferase levels and serum bilirubin levels are moderately elevated. Full histological recovery is the rule if the metabolic injury can be controlled.

Underlying cirrhosis

In ALF due to either tyrosinemia type I or Wilson disease, the pathological features will include pre-existing cirrhosis.

Recovery

Spontaneous recovery from ALF is usually associated with complete histological recovery, even when extensive necrosis is present. Recovery from massive confluent necrosis is distinctly unusual, but when it occurs, postnecrotic cirrhosis often remains.

Biochemistry

The serum aminotransferase levels (alanine aminotransferase (ALT), aspartate aminotransferase (AST)) are usually markedly elevated in children with ALF. Levels are almost always above 1000 IU/L and may reach values above 10,000. Rapidly falling aminotransferase values signify "exhaustion" of the hepatocyte mass and terminal hepatic failure, unless associated with evidence of functional recovery such as improved coagulation and reduced encephalopathy.

Marked jaundice is typically seen with severe hepatic necrosis. Serum bilirubin concentrations typically range from 200 to 1200 μmol/L. The rate of increase in serum bilirubin often exceeds that expected with a normal rate of production and zero clearance. Increased production may result from catabolism of hepatic heme proteins or from hemolysis. Early in the course, most of the serum bilirubin is in the conjugated form, indicating excretory dysfunction of viable hepatocytes. Later, most of the bilirubin may be unconjugated, indicating loss of conjugating ability. Children with paracetamol poisoning, fulminant hepatitis secondary to hepatitis B, and metabolic disease may be anicteric or only mildly jaundiced.

Pathogenesis

ALF leads to multisystem organ failure, particularly affecting the brain and kidney. The process leading to hepatic injury is not known, but it is multifactorial and dependent on the balance between the following factors:

- Susceptibility of the host – e.g., neonate who develops fulminant HBV.
- Severity and nature of hepatic injury – e.g., dose of paracetamol.
- The ability of the liver to regenerate.

Liver regeneration

The ability of the liver to regenerate is a crucial factor for survival. Many patients who die with ALF show some evidence of hepatic regeneration, while in others there is no sign of regeneration. The cause and mechanism of hepatocyte injury may also impact the pace of regeneration as patients with ALF due to paracetamol poisoning or hepatitis A have a better prognosis than those with indeterminate hepatitis.

Encephalopathy

Encephalopathy is a unique feature of ALF that occurs in the majority of children with ALF and it is well recognized that encephalopathy is not always clinically apparent in infants and younger children. It results from an indirect effect of hepatocyte failure on the function of the brain, although the neuropharmacological events that lead to clinical HE are complex and not yet completely understood. In ALF, the presumption is that hepatocyte dysfunction has progressed to a point following which the liver fails to produce appropriate amounts of neuroregulatory substances, and/or fails to eliminate neurotoxins, resulting in brain dysfunction. Over the years, there have been many candidates for potential neurotransmitters or neurotoxins; these have included ammonia, glutamine, short-chain fatty acids, amino acids, mercaptans, and octopamine, and γ-aminobutyric acid (GABA). No neuropathological abnormalities associated with acute HE are considered irreversible. Cerebral edema is a separate entity that complicates ALF and may be reversible in the early stages only.

Clinical manifestations

Clinical presentation

The clinical presentation varies depending on the etiology, but essentially there is hepatic dysfunction with hypoglycemia, coagulopathy, and encephalopathy. Jaundice may be a late feature, particularly in metabolic disease. The clinical onset may be within hours or weeks. Most pediatric patients who develop ALF are previously healthy, with no history of major medical problems and no clear exposure to hepatitis or toxins.

All forms of viral hepatitis have similar clinical features. There may be a prodromal illness, with a "flu-like" syndrome of malaise, myalgia, nausea, vomiting, and diarrhea, and subsequent jaundice. While these findings may be typical of acute viral hepatitis, they are not specific for a viral etiology. The disease may progress rapidly at this stage, or deterioration may occur after a period of improvement.

Warning signs of progressive disease are:

- Prolonged PT that is unresponsive to vitamin K (particularly at presentation).
- Persistent jaundice, with a rapid increase in bilirubin in association with a progressive decline in serum aminotransferase levels.
- Decreasing liver size.
- Increasing lethargy or occasionally hallucinations.
- Rarely, hemorrhagic diathesis and systemic collapse.

By the time the disease is established, the patient is deeply jaundiced and fetor hepaticus is often evident. Features of encephalopathy such as drowsiness, confusion, aggression, incontinence, and lack of response to painful stimuli are common. The liver size may be large, normal, or small, depending on the stage of the disease. The patient may bleed from needle puncture sites, the nose, or the gastrointestinal tract.

Laboratory evaluation will demonstrate:

- Marked conjugated hyperbilirubinemia. Occasional exceptions are observed – as in some cases of drug-induced hepatitis, fulminant hepatitis B, and in idiopathic anicteric fulminant failure.
- Aminotransferases (ALT, AST) may be very high (>1000 IU/L), or may have fallen precipitously since their last measurement (in concert with a decreasing liver size, reflecting severe necrosis and collapse of hepatic mass).
- Plasma ammonia is usually elevated by two to eight times (>100 μmol/L).
- Serum creatinine may be elevated secondary to renal complications, while the urea may be high (renal dysfunction, increased production from blood in the gastrointestinal tract, dehydration) or low (failure of hepatic synthesis).
- Hypoglycemia may be present and difficult to correct.
- Arterial blood gas analysis may show a wide spectrum of abnormalities, ranging from respiratory alkalosis to mixed respiratory and metabolic acidosis, usually in association with hypoxemia.
- Electrolyte abnormalities are associated with vomiting and dehydration.
- Coagulation profiles demonstrate deficiencies of clotting factors and often evidence of consumptive coagulopathy.
- The platelet count is often reduced, due to consumption or reduced production (aplastic anemia occurs in 10–20% of cases of indeterminate hepatitis).
- The white blood cell count varies from high (stress response, secondary bacterial infection) to low (aplastic anemia).

Diagnosis

The diagnosis is established by the combination of clinical and biochemical features and specific diagnostic tests (Table 18.3). A histological diagnosis by liver biopsy may guide patient management if a potentially treatable cause, such as immune-mediated liver injury, is identified. However, liver biopsy should be approached with caution since abnormal coagulation can increase the risk of post-biopsy bleeding. Transjugular biopsy to reduce this risk is technically possible in children and larger infants.

Management

There is no specific therapy for ALF except hepatic replacement. Management is therefore directed towards early consideration for liver transplantation, hepatic support, and the prevention and treatment of complications, while awaiting recovery or a suitable donor for liver transplantation. The key elements are medical support in the setting of an intensive care unit and rapid referral to a transplant center.

Table 18.3 Clinical stages of hepatic encephalopathy. (Adapted from Trey & Davidson 1970 [14]. Reproduced with permission from Elsevier.)

Stage	Asterixis	EEG changes	Clinical manifestations
I (prodrome)	Slight	Minimal	Mild intellectual impairment, disturbed sleep–wake cycle
II (impending)	Easily elicited	Usually generalized slowing of rhythm	Drowsiness, confusion, coma, inappropriate behavior, disorientation, mood swings
III (stupor)	Present if patient cooperative	Grossly abnormal slowing	Drowsy, unresponsive to verbal commands, markedly confused, delirious, hyperreflexia, (+) Babinski sign
IV (coma)	Usually absent	Appearance of delta waves, decreased amplitudes	Unconscious, decerebrate or decorticate response to pain present (IVA) or absent (IVB)

It is essential to take a full history from the parents, which would include establishing appropriate risk factors such as information on intravenous injections, infusions of blood products, drugs, foreign travel, or contact with jaundice. It is important to establish which medications family members are taking, and in adolescents to enquire about recreational drug exposure and sexual contact. Over-the-counter medications, particularly those containing paracetamol, should be queried specifically, as the family may not consider these to be significant enough to volunteer the information that they are being used. The use of herbal teas and availability of potentially toxic mushrooms should be included in the history.

The initial physical examination should establish hepatic, cerebral, cardiovascular, respiratory, renal, and acid–base status. The patient's conscious state and degree of coma should be established and a complete central nervous system examination performed.

Evidence of chronic liver disease or other signs that may indicate etiology, such as Kayser–Fleischer rings, cataracts, and needle marks, should be established. Liver size should be measured and marked on the abdomen.

The presence of impaired central nervous system function with acute liver disease is an indication for immediate hospitalization, independent of any other clinical or biochemical findings.

General measures

Management should be in an intensive care unit setting with routine intensive care monitoring. Until a diagnosis is made, it is assumed that all children are infectious and that all blood, excretions, and secretions are potentially capable of transmitting viral hepatitis. Enteric isolation procedures must be enforced (Box 18.3).

A central venous catheter is useful for the assessment of central venous pressure and volume status, but may require surgical placement with coagulation support. An indwelling arterial line for continuous measurement of blood pressure and for biochemical and acid–base monitoring is essential. A nasogastric tube is passed and placed to gravity, with regular gentle saline lavage to detect upper gastrointestinal hemorrhage. The urinary bladder is catheterized and strict output records are maintained to help in the evaluation of fluid status and renal function.

Baseline biochemical and other investigations should be performed (see Box 18.2) and management instigated as in Box 18.3. Frequency of monitoring will depend on the severity of illness, ranging from daily in mild cases to 4-hourly to 6-hourly in patients in stage III and IV coma, and should include:

- Complete blood count.
- Blood gases and electrolytes.
- Aminotransferases.
- PT.
- Daily monitoring of plasma creatinine, bilirubin, and ammonia.

Box 18.2 Investigations in acute severe hepatitis.

Baseline essential investigations

- Biochemistry
  - Bilirubin, transaminases
  - Alkaline phosphatase
  - Albumin
  - Urea and electrolytes
  - Creatinine
  - Calcium, phosphate
  - Ammonia
  - Acid–base, lactate
  - Glucose
- Hematology
  - Full blood count, platelets
  - PT, PTT
  - Factors V or VII
  - Blood group cross-match
- Septic screen
  - Omitting lumbar puncture
- Imaging
  - Chest radiograph
  - Abdominal ultrasound
  - Head CT scan or MRI
- Neurophysiology
  - EEG

Diagnostic investigations

- Serum
  - Paracetamol levels
  - Cu, ceruloplasmin (>3 years)
  - Autoantibodies
  - Immunoglobulins
  - Amino acids
  - Hepatitis A, B, C, E
  - EBV, CMV, HSV
  - *Leptospira* (if clinically relevant)
  - Other viruses
- Urine
  - Toxic metabolites
  - Amino acids, succinylacetone
  - Organic acids
  - Reducing sugars

CMV, cytomegalovirus; CT, computed tomography; EBV, Epstein–Barr virus; EEG, electroencephalography; HSV, herpes simplex virus; MRI, magnetic resonance imaging; PT, prothrombin time; PTT, partial thromboplastin time.

It is useful to take a chest radiograph to diagnose left ventricular failure or aspiration. An abdominal ultrasound may indicate liver size and patency of the hepatic and portal veins, particularly if liver transplantation is being considered.

Fluid balance

The aim of fluid balance is to maintain hydration and renal function while not provoking cerebral edema. Maintenance fluids consist of 10% dextrose in 0.45 or 0.90% NaCl solution with infusion rates equaling 80% of normal maintenance

Box 18.3 Management of acute liver failure.

- No sedation except for procedures
- Minimal handling
- Monitor
  - heart and respiratory rate
  - arterial BP, CVP
  - core/toe temperature
  - neurological observations
  - gastric pH (>5.0)
  - blood glucose (>4 mmol/L)
  - acid–base
  - electrolytes
  - PT, PTT
- Fluid balance
  - 80% maintenance
  - dextrose 10–50%
  - sodium (0.5–1.0 mmol/L)
  - potassium (2–4 mmol/L)
- Coagulation support only if required
- Drugs
  - vitamin K
  - H_2-antagonist
  - antacids
  - lactulose
  - *N*-acetylcysteine (only for paracetamol poisoning)
  - broad-spectrum antibiotics
  - antifungals
- Nutrition
  - enteral feeding (1–2 g protein/day)
  - parenteral nutrition if ventilated

BP, blood pressure; CVP, central venous pressure; FFP, fresh frozen plasma; PN, parenteral nutrition; PT, prothrombin time; PTT, partial thromboplastin time.

requirements is usually adequate and may also limit risk of cerebral edema. Potassium requirements may be large, 3–6 mmol/kg/day, as guided by the serum concentration. As patients may become hypophosphatemic, intravenous phosphate may be given as potassium phosphate.

Attempts should be made to maintain urinary output using loop diuretics in large doses (frusemide at 1–3 mg/kg every 6 h) and colloid/fresh frozen plasma (FFP) to maintain renal perfusion. Should profound oliguria occur, consideration should be given to hemofiltration or dialysis.

Anemia should be corrected, maintaining the hemoglobin concentration above 10 g/dL to provide maximum oxygen delivery to tissues. Coagulopathy should be managed conservatively; with FFP infusions used sparingly in the setting of invasive procedures or significant spontaneous bleeding.

Other therapy

It is usual to prescribe vitamin K (2–10 mg i.v.), although it is rarely effective. H_2-antagonists and antacids (see later) should be administered prophylactically to prevent gastrointestinal hemorrhage from stress erosions. The efficacy of *N*-acetylcysteine (NAC) in the management of ALF not associated with paracetamol toxicity was tested in a recent multicenter, randomized, placebo-controlled study, supported by the National Institutes of Health. In that analysis of 184 children with ALF, patients that received NAC were not more likely to survive and a younger subgroup of the treated patients had lower 1-year survival with native liver than patients receiving placebo [12].

Antibiotic therapy

The results of surveillance cultures can be used to guide antibiotic therapy in the event of suspected infection, but broad-spectrum antibiotics (amoxicillin, cefuroxime, metronidazole, and prophylactic fluconazole) are only prescribed if sepsis is suspected or liver transplantation is anticipated.

Nutritional support

The role of parenteral nutrition in the management of patients with ALF is controversial. The main aims of therapy are:

- To maintain blood glucose (>4 mmol/L) and ensure sufficient carbohydrates for energy metabolism. The glucose infusion rate required to maintain an acceptable serum glucose may vary between patients, but rates as high as 12–15 mg/kg/min can be required. A catheter placed in a central vein will be necessary to deliver such a concentrated glucose solution.
- To reduce protein intake to 1–2 g/kg/day, either enterally or parenterally.
- To provide sufficient energy intake to reverse catabolism, either enterally or parenterally.

Children who are mechanically ventilated should have parenteral nutrition, as it may be 7–10 days before a full normal diet is resumed following transplantation.

Central nervous system monitoring

A baseline electroencephalogram (EEG) is helpful for staging coma and providing information on the prognosis (Figure 18.3). Computed tomography (CT) scans are probably not useful early in encephalopathy, but may provide information on cerebral edema, hemorrhage, or irreversible brain damage later in the disease (Figure 18.4). The role of MRI is evolving and baseline studies performed at the onset of clinical encephalopathy may provide a useful comparison for later studies assessing early cerebral edema. Frequent evaluation of neurological function and blood ammonia is essential to follow the progress of HE. The role of intracranial pressure monitoring remains controversial. All forms of intracranial monitoring are potentially hazardous in patients with severe coagulopathy, but they may provide helpful information on changes in intracranial pressure and improve selection for liver transplantation.

Prevention and management of complications

The clinical course is dominated by the complications of hepatic failure, and therapy should be focussed on their prevention and management.

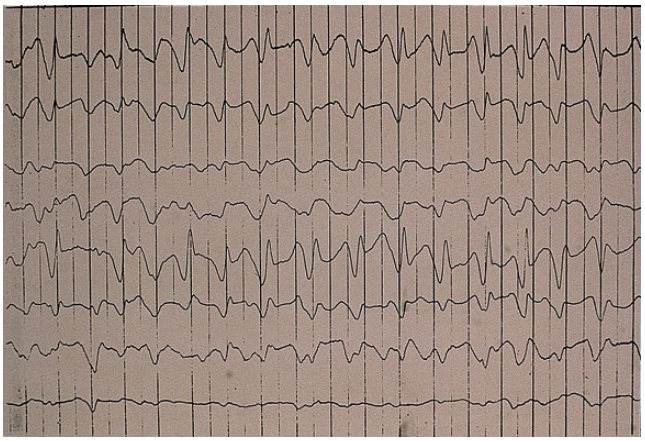
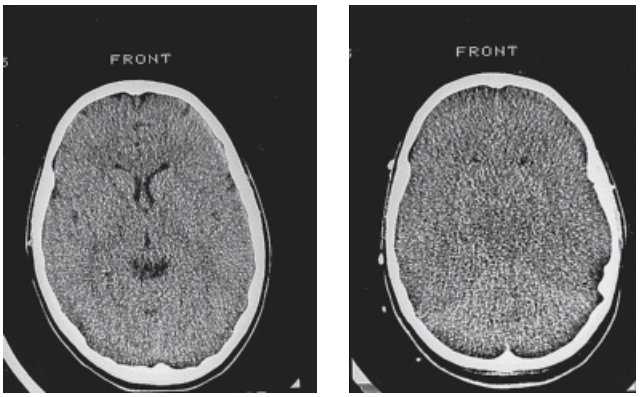


Figure 18.3 Acute hepatic encephalopathy may be difficult to detect in infants or older children. Electroencephalography demonstrates a slow rhythm and reduced amplitude, with characteristic triphasic waves. (From Hussain *et al.* 2014 [13]. Reproduced with permission of Wolters Kluwer Health.)



(A) (B)

Figure 18.4 The rapid development of cerebral edema in acute liver failure is a poor prognostic sign. This is best demonstrated by computed tomography, which shows a reduction in the size of the ventricles (A) and reversal of gray/white matter (B); however, it is a very late sign.

Hypoglycemia

Hypoglycemia (blood glucose <400 mg/L) develops in the majority of children. It may contribute to central nervous system impairment and other organ dysfunction. Factors contributing to hypoglycemia include:

- Failure of hepatic glucose synthesis and release.
- Hyperinsulinemia (due to failure of hepatic degradation).
- Increased glucose utilization (due to anaerobic metabolism).
- Secondary bacterial infection.

Frequent bedside monitoring of blood glucose concentrations (every 2–4 h) and intravenous administration of glucose (10–50% dextrose) are required to prevent this complication. Increased insulin production, secondary to excess glucose infusion, leads to increased glucose need, and can be avoided by permitting blood glucose to remain between 400 and 600 mg/L. Profound refractory hypoglycemia carries a grave prognostic implication and often heralds the imminent death of the patient.

Coagulopathy and hemorrhage

The management of coagulopathy and hemorrhage is a major part of the overall care of the child with ALF. Profound disturbances in hemostasis develop secondary to failure of hepatic synthesis of clotting factors and fibrinolytic factors, reduction in platelet numbers and function, or intravascular coagulation. The coagulation factors synthesized by hepatocytes include factors I (fibrinogen), II (prothrombin), V, VII, IX, and X, and a reduction in synthesis leads to the prolongation of prothrombin and partial thromboplastin time.

The PT is the most clinically useful measure of hepatic synthesis of clotting factors. Prolongation of the PT often precedes other clinical evidence of hepatic failure, such as encephalopathy, and may alert the clinician to the severity of acute hepatitis; it is a guide to the urgency of liver transplantation. Administering vitamin K parenterally ensures a sufficiency of this essential cofactor, but rarely improves coagulation in ALF.

The PT depends on the availability of factor VII, which has a shorter half-life than other factors and decreases more rapidly than other liver-derived clotting factors. As a result, measurement of factor VII may be a more sensitive indicator than the PT. Fibrinogen concentrations are usually normal unless there is also disseminated intravascular coagulation (DIC). The level of factor VIII may help differentiate between DIC and ALF, as it is synthesized by vascular endothelium and therefore in ALF is normal or increased, possibly as an acute-phase response or due to decreased utilization. Decreased levels of factor XIII may contribute to poor clot stabilization.

A reduction in platelet numbers (80×10^9/L) occurs in up to half of adult patients, although thrombocytopenia is less of a problem in pediatric experience. Severe thrombocytopenia, requiring platelet transfusion, suggests hypersplenism, intravascular coagulation, or aplastic anemia. The use of extracorporeal support devices may also contribute.

Intravascular coagulation, as detected by abnormal concentrations of fibrin degradation products, is present in almost all patients, indicating ongoing clot deposition and dissolution, most probably as a consequence of tissue necrosis in the liver. DIC is rarely significant, but can contribute to organ damage. The administration of commercial concentrates containing activated clotting factors may precipitate DIC.

Bleeding from needle puncture sites and line insertion is common, but significant pulmonary and gastrointestinal bleeding are uncommon. Intracranial hemorrhage is a rare, but frequently terminal event. Petechiae reflect decreased platelet function, disturbed vascular integrity, or DIC.

Although in the early stages of assessment prolongation of PT is a sensitive guide to prognosis and the need for liver transplantation, life-threatening bleeding complications may require treatment with FFP, cryoprecipitate, and platelets as needed. It is not necessary to maintain coagulation parameters (PT) in the normal range. In general, mild to

moderate coagulopathy (PT <30 s) requires no therapy except support for procedures. Significant bleeding complications typically occur only in the setting of severe coagulopathy (PT >40 s). Improvement of coagulation status with therapy (10–15 mL/kg of FFP every 6 h) may slow or resolve bleeding, but large volumes of blood products can increase the risk of cerebral edema. Therefore, this approach is reserved for life-threatening bleeding episodes or high-risk invasive procedures. Administration of recombinant factor VII (80 μg/kg) reliably corrects the coagulation defect in patients with ALF for a period of 6–12 h and may be useful in preparation for invasive procedures. Double-volume exchange transfusion or plasmapheresis can be used to temporarily improve coagulation, DIC and control hemorrhage. Hemofiltration may be necessary to control fluid balance if much coagulation support is required. Platelet counts should be maintained above 50×10^9/L by infusion of platelets. DIC is rarely severe enough to require heparin infusion.

Prevention of gastrointestinal hemorrhage

Gastrointestinal tract hemorrhage may be life threatening due to gastritis or stress ulceration. High-dose H_2-antagonists (ranitidine 1–3 mg/kg 8-hourly) or proton-pump inhibitors (omeprazole 10–20 mg/kg/day, pantoprazole 25 mg/m^2/day) should be administered intravenously, and sucralfate (1–2 g 4-hourly) may be given by nasogastric tube to reduce upper gastrointestinal tract bleeding.

Encephalopathy

Most children with ALF present with some signs of brain dysfunction. HE may be exacerbated by sepsis, gastrointestinal bleeding, electrolyte disturbances, or sedation, particularly benzodiazepine administration. Clinical manifestations and progression of HE are highly variable, but acute HE usually evolves over days through definable stages. It may progress rapidly, with coma developing within hours of the earliest detectable signs.

A scale for grading clinical encephalopathy is presented in Table 18.3. This scale is useful for assessing encephalopathy in older patients, but has less value in assessing neonates and infants, particularly in the early stages of encephalopathy. Although alterations in the EEG are not specific, the EEG is useful for monitoring progression in hepatic encephalopathy (see Table 18.3).

The earliest abnormalities may not be detectable by clinical assessment, but are apparent to family members. These include:

- Personality changes – reflecting forebrain dysfunction – include regression, irritability, apathy, and occasionally euphoria. Younger children are more likely to be irritable and apathetic.
- Sleep disturbances, such as insomnia or sleep inversion, are often observed. Intellectual deterioration, observed in stage I of chronic HE, is usually not evident in acute encephalopathy.
- Constructional apraxia related to disturbed spatial recognition may be present. Simple age-related tasks may be clinically useful tools for the day-to-day assessment of inattentiveness and apraxia. Subtraction of serial 7 s, recall of events (such as recently viewed videos), handwriting, and figure drawing are appropriate tasks that older children can be asked to repeat daily in order to assess early encephalopathy.
- Drowsiness and lethargy are readily apparent as the patient progresses into stage II HE.
- Asterixis develops and is a useful sign, but it cannot be elicited with regularity in children less than 8–10 years of age.
- Progressive motor impairment becomes evident, including ataxia, dysarthria, and apraxia. Other neuromotor disturbances that can be detected as patients progress to stage III encephalopathy include hyperreflexia and sustained clonus. EEG abnormalities are detectable at this stage. Infants exhibit increasing irritability and often produce high-pitched, ear-piercing screams. They may refuse to suckle or eat.
- Stage III HE is characterized by deepening somnolence and stupor. The patient is arousable by vigorous physical stimuli, but does not respond to commands. Patients are disoriented and often do not recognize family members. School-aged children and teenagers in deepening stage II and stage III coma often exhibit extreme agitation and rage. Seizures may develop. Neurological findings are more profound (see Table 18.3).
- Progression into stage IV HE is heralded by the onset of coma. The patient responds only to painful stimuli. At first, the patient is flaccid, but in deeper stage IV the patient will assume decerebrate posturing, and brainstem reflexes are lost.

Acute HE is completely reversible after resolution of the hepatic dysfunction. Although the role played by ammonia in the development of encephalopathy is controversial, therapy to reduce ammonia production or accumulation is indicated. The components of therapy are:

- Restriction of dietary protein.
- Enteral antibiotics.
- Enteral lactulose.
- Controlling the complications of ALF that contribute to ammonia accumulation.

In the early stages of HE, conventional measures are taken to minimize the formation of nitrogenous substances by the intestine. A cathartic, such as sodium-free magnesium sulphate and/or a non-absorbable disaccharide (lactulose 1–2 mL/kg every 4–6 h) may be administered orally or via the nasogastric tube. Neomycin (50–100 mg/kg/day) or rifaximin may also be used to prevent ammonia production if diarrhea secondary to lactulose is a problem. Protein intake should be limited to 1–2 g/kg/day and may be administered enterally or parenterally to limit the production of ammonia. Caloric intake is maintained in the early stages with glucose

polymers and supplemented by infusion of 10% dextrose solution, with frequent monitoring of blood glucose.

The older patient with aggressive delirium is at risk for self-injury, as well as being a risk to care providers. Sedation is not usually needed, except in violent patients. Elective ventilation should be considered if the encephalopathy progresses. If sedation is required, either for restraint or during procedures, short-acting barbiturates or opiates can be safely utilized, but benzodiazepines should be avoided. There are potential therapeutic implications related to the GABA receptor, which has been implicated in encephalopathy. Flumazenil, a benzodiazepine antagonist, may produce temporary reversal of HE, but clinical application is limited since the effect is transient.

Many of the complications of ALF, such as gastrointestinal hemorrhage, increase the potential for ammonia accumulation and its consequent neurotoxicity. Measures should be taken to prevent and control hemorrhage. Dehydration, electrolyte, and acid–base disturbances should be corrected, and blood glucose concentration should be maintained by administering a 10–25% glucose solution.

Cerebral edema

Brain death associated with cerebral edema is a frequent cause of death in ALF and contributes to reduced survival after liver transplantation. Every effort should be made to prevent this complication, since the prognosis is poor once it is evident.

Cerebral edema may develop between stage III and stage IV HE and present within hours of the onset of coma. It is heralded by changes in the neurological examination – abnormally reacting or unequal pupils, muscular rigidity and decerebrate posturing, mild clonus and/or focal seizures, and loss of brainstem reflexes. There may be alteration of respiratory pattern, bradycardia, and an increase in blood pressure. It is associated with a rise in intracranial pressure (ICP) >30 mmHg. CT or MRI scans of the brain will show flattening of the gyri and reduction of the size of the ventricles, but they are not helpful for early diagnosis (see Figure 18.4). Loss of the definition of gray/white matter is an ominous sign. Fixed, dilated pupils indicate brainstem coning and irreversible brain damage, but care in interpretation is required if sedative drugs have been used.

The etiology of cerebral edema is not known, but iatrogenic factors may contribute. These include: fluid overload from therapeutic efforts to improve coagulopathy and hypotension; failure to maintain blood glucose concentrations, leading to anaerobic brain metabolism, which can result in cerebral fluid shifts; and failure to maintain systemic blood pressure, which can lead to cerebral ischemia and secondary edema.

Management

Current treatment for cerebral edema in ALF is inadequate, so every effort must be made to prevent it. The key strategy is fluid restriction (<80% of maintenance), maintaining circulating volume with colloid as possible. The intravenous infusion of mannitol (0.5 g/kg every 4–6 h) helps control acute increases in ICP and may reverse acute neurological changes. Serum osmolarity should be monitored during mannitol therapy and should not exceed 320 mOsmol/L. Hemofiltration to prevent or remove fluid overload is an important strategy, particularly while awaiting a suitable donor, if diuretic therapy is ineffective or hepatorenal failure develops.

Elective ventilation should be performed if cerebral edema is suspected. Intubation may be carried out using a short-acting agent neuromuscular blocking agent (atracurium, eliminated by Hofmann degradation, or rocuronium, 80% eliminated by hepatic metabolism) to prevent the rise in ICP associated with the gag reflex. Further sedation may be required with physiotherapy and suction. Hyperventilation ($PCO_2 < 3.5$ kPa) may have a temporary effect in reducing ICP, but metabolic compensation limits this to 24 h. Diligent efforts should be made to maintain cerebral perfusion pressure (mean arterial pressure minus ICP) by administering blood products, albumin, and inotropic agents (epinephrine or norepinephrine). Mild hypothermia or controlled normothermia also shows promise in preventing and perhaps treating cerebral edema in ALF.

Corticosteroids are of no value in preventing or reducing cerebral edema. Barbiturate coma (thiopentone 0.5–1.0 mg/kg i.v. followed by an infusion of 0.5–3.0 mg/kg/h) may maintain cerebral perfusion while a donor liver is awaited, but has no proven value in ALF. Convulsions should be treated promptly. Monitoring ICP is controversial, as it has no therapeutic role and does not improve overall outcome; however, it may improve selection for liver transplantation.

Electrolyte and acid–base disturbances

Disturbances in sodium homeostasis – either hyponatremia and/or hypernatremia – are observed in virtually all children. Hyponatremia is more common, despite sodium retention by the kidney. It may result from decreased water excretion, increased antidiuretic hormone, disturbances in the sodium/potassium pump, or the excess administration of hypotonic saline. Hypernatremia is less common, but is related to the administration of sodium-rich intravenous fluids and the vigorous use of lactulose or mannitol.

Hypokalemia occurs secondary to increased retention of sodium by the kidney from secondary hyperaldosteronism, the vigorous use of diuretics, excessive vomiting, or nasogastric suction. Occasionally, hyperkalemia is observed in patients with massive hepatic necrosis and/or hemolysis. Hypocalcemia and hypomagnesemia frequently occur and should be corrected.

Acid–base disturbances are common and may be secondary to liver failure, sepsis, or the underlying disease. Respiratory alkalosis is observed in the early stages of encephalopathy, due to central hyperventilation. Metabolic

alkalosis is seen with hypokalemia and vigorous use of diuretics, particularly furosemide. Metabolic acidosis may be multifactorial and develop secondary to metabolic liver disease, with accumulation of organic acids, including lactate and free fatty acids, although ketosis is usually minimal. Other factors include: administration of blood preserved with citrate, tissue hypoxia and anaerobic metabolism, renal failure, or paracetamol poisoning. Respiratory failure and respiratory acidosis develop as coma deepens, requiring mechanical ventilation.

Renal dysfunction

Renal insufficiency complicates the course in 75% of children, and may be due to prerenal uremia, acute tubular necrosis, and functional renal failure.

Prerenal uremia may be due to dehydration or gastrointestinal bleeding because of absorption of nitrogenous substances from the gut. A marked increase in the blood creatinine concentration may develop from decreased glomerular filtration and/or increased muscle breakdown.

Acute tubular necrosis is seen in a minority of patients and may occur due to hypovolemia or dehydration related to mannitol infusion or diuretic therapy. Features include: abnormal urinary sediment; urinary sodium concentration >20 mmol/L, reduction in creatinine clearance (urine : plasma creatinine ratio <10); and oliguria (urine output <0.5 mL/kg/h).

Functional renal failure (hepatorenal syndrome) is the commonest cause of renal insufficiency. Features include sodium retention (urinary sodium concentration <20 mmol/L), normal urinary sediment, and reduced urinary output (<1 mL/kg/h). The etiology is multifactorial, and electrolyte imbalance, sepsis, and hypovolemia all play a part. Endotoxemia may contribute to renal injury.

The aim of management is to maintain circulating volume to prevent prerenal hypovolemia and ensure that urine output is >0.5 mL/kg/h. A fluid challenge (10 mL/kg) may be successful unless central venous pressure indicates fluid overload (>8–10 cmH_2O), when the use of furosemide (1–2 mg/kg i.v. or 0.25 mg/kg/h by infusion) may be effective. Established renal failure requires hemodialysis or filtration for fluid overload.

While functional renal failure recovers quickly after liver transplantation, acute tubular necrosis may severely complicate the postoperative management. Although many patients require hemodialysis or hemofiltration support, renal function returns to normal after successful liver transplantation.

Ascites

The use of ultrasound in the pretransplant assessment has demonstrated excessive peritoneal fluid in many patients, probably due to acute portal hypertension, from lobular collapse, vasodilation, poor vascular integrity, and reduced oncotic pressure. Clinically evident ascites occurs in less than half the patients, but may be a site for secondary bacterial or fungal infection, indicating the necessity for paracentesis in septic patients without an obvious focus of infection. Therapy is not indicated, other than for correction of oncotic pressure with albumin infusion and general fluid management.

Cardiovascular and pulmonary complications

Cardiac output is increased secondary to reduced vascular resistance and arteriovenous shunting. Reduced vascular resistance may be due to gut-derived endotoxin or to substances released from the necrotic liver, as removal of the liver has improved hemodynamic stability in a few cases. Patients frequently exhibit clinical evidence of warm extremities, facial flush, and erythema of the palms and soles despite profound hypotension ("warm shock").

Hypotension due to hemorrhage, bacteremia, or increased capillary permeability is a frequent event and may be refractory to volume replacement and to administration of pressor agents.

Sinus tachycardia is present in 75% of patients, while inappropriate bradycardia is a late sign that may be associated with a rise in ICP, suggesting a failure of central regulatory mechanisms, which may occur in the absence of clinically evident cerebral edema.

The combination of hypotension, evidence of peripheral vasodilation, and metabolic acidosis (or elevated blood lactate) is an indication of imminent death.

Respiratory problems

Defective ventilation and ventilatory response to chemical stimuli are commonly observed. Hyperventilation often accompanies stage II–III encephalopathy and results in respiratory alkalosis. Patients in stage IV coma develop hypoventilation, hypoxia, and hypercapnia. Arterial blood gas analysis usually reveals a mixed respiratory–metabolic acidosis. Although these patients may increase ventilation in response to transient hypoxia, ventilation is not maintained if hypoxia is prolonged. Elective mechanical ventilation guided by arterial blood gas analysis should be initiated at the first sign of respiratory failure. Unfortunately, positive-pressure ventilation, with positive end-expiratory pressure, may reduce hepatic perfusion and exacerbate metabolic acidosis.

Poor oxygenation despite adequate (mechanical) ventilation can be the result of intrapulmonary shunting of blood – a secondary ventilation–perfusion mismatch due to microvascular dilation. Necropsy findings include diffuse dilation of the pulmonary vascular bed and occasional spider nevi. Intrapulmonary shunting resolves promptly after liver transplantation or spontaneous recovery.

About one-third of adult patients with ALF demonstrate clinical and/or radiographic evidence of pulmonary edema, which is higher than has been observed in children with ALF. It may be due to vasodilation and loss of vascular integrity and can respond to diuretics and correction of plasma oncotic pressure.

Pulmonary infection from *Staphylococcus aureus*, Gram-negative bacteria, *Pseudomonas*, and *Candida* often complicates the course. Risk factors include pulmonary edema, intubation, mechanical ventilation, and immunodeficiency. The use of prophylactic antibiotics is not standardized across pediatric centers, but institution of broad-spectrum antibiotics should be considered in the setting of clinical or radiographic evidence of new consolidation. Positive endotracheal tube cultures should not be treated unless accompanied by clinical or radiographic evidence of pulmonary infection. Other complications include aspiration pneumonia and pleural effusions. Pulmonary hemorrhage is a terminal event.

Secondary bacterial and fungal infections

The majority of adults and 50% of children will develop significant infection, which may be related to impairment of cellular and humoral immune systems. The organisms most often implicated are Gram-positive (*S. aureus*, *S. epidermidis*, and streptococci), presumably of skin origin. Occasionally, Gram-negative bacteria or fungal infection are observed. Urinary tract infections from indwelling catheters and pulmonary infection, particularly in ventilated children, are common.

Management includes surveillance cultures from indwelling catheters, urine cultures, and surface swabs. Broad-spectrum antibiotics should be started with the suspicion of sepsis, as the signs may be subtle. For example, a rise in heart rate, the difference in the core/toe temperature gradient, a fall in blood pressure or urine output, the rapid development of hypoglycemia, hypothermia, or a deterioration in the mental state. Selection of antimicrobial therapy should be guided by the spectrum of organisms common in ALF and local susceptibility patterns. Positive cultures in the absence of clinical infection should result in removal or replacement of the infected catheter and administration of the appropriate antimicrobials, with close attention to the possibility of additional, perhaps opportunistic infection. Aminoglycoside antibiotics should be avoided, if possible, because they can contribute to renal failure.

Pancreatitis

Pancreatic lesions consistent with acute pancreatitis have been found at autopsy in a significant proportion of adults with ALF, but this is rarely clinically evident. Children with valproic acid toxicity may have significant pancreatic lesions, with pain, hypotension, and disturbed calcium homeostasis.

Aplastic anemia

Bone marrow failure is a potentially fatal complication of hepatitis of indeterminate cause, with or without ALF. It may not be evident before transplantation, or may develop after improvement in liver function and is associated with a high mortality. Stem cell transplantation, administration of granulocyte–colony-stimulating factor may be therapeutic options.

Specific therapies

Paracetamol ingestion

The standard emergency management methods for ingested poison (gastric lavage, forced diuresis, etc.) are no longer used. Early detection of paracetamol toxicity by estimating levels and/or metabolites is important. Treatment with NAC (140 mg/kg initially with 70 mg/kg 4-hourly) should start within 24 h of ingestion and then continue for at least 72 h or until liver failure has resolved and coagulation parameters are back to normal.

Amanita phalloides poisoning

Benzylpenicillin (10,000,000 U/kg/day) may reduce hepatic uptake of amatoxin, whilst thioctic acid (300 mg/kg/day i.v. infusion) may reduce hepatic damage. Hemodialysis or hemofiltration or the Molecular Adsorbent Recirculating System (MARS®) may also remove the amatoxin (see later).

Hepatic support

Many different measures have been used to support the liver while awaiting regeneration or transplantation, including a variety of experimental drugs such as prostaglandin E, insulin, or glucagon, which have not been shown to be effective.

Methods for removing potential neuroactive toxins include double-volume exchange transfusion, plasmapheresis, albumin dialysis (MARS), liver-assist devices containing chemical scrubbers or cultured hepatocytes, and extracorporeal perfusion through human or animal livers. Although these therapeutic maneuvers may provide support during liver regeneration or while awaiting a donor, none has been shown to have any benefit with regard to survival.

Double-volume exchange transfusion (in children <15 kg) and plasmapheresis in older children may produce a transient improvement in coagulopathy and neurological state, but may contribute to hemodynamic instability [15].

Molecular adsorbent recirculating system

MARS is an alternative form of hemodialysis that uses a specific filter to remove toxic products, but not albumin. It has a role in the management of both ALF and acute-on-chronic liver failure in adults. Its use in the management of children is anecdotal, and it is no longer widely available.

Liver transplantation

Liver transplantation should be considered in all children who develop stage III or IV hepatic coma, as the mortality in this group exceeds 70% [16]. Transplantation is appropriate to treat patients with ALF caused by viral hepatitis (including hepatitis B), drug-induced liver injury, halothane hepatitis, and mushroom poisoning, as well as those with an indeterminate cause. It is also effective for certain forms of inborn errors of metabolism – for example, Wilson disease and tyrosinemia type I, although it is contraindicated in children with multisystem disease or mitochondrial deletions.

As a successful outcome following liver transplantation is less likely than with other forms of liver disease, selection is critical, and is based on previous experience of mortality in the pretransplant era. Transplantation using a living donor can accelerate the process with good outcomes in the setting of ALF [17].

The etiology of ALF is an important factor in determining whether transplantation is appropriate. The highest mortality is seen in children with hepatitis of indeterminate cause, particularly those with a delayed onset of coma and rapid progression to stage III or IV hepatic coma, a shrinking liver and falling transaminases associated with an increase in bilirubin, and coagulopathy. These children should be immediately considered for transplantation. Children with fulminant Wilson disease are unlikely to recover with medical treatment and require transplantation [18].

In contrast, children with hepatitis A and children with drug-induced liver disease, particularly paracetamol poisoning, may make a complete recovery with intensive medical therapy. Careful monitoring for poor prognostic factors is thus required before selection.

In practical terms, it is appropriate to list for emergency liver transplantation all children who have reached stage III hepatic coma, as the shortage of donor organs may mean a considerable wait for transplantation or death on the waiting list.

As the development of irreversible brain damage is a major contraindication to transplantation, it is essential to be certain that severe, irreversible brain damage has not occurred prior to the operation. Current techniques are inadequate, but include monitoring ICP, the identification of cerebral infarction or intracranial hemorrhage by cerebral CT or MRI scans, and looking for evidence of midbrain coning, such as fixed, dilated pupils.

Auxiliary transplantation, in which the recipient liver is left in situ to regenerate, is a controversial treatment for ALF, but may have the benefit that the graft may be removed if the original liver regenerates. It is not suitable for transplantation for ALF secondary to metabolic or autoimmune liver disease, as there is no potential for these livers to recover and there may be a risk of hepatoma in the cirrhotic liver.

Family support

Families of children with ALF are naturally devastated by the development of potentially fatal ALF in their child. These families require a considerable amount of psychological support and counseling as they assimilate information and grasp the seriousness of their child's condition and the implications of liver transplantation. Psychological problems in both the family and the recipient of the liver transplant are common and will need addressing following the operation. The particular problems of self-poisoning in adolescents may need additional psychiatric help.

Prognosis

Prognosis for individual conditions are discussed in the Etiology section. Three outcomes are associated with ALF: death, survival with native liver, and liver transplantation. Death and survival are the only two natural outcomes, while liver transplantation is an intervention performed in individuals considered to be at a high risk of death or irreversible neurological injury. The outcome of death is not the same as the outcome of liver transplantation as the latter likely includes individuals who would have survived or would have died without liver transplantation given that the natural history was interrupted by the procedure. These three outcomes occur within all age groups, diagnostic categories, grades of encephalopathy whether on admission or at their peak, and degree of INR prolongation. While cohorts of patients are more likely to survive with their native liver (e.g., acute paracetamol toxicity, hepatitis A) while others have a high risk of death (e.g., neonatal herpes simplex, GALD), predicting the outcome for an individual patient remains very challenging.

In the absence of liver transplantation, a child with ALF will either survive or die. An ideal prognostic tool would be one which serve three functions: (1) reliably predict death early enough in the clinical course for liver transplantation to be not only lifesaving, but also avoid irreparable neurological injury; (2) reliably predict patient survival with their native liver to minimize unnecessary liver transplantation with its associated morbidity and mortality as well as enable proper stewardship of liver donation; and (3) incorporates the dynamic nature of PALF. Unfortunately, the ideal tool has not yet been identified.

Kings College Hospital Criteria (KCHC) were developed utilizing 588 adult patients (1973–1985), of whom 570 progressed to stage IV encephalopathy, and validating the criteria in a cohort of 175 patients (1986–1987) [16]. The number of children included in either cohort was not reported. There were 54 patients with non-paracetamol ALF in the validation cohort and the 12 patients who underwent liver transplantation were grouped with those who died in the analysis. The positive predictive value for mortality in non-paracetamol-induced ALF was 97%, indicating a high risk of death if criteria were met. The KCHC have served as the "gold standard" to predict outcome in paracetamol and non-paracetamol ALF with similar findings noted in some, but not all, studies in adult patients.

Important differences between adults and children with ALF in children include definition, etiology, and outcome. For children with PALF in the current era where liver transplantation is an option, transplant interrupts the natural history of PALF and limits any ability to reconstruct a "natural history" cohort to test a predictive model. In an effort to construct a semblance of such a natural history cohort, the PALF study group applied KCHC to 522 participants who either died or survived to 21 days with their native liver, excluding those who received a

liver transplant [19]. Among the 163 participants who met KCHC for non-paracetamol ALF and would be predicted to die, only 54 (33%) died within 21 days of enrollment in the PALF study. Among those expected to live by not meeting KCHC (n = 289), 34 (88%) survived. Thus, meeting KCHC did not reliably predict death in PALF. Conversely, not meeting KCHC was much better at predicting survival.

Virtually all reports seeking to identify a mortality risk score in PALF suffer from the same flaw of grouping death and liver transplant into the same statistical category. The Liver Injury Unit (LIU) score, utilizing peak bilirubin, PT or INR, and ammonia was developed and validated at single site [20]. However, when the LIU score was applied to the PALF study cohort for each of with all three outcomes analyzed separately, utilization of the LIU score predicted liver transplantation better than it predicted death [21]. Utilization of the pediatric end-stage liver disease score, the pediatric risk mortality score, as well as a computational artificial neural network are similarly handicapped [22].

Most prognostic tools utilize clinical values at or near the time of admission given the rapid progression of disease for some individuals who die or receive a liver transplant within 1–2 days. Incorporating serial testing into the prognostic model, however, allows for day-to-day assessment of deterioration or improvement which would inform liver transplant decisions. Modeling of serial measurements of 17 markers of inflammatory and immune responses collected for up to 7 days following enrollment into the PALF study found unique immune/inflammatory networks that differentiated death from survival with native liver [11]. Those who received a liver transplant had a dynamic network that had features of both survival and death suggesting underlying immune or inflammatory responses may drive outcome regardless of diagnosis.

A study from New Delhi, where liver transplantation was not performed and diagnoses differ from Western countries, utilized clinical variables that included HE (grade >2), INR (≥5), arterial ammonia (≥123 μmol/L), and serum bilirubin (≥15 mg/dL) at admission. A weighted score was assigned for persistence or worsening of these parameters over 3 days. The resulting ALF early dynamic (ALFED) model had a high positive predictive value (85%) and negative predictive value (87%) [23].

Improvement in current models to predict death and survival in PALF will improve liver transplant decisions. Clinical judgement and experience, however, remain an intangible yet important factor in the final decision to proceed to liver transplantation.

Outcomes

In the pre-transplant era, mortality in children presenting with ALF and encephalopathy was in excess of 70%. The major causes of death in children before viable liver transplantation were: sepsis, 15%; hemorrhage, 50%; renal failure, 30%; and cerebral edema, 56% [6]; these continue to be the main causes of death [24]. For patients meeting entry criteria for the PALF study, 21-day outcome of survival, death, and liver transplant have been approximately 50%, 14%, and 36%, respectively. Compared to other interventions such as plasmapheresis or steroids, only liver transplantation has an appreciable effect on the mortality, although the long-term survival is lower than with transplantation for other forms of liver disease.

An analysis of data from the Studies of Pediatric Liver Transplantation (SPLIT) Registry revealed a 4-year post-transplant patient survival rate of 69% for children with ALF, in comparison with 86% for all other patients [25]. With improving surgical and supportive management, post-transplant outcome for PALF is beginning to compare favorably with transplantation for other conditions. Cerebral dysfunction or brain death following transplantation is an important cause of death, indicating the importance of not transplanting patients with irreversible brain damage.

In patients with spontaneous recovery of liver function, the long-term outlook is excellent for most patients. Complete clinical and biochemical resolution occurs, although postnecrotic cirrhosis may develop in some survivors. Exceptions to the general rule of full recovery are those who develop aplastic anemia, recurrent ALF associated with genetic predispositions, and evolution of clinical manifestations of systemic mitochondrial disease.

Encephalopathy, hyperammonemia and varying degrees of acute neurological dysfunction are associated with PALF. With improved survival for those who recover spontaneously as well as following liver transplantation, data are emerging regarding functional outcomes following ALF. A recent cross-sectional analysis of 36 children, 23 of whom received a liver transplant, found long-term survivors had an average IQ and visual spatial ability, but greater than expected impairments in motor skills, executive function, health-related quality of life, and fatigue [26]. Future studies will hopefully identify opportunities to improve management strategies that will reduce morbidities associated with PALF.

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SECTION 8

Metabolic Liver Disease

CHAPTER 19

Metabolic Liver Disease in the Infant and Older Child

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Key points

- Inherited metabolic disorders are important causes of liver disease in infancy and childhood.
- Many inherited metabolic disorders are treatable, and urgent investigation, diagnosis, and initiation of treatment are essential to ensure a good outcome.
- Inherited metabolic disorders that present with liver disease may manifest as non-immune hydrops fetalis, acute liver failure, cirrhosis, cholestasis, and hepato(spleno)megaly, and must be included in the differential diagnosis for these presentations.
- Some inherited metabolic disorders do not have specific treatment available; for these, establishing a diagnosis is important for prognosis and genetic counseling.
- A high index of suspicion is the key to diagnosis. Discussion with a metabolic specialist center and laboratory is important in order to enable urgent diagnosis and therapy.
- Research into treatment of many inherited metabolic disorders is ongoing and should enable effective therapies to be developed in the near future.

There are a large number of inborn errors of metabolism (IEMs), many of which present with liver disease. Although most patients present in the neonatal period with cholestasis or an acute illness (Chapters 8 and 9), for many the condition only becomes manifest later in infancy or childhood with a broad range of manifestations. Recognition of a specific diagnosis is important for appropriate management, for the prognosis, and for genetic counseling. Diagnosis of these disorders may be complicated, as non-specific liver dysfunction may lead to secondary biochemical disturbances suggestive of a metabolic disorder.

The differential diagnosis of metabolic liver disease presenting in infancy and in older children varies with the type of clinical presentation.

Pathophysiology of liver dysfunction in inborn errors of metabolism

Most inborn errors involve abnormalities in enzymes and transport proteins. In some disorders, the basic defect involves only one functional system such as the endocrine system, immune system, coagulation system, etc. Others involve a basic biochemical dysfunction that is common to many organs or tissues; this group includes disorders of energy metabolism and intermediary metabolism. The liver may often be involved together with other organ systems.

From a pathophysiological perspective, disorders can be classified into three main groups:

- Disorders that involve the synthesis or catabolism of complex molecules, of which the lysosomal and peroxisomal disorders are examples (see Chapter 9). The affected organs, including the liver, are those in which the partly degraded molecules accumulate and cause disruption to the organ function.
- Disorders caused by the accumulation of "toxic" compounds arising as a consequence of the enzyme defect, as occurs in galactosemia and tyrosinemia type I.
- Energy metabolism disorders, caused by a deficiency in energy production and its consequences. Included in this group are the glycogen storage disorders, fat oxidation, and mitochondrial disorders.

For several IEMs, liver dysfunction therefore arises as a primary effect of the IEM itself. However, for some disorders

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such as urea cycle defects and organic acidurias, although the defect (such as enzyme deficiency) is expressed mainly in the liver cells, liver dysfunction is not the primary manifestation and the main clinical effects are seen in other organs.

Clinical presentation of liver metabolic disease [1, 2]

The differential diagnosis of metabolic liver disease varies with the clinical presentation and should be considered in the following clinical scenarios.

Hydrops fetalis

Hydrops fetalis and neonatal ascites occur due to a range of fetal and placental malformations, immunological disorders, infections, and a number of IEMs including the lysosomal and peroxisomal disorders, congenital disorders of glycosylation (CDG), and glycogen storage disease type IV. Although the prognosis is poor, it is important to establish the diagnosis for genetic counseling.

Acute liver failure

Acute liver dysfunction due to IEMs causes variable degrees of jaundice, edema, ascites, coagulopathy, and encephalopathy. The differential diagnosis includes galactosemia, tyrosinemia type I, fatty acid oxidation defects, urea cycle defects, HFI, and respiratory chain disorders. Many of these disorders are amenable to specific treatment and the investigation protocol must include a wide range of metabolic investigations.

Chronic cholestasis

The majority of these cases present in the neonatal period (see Chapters 8 and 9), and the differential diagnosis includes IEMs such as α_1-antitrypsin deficiency, inborn errors of bile acid synthesis, Niemann–Pick disease type C, inborn errors of peroxisomal function, citrullinemia type II, and CDG. The presence of failure to thrive, hypoglycemia, acidosis, and encephalopathy are suggestive of an underlying IEM.

Hepatomegaly or hepatosplenomegaly

A palpable liver with a firm or hard consistency may suggest cirrhosis, and conditions such as galactosemia, HFI, tyrosinemia type I, α_1-antitrypsin deficiency, Wilson disease, glycogenosis type IV, and lysosomal storage disorders (LSDs) should be considered in the differential diagnosis. When the consistency of the liver is soft or normal and there is associated splenomegaly, LSDs should be considered.

Associated features such as a coarse facial appearance, joint stiffness, corneal opacities, skeletal deformities, cardiomyopathy, oculomotor apraxia, and neurodevelopmental regression may suggest an underlying LSD.

Glycogen storage disease, Fanconi–Bickel syndrome, or fructose-1,6-bisphosphatase deficiency are usually associated with hypoglycemia and lactic acidosis. Other conditions that may be associated with isolated hepatomegaly include argininosuccinic aciduria, cholesterol ester storage disease, and CDG.

Clinical evaluation

Diagnostic evaluation should begin with a complete history and physical examination. Important pointers to an IEM from the history include:

- A positive family history and/or parental consanguinity.
- Sudden, unexplained death in a previous sibling.
- Acute fatty liver of pregnancy and the hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome during pregnancies with affected fetuses are associated with some fatty acid oxidation defects.
- Recurrent episodes of clinical disease at times of catabolic stress can occur with fatty acid oxidation defects, urea cycle defects, and organic acidemias.
- A history of specific dietary avoidance may be suggestive of fructose intolerance or a urea cycle defect.

The physical examination should include:

- Assessment of growth and development.
- Examination for dysmorphism, skeletal features, joints, skin, and hair.
- A full systems examination to include cardiac, abdominal, respiratory, and neurological examination.

Investigations to assess multisystem involvement must also be included:

- Cardiac and renal assessment.
- MRI/MRS, audiology, and neurophysiological studies to define the extent and nature of neurological disease.
- Detailed ophthalmologic evaluation as specific eye signs may be present in the lysosomal, peroxisomal, and respiratory chain disorders.

Initial investigations for metabolic liver disease

It is usually difficult to distinguish clearly between liver disease that is due to an underlying metabolic defect and liver disease that is due to other non-IEM causes; therefore, initial investigations for liver dysfunction due to any cause must include tests for the common and treatable metabolic conditions. Occasionally, however, a result can be suggestive of a particular disease and guide further investigations–e.g., disproportionately elevated alkaline phosphatase in tyrosinemia type I, or low γ-glutamyltransferase (GGT) in bile acid synthesis disorders.

Clinical indications must be provided on the request forms, including feeding status/diet and drug and transfusion

histories, as these may confound the results of some metabolic tests and give misleading results. More specific investigations such as enzyme assays on skin or liver biopsies and DNA mutation analysis are usually indicated if the clinical features or the initial investigations point to a specific diagnosis or group of conditions; however in an acute life-threatening situation any opportunity to obtain these specimens must not be lost. Recent advances in molecular genetics have resulted in dramatic reductions in the cost and the turnaround times for DNA mutation analyses and increasingly, next generation sequencing (NGS) can be used as a first-line investigation to look for specific conditions (such as Niemann–Pick disease type C or glycogen storage diseases) or as part of a standard panel (such as for cholestasis).

Laboratory investigations for metabolic disorders often require special methods of specimen collection, storage, and transport, and it is best to plan such investigations in consultation with a specialist metabolic laboratory before taking any specimens.

Initial investigations should include tests for the more common metabolic conditions according to the presenting features (Table 19.1); more specific tests for individual conditions are detailed in Table 19.2.

Table 19.1 Initial investigations for inborn errors of metabolism.

Presenting feature	Investigations
Hydrops fetalis	**Urine** glycosaminoglycans, oligosaccharides, organic acids **Plasma** acylcarnitines, I-cell disease screen, sterols, very-long-chain fatty acids, transferrin electrophoresis, lactate, ferritin, Niemann–Pick disease biomarkers **Whole blood** for DNA extraction and storage, lysosomal enzymes, hemoglobinopathies, erythrocye enzymes **Skin biopsy** for fibroblast culture and storage **Bone marrow** biopsy or aspirate for storage cells and inclusions
Liver failure	Erythrocyte galactose-1-phosphate uridyltransferase (Beutler test) Plasma and urine amino acids Urine organic acids including succinylacetone Urine and plasma bile acids Plasma α-fetoprotein Plasma glucose, lactate Plasma ammonia Plasma/blood spot acylcarnitines Plasma ferritin, total iron binding capacity Serum α_1-antitrypsin and phenotype Plasma Niemann–Pick disease biomarkers
Cholestasis	As for liver failure, *plus:* Plasma very-long-chain fatty acids Plasma transferrin isoforms Vacuolated lymphocytes in peripheral blood Storage cells in liver/bone marrow biopsy Specific enzyme assay in leukocytes/fibroblasts Cholestasis DNA gene panel if available
Hepatomegaly or hepatosplenomegaly	Plasma urate Urine organic succinylacetone Plasma lipids Urine oligosaccharides Urine glycosaminoglycans Liver histology Specific enzyme analysis on liver/leukocytes Vacuolated lymphocytes Storage cells in liver/bone marrow Plasma chitotriosidase Plasma transferrin isoforms DNA gene panel if available for glycogen storage disease or hepatomegaly

Disorders of carbohydrate metabolism

Glycogen storage diseases [3, 4]

Glycogen is the main storage form of carbohydrate in animals and is most abundant in the liver and muscle. Liver glycogen controls the export of glucose, which maintains blood glucose concentrations between meals, and is almost completely depleted within a few hours of fasting. Muscle glycogen provides muscles with readily available energy via glycolysis. The synthesis (glycogenesis) and breakdown (glycogenolysis) of glycogen are catalyzed by a number of different enzymes, which in turn are activated or inactivated by hormones. The glycogen storage diseases (GSDs) are due to defects of glycogen synthesis or breakdown, each caused by a specific enzyme defect, and result in abnormal storage and/or deficient mobilization of glycogen. Some enzyme defects are confined to the liver and are associated with hepatomegaly and hypoglycemia, whereas others affect only muscle glycogen metabolism and result in muscle cramps, weakness, and myopathy. Traditionally, the GSDs have been denoted by a number relating to the historical sequence in which they were described; nowadays, they are also known by the specific enzyme deficiencies. The overall frequency of the combined group is believed to be around 1 in 20,000 to 1 in 25,000 live births. Here only GSDs that affect the liver are described.

Glycogen storage disease type I

GSD I is due to a defective breakdown of glucose-6-phosphate, resulting in decreased hepatic production of glucose and accumulation of glycogen in liver, kidney, and intestine. Two subtypes of GSD I are recognized: GSD Ia, due to glucose-6-phosphatase deficiency, and GSD Ib, due to defects of the glucose-6-phosphate transporter.

GSD Ia: glucose-6-phosphatase deficiency, von Gierke disease

GSD Ia is inherited as an autosomal recessive trait. The glucose-6-phosphatase gene has been mapped to chromosome 17q21. Over 110 mutations have been identified (in the Human Gene Mutation Database), and a number

Table 19.2 Specific investigations for metabolic liver disease.

Presenting feature	Metabolic conditions	Investigations
Liver failure	Galactosemia	Erythrocyte galactose-1-phosphate uridyltransferase
		Erythrocyte galactose-1-phosphate
		DNA mutation analysis
	Tyrosinemia type 1	Plasma and urine amino acids
		Urine organic acids
		Urine succinylacetone
		Erythrocyte porphobilinogen synthetase
		α-Fetoprotein
	Hereditary fructose intolerance	Plasma lactate
		DNA mutation analysis
		Enzyme analysis on liver biopsy
	Mitochondrial respiratory chain defects	Plasma and cerebrospinal fluid lactate, mitochondrial DNA analysis (blood)
		Muscle biopsy for DNA, histology, histochemistry and enzyme analysis
		Nuclear gene sequencing (e.g., known mitochondrial DNA depletion genes)
	Long-chain fatty acid oxidation defects (usually with associated hypoglycemia)	Urine organic acids
		Plasma/blood spot acylcarnitines
		DNA mutation analysis
	Neonatal hemochromatosis (see Chapters 9 and 10)	Plasma ferritin, total iron binding capacity
		Liver or lip biopsy, MRI of brain and abdomen
	α_1-Antitrypsin deficiency	Serum α_1-antitrypsin and phenotype
	Urea cycle defects and organic acidurias	Plasma ammonia
		Plasma lactate
		Urine organic acids
		Plasma and urine amino acids
		Plasma/blood spot acylcarnitines
		DNA mutation analysis
		Skin fibroblast enzymes
Cholestasis (neonatal or later)	As for liver failure, *plus*:	–
	DNA analysis for specific disorders or on "cholestasis panel" if available	–
	Peroxisomal disorders	Plasma very-long-chain fatty acids, DHAPAT, phytanic/pristanic acid, plasmalogens
		Peroxisomal morphology in liver/fibroblasts
	Congenital disorders of glycosylation	Plasma transferrin electrophoresis
	Lysosomal storage disorders	Vacuolated lymphocytes
		Urine oligosaccharides and glycosminoglycans
		Plasma chitotriosidase
		Urine/plasma oxysterols
		Storage cells in liver/bone marrow biopsy or skin fibroblasts
		Specific enzyme assay in leukocytes/fibroblasts
		Storage cells in bone marrow/liver
		Filipin staining of skin fibroblasts for Niemann–Pick disease type C
		DNA analysis for Niemann–Pick disease type C
	Bile acid synthesis defects	Urine and plasma bile acids
	Citrin deficiency	DNA analysis
Hepatomegaly or hepatosplenomegaly	Glycogen storage diseases	Plasma glucose, lactate
		Plasma urate
		Plasma lipids
		Urine oligosaccharides
		Liver histology
		DNA analysis for glycogen storage disorders
		Enzyme analysis on liver/muscle/skin fibroblasts
	Lysosomal storage disorders	As for "cholestasis"
	Congenital disorders of glycosylation	Plasma transferrin isoforms

DHAPAT, dihydroxyacetone phosphate acyltransferase.

of ethnic-specific mutations have been described: R83C and Q347X in white people; R83C in Ashkenazi Jews; G727T in Chinese and Japanese; and V166G in Arabs. The diagnosis of GSD Ia can be established by mutation analysis, which can be used as the first-line investigation with liver enzyme analysis used in the cases where gene sequencing result is equivocal.

Presentation

Patients typically present in the neonatal period or early infancy with hepatomegaly, hypoglycemia, hyperlactacidemia, and tachypnea secondary to metabolic acidosis. Older children tend to have a doll-like face, thin extremities, short stature, and a protuberant abdomen due to massive hepatomegaly. The kidneys are also enlarged, but there is no cardiac involvement or splenomegaly. Recurrent vomiting, diarrhea, and skin xanthomas may also occur.

Children have a short tolerance to fasting and typically become hypoglycemic if a feed is delayed or if intake is reduced during intercurrent illness. Symptoms of hypoglycemia are usually accompanied by tachypnea secondary to metabolic acidosis (lactic acidosis). Exceptional patients are able to tolerate prolonged fasting without becoming hypoglycemic, possibly due to residual glucose-6-phosphatase activity.

Biochemical abnormalities are characterized by:

- *Hypoglycemia*, which occurs soon after dietary sources of glucose are exhausted. The degradation of glycogen to pyruvate remains intact, with resultant increases of blood lactate and pyruvate concentrations.
- *Hyperlacticacidemia*. Lactate can serve as an alternative fuel for the brain, and its overproduction is helpful unless pathological metabolic acidosis develops. Osteopenia may result from chronic lactic acidosis, as bone is believed to play an important role in buffering chronic acidosis.
- *Hyperlipidemia*. Increased activity of the glycolytic pathway results in increased acetyl-coenzyme A (CoA) production. Excess acetyl-CoA that is not used for energy production is converted to malonyl-CoA, an inhibitor of fatty acid oxidation and a potent stimulant of fatty acid synthesis, resulting in increased plasma triglycerides and to a lesser extent cholesterol.
- *Hyperuricemia* is caused both by reduced clearance of urate by the kidneys, due to competition with lactic acid, and by increased production of uric acid from adenine nucleotide degradation.

Diagnosis

Characteristic laboratory findings are:

- Fasting hypoglycemia (<2.5 mmol/L).
- Blood lactate >5 mmol/L.
- Hyperlipidemia (cholesterol >6 mmol/L and triglycerides >3 mmol/L).
- Hyperuricemia (>350 µmol/L, age dependent).
- Mildly elevated plasma aminotransferases.
- Normal plasma bilirubin, albumin, coagulation.

Liver histology demonstrates uniform distension of hepatocytes with glycogen and prominent lipid vacuoles. The glycogen content of the liver is raised (normal <6%). Normal liver architecture may be obscured by the distended hepatocytes, but there is no fibrosis or cirrhosis (Figure 19.1). The stored material stains strongly positive on periodic acid–Schiff (PAS) and is digestible by diastase. Histochemical stains for glucose-6-phosphatase are negative.

The diagnosis can be confirmed either by mutation analysis of the *G6PC* gene or by enzyme analysis on a liver biopsy specimen.

Antenatal diagnosis by chorionic villus sampling is possible if the mutation has been identified; otherwise, enzyme analysis on fetal liver biopsy is the only option.

Management and outcome

The main aim of therapy is to prevent hypoglycemia and suppress the secondary metabolic derangements. This is achieved by providing a continuous supply of exogenous glucose, which maintains normal blood glucose concentrations and inhibits counter-regulatory responses. The glucose requirement has been estimated to be 8–10 mg/kg/min in infants and 5–7 mg/kg/min in older children [1]. In infants, this is best achieved by frequent daytime feeding. Continuous nocturnal enteral glucose feeds are usually required initially. In older children and adults, use of oral uncooked cornstarch, which is hydrolyzed in the gut to release glucose slowly over hours, may reduce the need for continuous or nocturnal feeding. The dosage of cornstarch required to ensure metabolic stability varies greatly, but usually 1–2 g/kg/dose of cornstarch is given 4–12-hourly, depending on individual fasting tolerance. Tests to estimate fasting tolerance and monitoring of preprandial and postprandial

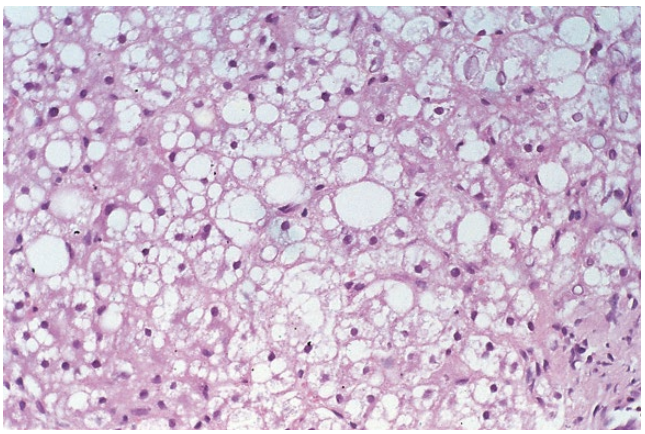


Figure 19.1 Glycogen storage disease types Ia and I non-a are characterized by increased glycogen storage. The hepatocytes are swollen with glycogen and steatosis is prominent. Glycogen-6-phosphatase is either deficient or functionally abnormal.

glucose and lactate concentrations are useful in making decisions about dietary adjustments. With increasing age, the tendency towards hypoglycemia becomes less – perhaps as a result of the natural decrease in metabolic rate.

Adequate dietary treatment can result in correction/reduced severity of many of the metabolic abnormalities and result in greatly reduced morbidity. Some long-term complications such as hypoglycemic brain damage and poor growth respond well to dietary management, although patients tend to be shorter than their peers. The onset of puberty is often delayed. Most females have ultrasound evidence of polycystic ovaries, though other features of polycystic ovarian disease are rare and the effects on fertility are unknown.

Chronic hyperuricemia can lead to gout, renal stones, and osteopenia, resulting in pathological fractures and may require treatment with allopurinol. Early glomerular hyperfiltration slowly progresses to microalbuminuria, proteinuria, and eventually a reduced glomerular filtration rate secondary to focal glomerulosclerosis and interstitial fibrosis. Declining renal function and hypertension eventually occur, and renal dialysis and transplantation may be necessary. Other renal abnormalities that have been described include a Fanconi-like syndrome, distal tubular dysfunction, kidney stones, and amyloidosis. It is unclear whether dietary management can prevent long-term renal dysfunction and osteopenia, but it was suggested that poor metabolic control results in reduced muscle strength and low bone mass.

Atherosclerosis is rare, despite the atherogenic profile of plasma lipids. There is an increased risk of pancreatitis secondary to the lipid abnormalities. Hypertriglyceridemia sometimes requires treatment; fish oil supplements and prevention of hypoglycemia have been effective in lowering plasma triglycerides and cholesterol.

Pulmonary hypertension is a rare fatal complication. The exact cause is unknown, but vasoconstrictive stimuli such as severe metabolic acidosis, hypoxia, and abnormal hepatic clearance of circulating vasoactive agents have been proposed as possible mechanisms.

Epistaxis and easy bruising following minor trauma are commonly observed; bleeding times are prolonged and associated with abnormal platelet adhesion and aggregation. Surgical procedures should not be undertaken without first evaluating bleeding time and establishing good metabolic control. Intensive intravenous glucose therapy for 24–48 h before surgery can normalize abnormal bleeding times; 1-deamino-8-D-arginine vasopressin (DDAVP) has also been reported to reduce bleeding complications. Successful pregnancies have been reported.

Liver function is typically normal and cirrhosis does not develop. Hepatic adenomas are commonly present by the second or third decades of life, especially in suboptimally treated individuals. The adenomas may be single or multiple, and have the potential for severe hemorrhage and malignant transformation. Regular ultrasound examinations and serum α-fetoprotein determinations are therefore essential. Hepatic adenomas have been shown to regress following intensive dietary treatment. Recent long-term studies indicate that although adenomas may occur despite early continuous glucose therapy, improved control of hypoglycemia may reduce their incidence.

Liver transplantation is rarely undertaken, as the disease should be adequately controlled with dietary management. However, liver transplantation may be indicated for symptomatic multiple hepatic adenomas at risk of malignant transformation and/or poor metabolic control [5]. Successful liver transplantation restores normal metabolic balance, allows catch-up growth, and improves quality of life. However, it may not prevent the development of renal dysfunction with focal segmental glomerulosclerosis, and this needs to be considered when treating with nephrogenic immunosuppressive drugs. Although successful hepatocyte transplantation been reported, this treatment has been shown to only have temporary effect.

Glycogen storage disease type Ib (also known as type non-a, Ic, and Id)

GSD I non-a is caused by homozygous or compound heterozygous mutations in *G6PT1* that encodes glucose-6-phosphate transporter. Thus despite the presence of glucose-6-phosphatase patients have functional enzyme deficiency as glucose-6-phosphate cannot be transported to the glucose-6-phosphatase site of action at the inner wall of the endoplasmic reticulum (ER). Although it was originally believed that different proteins are deficient in GSD types Ib, Ic, and Id, the evidence suggests that GSD types Ic and Id do not differ from GSD Ib clinically, enzymatically, or genetically. These conditions are therefore now categorized as GSD I non-a or GSD Ib. The prevalence of GSD Ia relative to GSD I non-a is estimated to be around 5–10 to 1.

The glucose-6-phosphatase transporter gene has been localized to chromosome 11q23. Several mutations have been reported; G339C and 1211delCT appear to be common in white patients, while W118R appears to be common amongst Japanese patients.

Presentation

The clinical presentation, metabolic derangements, and complications are the same as in GSD Ia, with the additional finding of neutropenia and impaired neutrophil function. Neutropenia becomes apparent in infancy or early childhood and is usually intermittent. Inflammatory bowel disease resembling Crohn disease may also occur, often preceded or accompanied by oral, perioral, and perianal ulcers, infections, abscesses, and fistulas.

Diagnosis

Biochemical abnormalities and liver histology are indistinguishable from GSD Ia (Figure 19.1). Diagnosis requires DNA analysis or demonstration of deficient glucose-6-phosphatase

activity in fresh liver biopsy tissue in which hepatocytes and microsomes are intact. When the cell is disrupted by freezing, measured glucose-6-phosphatase activity is normal, as the substrate has free access to the enzyme.

Antenatal diagnosis is possible by mutation analysis in chorionic villus tissue if the mutations are known; otherwise, fetal liver biopsy is required.

Management and outcome

Management of GSD Ib patients is the same as in GSD Ia. Dietary metabolic control has no effect on neutropenia and neutrophil dysfunction, but treatment with granulocyte–colony-stimulatingfactor(G-CSF)orgranulocyte–macrophage–colony-stimulating factor (GM-CSF) successfully corrects neutropenia, decreasing the frequency of bacterial infections and improving chronic inflammatory bowel disease in these patients. Splenomegaly is an important short-term complication of G-CSF therapy in these patients, but usually does not result in clinically significant thrombocytopenia.

As Sweet syndrome (acute febrile neutrophilic dermatosis), acute myelogenous leukemia, and renal carcinoma have been reported in patients receiving G-CSF treatment, close follow-up with annual bone marrow aspiration and imaging studies is advisable. Prophylactic antibiotic therapy may be an option for patients with neutropenia who do not have inflammatory bowel disease.

Liver transplantation has been successfully carried out in a few patients with GSD Ib. Reported benefits include improved metabolic control and growth, as well as decreased hospitalization. However, improvement in neutropenia has been variable and patients have continued to require G-CSF treatment after hepatic transplantation. Bone marrow transplantation for neutropenia and recurrent infections in patients with GSD Ib have successfully reduced the rate of infection and need for G-CSF, improved growth, and reduced gastrointestinal symptoms but fasting intolerance persists.

Glycogen storage disease type III

GSD type III is caused by deficiency of the debrancher enzyme amylo-1,6-glucosidase, resulting in accumulation of partially broken-down glycogen molecules (limit dextrin). Patients with liver and muscle involvement (GSD IIIa) have a generalized debrancher deficiency that affects the liver, muscle, fibroblasts, cardiac muscle, and erythrocytes, whereas patients with GSD IIIb have debrancher deficiency confined to the liver. Type IIIa is more common, occurring in about 80% of patients with GSD III.

The inheritance of GSD III is autosomal recessive. The gene is located on chromosome 1p21 and 170 mutations have been reported. Muscle and liver isoforms of the enzyme are encoded by the same gene, although certain mutations (those associated with GSD IIIb) appear to be associated with retention of debrancher activity in muscle, but not in liver.

Presentation

The presentation may be indistinguishable from that of GSD I, but milder. The main clinical features are hypoglycemia, hepatomegaly, short stature, skeletal myopathy, hyperlipidemia, and cardiomyopathy. There is wide variability in clinical and biochemical phenotypes, depending on the extent and localization of the enzyme defect.

In contrast to GSD I, however, renal enlargement is not present.

Patients who have muscle involvement often develop slowly progressive skeletal myopathy and wasting, progressing from minimal signs in childhood to severe muscle weakness by the third or fourth decade of life. Muscular involvement can be very variable and range from mild to severe and life-threatening. Left ventricular hypertrophy is common in patients with muscular involvement, and may lead to significant cardiac dysfunction in the long term.

Diagnosis

Patients characteristically have:

- Fasting hypoglycemia with ketosis/ketonuria.
- Hyperlipidemia (cholesterol >6 mmol/L with normal triglycerides <3 mmol/L).
- Uric acid is usually normal.
- Lactate is moderately increased (2.5–5.0 mmol/L) or normal.
- Elevated hepatic aminotransferases.
- Increased creatine kinase (type IIIa); it should be noted that normal levels do not rule out muscle involvement.

Liver histology is similar to GSD I. Two distinguishing features in GSD III are the presence of fibrosis and a relative paucity of steatosis. The diagnosis is confirmed by identifying the deficient enzyme in leukocytes or hepatic tissue, or by DNA analysis.

Antenatal diagnosis is possible by enzyme assay in chorionic villus samples or cultured amniotic fluid cells, as well as by mutation analysis in informative families.

Management and outcome

The general principles of treatment and prevention of hypoglycemia are the same as for GSD I. Adequate dietary management is associated with catch-up growth, decreased liver size, and improved liver function. A high protein intake may help in improving glycemic control and symptoms of myopathy and cardiomyopathy, as protein can be used for gluconeogenesis, a pathway that is intact in GSD III.

With age, hepatomegaly, hepatic function, and fasting tolerance improve and may completely resolve after puberty. However, progressive liver dysfunction and liver failure can occur. Hepatic adenomas have been reported in up to 25% of patients, but malignant transformation is rare. Hepatocellular carcinoma in association with advanced liver cirrhosis can occur. Liver transplantation may be indicated for cirrhosis, end-stage liver failure, and/or hepatocellular carcinoma.

The long-term outlook is favorable for patients without muscle involvement (GSD IIIb). For those with GSD IIIa, the prognosis depends on the severity of neuromuscular and cardiac disease. At present, there appears to be no satisfactory way of preventing progressive myopathy [1].

Successful pregnancies have been reported.

Glycogen storage disease type IV

This rare disease is due to a defect in the enzyme required for normal branching of the glycogen molecule (α1,4-glycan-6-glycosyltransferase). The glycogen that accumulates is abnormal and poorly soluble, with fewer branch points than normal glycogen. Accumulation is generalized and occurs in the liver, heart, muscle, skin, intestine, brain, and peripheral nervous system.

Inheritance is autosomal recessive. The hepatic and neuromuscular forms of GSD IV are caused by mutations on the same gene, which has been localized to chromosome 3p12.

Presentation

The most common presentation is in infancy, with liver dysfunction and failure to thrive. Initial hepatomegaly progresses to cirrhosis and portal hypertension with splenomegaly, ascites, and variceal bleeding, leading to death by the age of 5 years. Hypoglycemia is rare, except as a feature of liver failure. Some patients appear to have non-progressive liver disease, and hepatocellular carcinoma has been reported in one such individual.

Brancher enzyme deficiency may also present with neuromuscular symptoms without hepatic involvement. These individuals may present in the neonatal period with severe hypotonia and neurological involvement, leading to death in infancy, in late childhood with myopathy and/or cardiomyopathy, or in adulthood with diffuse central and peripheral neurological symptoms associated with polyglucosan body storage in the nervous system. In extreme cases, prenatal onset of symptoms may result in hydrops fetalis or fetal akinesia deformation sequence.

Diagnosis

Clinical features of the hepatic form are indistinguishable from other causes of liver disease in infancy, and the diagnosis is usually suspected from liver histology. Pale, amphophilic hyaline deposits along with large lipid vacuoles are seen on light microscopy, with fibrosis or cirrhosis. The abnormal glycogen can be demonstrated as large PAS-positive, diastase-resistant deposits in hepatocytes (Figure 19.2) and with special stains such as Lugol's iodine or colloidal iron phosphate. Similar histological findings may be demonstrable on cardiac and skeletal muscle biopsy. Confirmation of the diagnosis requires DNA sequencing of enzyme assay in liver, muscle, leukocytes, or fibroblasts.

Antenatal diagnosis is possible by enzyme assay on cultured amniocytes or chorionic villus tissue, as well as by DNA analysis if the mutation(s) are known.

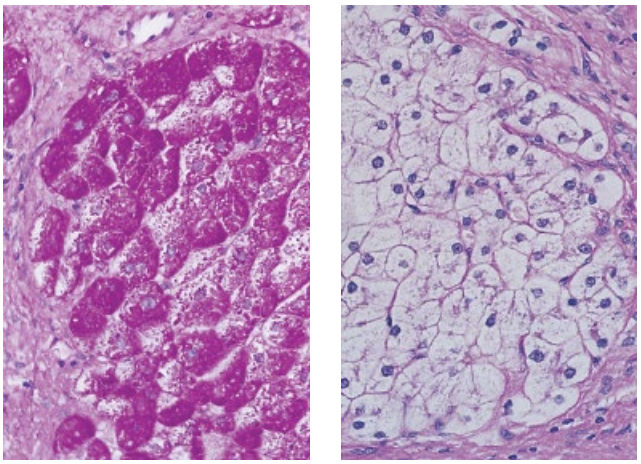

(A) (B)

Figure 19.2 Glycogen storage type IV typically demonstrates cytoplasmic inclusions of the abnormal glycogen (amylopectin) (A), which is completely removed by diastase (B).

Management and outcome

Dietary management with continuous nasogastric feeding and/or cornstarch may help improve growth and muscle strength. Liver transplantation is an effective treatment for those patients who develop progressive liver failure. To date, 18 patients with GSD IV are reported to have undergone liver transplantation, mortality was due to sepsis, hepatic artery thrombosis, and cardiomyopathy. Two patients required a second transplant. The survivors of the liver transplantation have not developed any neurological, muscular, or cardiac complications up to 13.5 years after transplantation, and some have had a progressive reduction in myocardial amylopectin storage.

Deficiencies of the liver phosphorylase system: glycogen storage diseases types VI and IX

Defects of the phosphorylase system are either due to deficient phosphorylase enzymes or to defects of the phosphorylase-activating system. These systems are enzymatically distinct in the liver and skeletal muscles. Muscle phosphorylase deficiency (GSD VI) presents in adulthood with muscle cramps and exercise intolerance; it does not involve the liver and will not be discussed further. Of the liver phosphorylase system defects, phosphorylase kinase defects (GSD IX) are much more common than liver phosphorylase deficiency (GSD VI).

The liver phosphorylase gene is located on chromosome 14q21–22. The genetics of the phosphorylase kinase system is very complex, as the enzyme consists of four subunits encoded on different genes (X chromosome as well as autosomes), which are differentially expressed in different tissues. The clinical expression of individual enzyme deficiencies and isoforms is variable and depends on both the severity and the distribution of the enzyme defect.

Presentation

Patients with GSD VI usually present with hepatomegaly and growth failure in early childhood. Symptoms of hypoglycemia, hyperlipidemia, and hyperketosis are mild if present. Plasma lactate and urate concentrations are usually normal. There is no cardiac or skeletal muscle involvement, and the condition has a benign course, with a reduction in hepatomegaly after puberty.

Phosphorylase kinase deficiency (GSD IX) is clinically and genetically more heterogeneous. Seventy-five percent of the patients have X-linked liver phosphorylase deficiency (type IXa), which manifests between the ages of 1 and 5 years with hepatomegaly, growth retardation, mild hypoglycemia, and mild elevation of hepatic transaminases, cholesterol, and triglycerides. Hepatomegaly and growth retardation usually resolve after puberty.

Autosomal recessive forms of phosphorylase kinase deficiency (GSD IXb, c) present with more severe liver disease, which may progress to cirrhosis, with or without skeletal myopathy. Rare variants with isolated muscle and cardiac involvement have also been described.

Diagnosis

Liver histology reveals nonuniform distension of hepatocytes with fibrosis and small fat droplets. On electron microscopy, the cytoplasmic glycogen particles are seen in rosette formation, less compact than normal, with a frayed pattern. Definitive diagnosis of phosphorylase and phosphorylase kinase deficiencies rely on specific enzyme assays in affected tissues, i.e., liver or muscle. Enzymes can be measured in leukocytes and erythrocytes, but the presence of different isoenzymes can make interpretation difficult. DNA analysis is possible and sequencing of the GSD gene panel makes the diagnosis easier to achieve.

Management and outcome

Treatment for these conditions is symptomatic. Frequent feeds and overnight feeding, with or without cornstarch, may be necessary for more severely affected patients, but many do not require any specific intervention. The tendency to hypoglycemia diminishes with age, and catch-up growth usually occurs without any specific treatment.

Fanconi–Bickel syndrome (glycogen storage disease type XI)

This rare disorder is characterized metabolically by hepatorenal glycogen accumulation and fasting hypoglycemia, postprandial hyperglycemia, and hypergalactosemia. The disorder is due to defective function of the GLUT2 transporter, which is the most important glucose transporter in hepatocytes, pancreatic β-cells, enterocytes, and renal tubular cells. Deficiency results in impaired import and export of glucose and galactose in affected tissues. Hypoglycemia is due to impaired glucose transport from the liver and defective renal reabsorption of glucose and galactose. Hepatic and renal glycogen accumulation results, leading to impaired tubular function, Fanconi nephropathy, and rickets. Over 100 patients are currently known.

GLUT2. The *GLUT2* gene has been localized to chromosome 3q26.1-q26.3, and 69 different mutations have been reported. Parental consanguinity is commonly observed.

Presentation

The main clinical features are hepatomegaly secondary to glycogen storage, and renal tubular dysfunction. Presentation in infancy includes recurrent vomiting, fever, failure to thrive, and hypophosphatemic rickets, while in early childhood, short stature, protuberant abdomen, hepatomegaly, moon-shaped facies, and fat deposition around the shoulders and abdomen is usual. Fasting hypoglycemia is a frequent finding, although symptomatic hypoglycemia is rare. Chronic diarrhea due to sugar malabsorption may occur. Rickets and osteoporosis lead to pathological fractures. Mild abnormalities of liver function are common, but hepatic adenomas and malignancies have not been reported.

Diagnosis

Characteristic findings on investigation include:

- Postprandial hyperglycemia and hypergalactosemia.
- Fasting hypoglycemia.
- Mildly abnormal liver function tests.
- Generalized renal tubular reabsorption defects: glycosuria, galactosuria, generalized aminoaciduria, phosphaturia, hypercalciuria, hyperuricosuria, mild proteinuria.
- Mild metabolic acidosis related to renal bicarbonate loss.
- Increased liver glycogen content on biopsy specimens.

The diagnosis can be confirmed by DNA mutation analysis. Antenatal diagnosis can be performed by DNA analysis.

Management and outcome

There is no definitive therapy available. Treatment is supportive, and includes replacement of water and electrolytes, alkalinization with bicarbonate solutions, vitamin D and phosphate supplementation, galactose restriction, and frequent small meals. Uncooked cornstarch may be useful. Fructose may be used as an alternative carbohydrate source in patients with malabsorption, as its absorption is not mediated by GLUT2.

The prognosis is good, and many patients reach adulthood in a stable condition, including the original patient described by Fanconi and Bickel; short stature appears to be the major subjective long-term problem.

Galactosemia

Galactosemia usually results in severe liver dysfunction in the neonatal period or early infancy, and is discussed in detail in Chapter 9.

Hereditary fructose intolerance [6]

Fructose is an important dietary source of carbohydrate. It is metabolized in the liver, renal cortex, and small intestine by three enzymes: fructokinase, aldolase B, and triokinase. HFI is caused by deficiency of aldolase B, resulting in an inability to convert fructose-1-phosphate into dihydroxyacetone phosphate and glyceraldehyde. Ingested fructose accumulates as fructose-1-phosphate, which has two major consequences:

1. Hypoglycemia, resulting from inhibition of the glycogenolytic enzyme glycogen phosphorylase and impaired gluconeogenesis due to an inability to condense glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.
2. Depletion of the nucleotides adenosine triphosphate (ATP) and guanosine triphosphate (GTP), as a consequence of their high utilization and sequestration in the formation of large amounts of fructose-1-phosphate. ATP is believed to lead to impaired protein synthesis and ultimately to liver and renal dysfunction.

The incidence may be as high as 1 in 23,000 live births in the UK. HFI is inherited as an autosomal recessive trait, caused by mutations in the *ALDOB* gene. About 20 mutations are known; three common mutations, A149P, A147D, and N334K, account for around 84% of *ALDOB* mutations in Europe.

Presentation

Infants and adults with HFI are asymptomatic until fructose, sucrose, or sorbitol is ingested. The age of presentation depends on the timing of the introduction of these sugars. Typically, the first symptoms occur during weaning when fruits and vegetables are introduced into the diet and include gastrointestinal discomfort and hypoglycemia following fructose-containing meals. Nausea, vomiting, pallor, sweating, lethargy, tremors, and seizures may occur. If the condition remains unrecognized and fructose intake continues, failure to thrive, signs of liver disease (hepatomegaly, jaundice, coagulopathy), and proximal renal tubular dysfunction (renal tubular acidosis, hypophosphatemic rickets) develop. Younger infants and children may be at risk of death from liver and renal failure.

HFI patients who survive beyond infancy develop an aversion to sweet foods and self-select a low-fructose diet. School-age children may avoid social situations that require them to ingest sugar-containing foods, which can be misinterpreted as psychotic behavior. Characteristically, patients with HFI have caries-free teeth, and the diagnosis may be suspected by dentists. Some individuals are diagnosed only during family testing or for investigation for growth retardation or isolated hepatomegaly; others are recognized only after receiving inadvertent fructose- or sorbitol-containing infusions, sometimes with fatal results.

Diagnosis

Characteristic findings are:

- Increased conjugated bilirubin.
- Hypoalbuminemia.
- Increased hepatic aminotransferases.
- Hypoglycemia.
- Lactic acidosis.
- Low plasma phosphate.
- Plasma tyrosine and methionine may be elevated secondary to liver dysfunction.
- Anemia, acanthocytosis, and thrombocytosis.
- Positive urine-reducing substances, fructosuria, proteinuria, generalized aminoaciduria.
- Abnormal renal tubular function tests.

The diagnosis is confirmed by:

- Mutation analysis.
- Enzymatic deficiency (liver or intestinal mucosal biopsy).

Hepatic pathology varies from hepatic necrosis in infants, who present with acute liver failure, to diffuse steatosis, periportal or lobular fibrosis, or cirrhosis (Figure 19.3). Electron microscopy demonstrates the pathognomonic punched-out areas between cytoplasmic organelles known as "fructose holes."

Management and outcome

Management consists of eliminating fructose, sucrose, and sorbitol from the diet for life. Sucrose and sorbitol are frequently used as sweeteners in syrups and suspensions, as well as in tablet coatings and toothpaste; the suitability of all medications must be checked before prescribing. Fructose elimination usually results in a dramatic improvement in hepatic function, with regression of fibrosis and prevention of cirrhosis, as well as improvement in renal function. Provided liver and renal disease are not advanced, full restoration of normal health, growth, and development may be expected, although hepatomegaly may persist for years after adequate treatment. Life-threatening fulminant hepatic failure may develop on the reintroduction of fructose, sucrose, or sorbitol. The development of hepatoma has been reported.

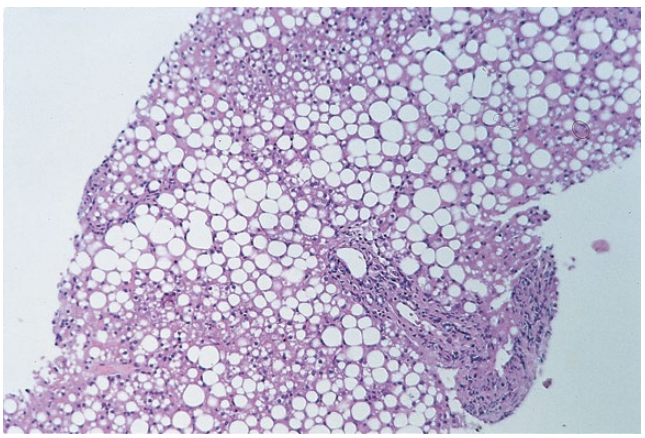


Figure 19.3 Hereditary fructose intolerance is associated with severe steatosis on liver histology. Persistent ingestion of fructose leads to cirrhosis.

Fructose-1,6-bisphosphatase deficiency [7]

Fructose-1,6-bisphosphatase deficiency results in impaired gluconeogenesis from all precursors, including fructose. Maintenance of normal glucose concentrations therefore depends on dietary glucose and galactose and on hepatic glycogenolysis. Hypoglycemia results when these sources of glucose are exhausted. Accumulation of the gluconeogenic precursors lactate, alanine, and glycerol occurs. It is a rare disorder and its incidence is unknown.

The condition is inherited as an autosomal recessive trait. Liver and muscle express distinct enzymes, and the muscle enzyme is not involved in the disorder. The liver enzyme is encoded for by the *FBP1* gene, and several mutations have been described.

Presentation

Fructose-1,6-bisphosphatase deficiency is a life-threatening condition; about half of the affected patients become symptomatic in the newborn period, with lactic acidosis and hypoglycemia. Presenting symptoms include hyperventilation secondary to profound lactic acidosis, irritability, hypotonia, somnolence, apneic spells, coma, convulsions, and hepatomegaly. Other patients may present in infancy or early childhood with hypoglycemia and acidosis triggered by a febrile illness. Subsequent attacks may be triggered by intercurrent illnesses, though patients remain very well between attacks. The condition can be misdiagnosed as mitochondrial respiratory chain disease or GSD. Ingestion of large quantities of fructose or sucrose is known to precipitate acute decompensation, although in contrast to HFI, children do not avoid sweet foods.

Diagnosis

Characteristic findings are:

- Hypoglycemia.
- Increased plasma lactate and ketones.
- Metabolic acidosis.
- Increased free fatty acids.
- Hyperuricemia.
- Normal liver and renal function.

The diagnosis is confirmed by DNA mutation analysis of the *FBP1* gene or by demonstrating the enzyme deficiency in leukocytes, lymphocytes, or liver biopsy. The enzyme is not expressed in skin fibroblasts or amniotic cells. Antenatal diagnosis is possible by mutation analysis.

Management and outcome

Treatment of acute attacks consists of infusions of high-concentration glucose and bicarbonate to correct hypoglycemia and acidosis. The basic principle of long-term management is avoidance of fasting, particularly during intercurrent illnesses; overnight gastric drip feeding may be required in very young infants. Dietary fructose and sucrose may have to be restricted, particularly during febrile illnesses. After diagnosis and institution of adequate management, the condition follows a relatively benign course with normal growth and development. Fasting tolerance improves with age, and may be normal in adults.

Transaldolase deficiency [8]

The pentose phosphate pathway is essential for the production of the 5-carbon sugar, ribose, for neucleotide and nucleic acid synthesis, and also for the reduction of nicotinamide adenine dinucleotide phosphate (NADP) to the reduced form (NADPH), which is important in combating cellular oxidative stress. Three defects in this pathway have been described, and one of these, transaldolase deficiency (TALDO), is associated with significant liver pathology.

Presentation

A broad range of phenotypes has been described in this rare and recently described disorder, but most patients have antenatal or neonatal presentation with hydrops fetalis, intrauterine growth retardation, neonatal hepatosplenomegaly, cholestasis, bleeding diathesis, and liver failure. Liver cirrhosis and fibrosis are seen in older patients. Multisystem involvement with congenital cardiac malformations, facial dysmorphism, cutis laxa, and renal disease (tubulopathy, nephrocalcinosis) have been described. Neurological involvement is usually mild. Early-onset hepatocellular carcinoma has been reported in one patient.

Diagnosis

The diagnosis is established by urine analysis for polyols (ribitol, erythritol, arabitol) and seven-carbon sugars (such as sedoheptulose) and confirmed by genetic analysis of the *TALDO1* gene for pathogenic mutations. Plasma analysis may not reveal typical abnormalities. Krebs cycle intermediates may be found on urine organic acid analysis. Prenatal diagnosis is possible by amniotic fluid analysis for polyols or by mutation analysis.

Management and outcome

There is no specific treatment currently available apart from supportive management. Outcome is determined by the severity of neonatal liver failure. Liver manifestations may improve in patients who survive the neonatal period.

Lysosomal storage disorders [9–11]

The lysosomes are intracellular organelles containing a large number of different enzymes at acid pH whose main function is the degradation of macromolecules. However many other functions have more recently been ascribed to lysosomes including secretion, nutrient sensing, and cell signaling. The LSDs are each due to a specific enzyme deficiency resulting in abnormal storage of partially degraded macromolecules in the lysosomes. They can be considered as three groups (Table 19.3).

Table 19.3 Lysosomal storage disorders associated with hepatosplenomegaly.

Disorder	Enzyme defect	Clinical features	Hepatosplenomegaly	Outcome
Sphingolipid and lipid storage disorders				
G_{M1} gangliosidosis	β-galactosidase	Neurodegeneration, dysostosis multiplex, coarse features, hepatosplenomegaly, cherry-red spot. Infantile, juvenile and adult forms recognized	Infantile form: ++ Juvenile forms: – or + Adult form: –	Infantile: death by 2 years Juvenile: death 3–10 years Adult: onset 2nd–4th decade, slow neurodegeneration
Gaucher	β-glucosidase	Hepatosplenomegaly, bone and lung infiltration. Neurological (type II), non-neurological (type I) and intermediate (type III) forms	++ to +++	Type I (common): prolonged survival, death due to pulmonary or hematological complications Type II: death in infancy Type III: variable survival into childhood; enzyme replacement therapy available
Niemann–Pick A and B	Sphingomyelinase	Hepatosplenomegaly, lung infiltration, neurological (A) and non-neurological (B) types	++ to +++	Type A: death in infancy Type B: relatively normal lifespan
Niemann–Pick C	Cholesterol and lipid trafficking defect	Neonatal liver failure or cholestasis, hepatosplenomegaly, vertical ophthalmoplegia, ataxia, later neurodegeneration	++ to +++	Death 1–3 decades after onset of neurodegeneration
Wolman disease and cholesterol ester storage disease	Acid esterase	Hepatosplenomegaly, steatorrhea, failure to thrive, adrenal calcification, neurodegeneration. Cholesterol ester storage disease a mild variant, causing hepatic fibrosis in adults	++	Wolman: death in infancy. Cholesterol ester storage disease: death from liver failure in adulthood
Farber disease	Ceramidase	Psychomotor deterioration, subcutaneous nodules, painful and deformed joints	+ to ++	Death in infancy; late-onset variants known
Mucolipidoses and glycoprotein storage disorders				
Mucolipidosis I (sialidosis)	α-Neuraminidase	Myotonic seizures, cherry-red spot, psychomotor retardation, hepatosplenomegaly, and dysostosis multiplex	– to ++	Severe cases: death in early childhood Milder cases: survival into adulthood, severely retarded
Mucolipidosis II (I-cell disease)	*N*-acetylglucosamine-1-phosphatase (defective enzyme transport into lysosomes)	Coarse facies, kyphoscoliosis, joint contractures, gingival hyperplasia, cardiomyopathy, dysostosis. Onset in infancy	++	Death by age 4–6 years from cardiopulmonary disease
Mucolipidosis III	Milder mutations in the same gene as in mucolipidosis II	Stiff joints, kyphoscoliosis, short stature, low–normal intelligence. Presentation by 3–4 years	+ to ++	Usually survive into adulthood with severe orthopedic problems and mild cardiac involvement
Galactosialidosis	Neuraminidase and β-galactosidase	Combined features of mucolipidosis I and GM1 gangliosidosis, onset usually in late childhood	++	Survival into adulthood usual, with variable degree of mental retardation
Fucosidosis	α-Fucosidase	Psychomotor retardation, mild dysostosis multiplex, angiokeratoma, visceromegaly. Onset in early childhood	++	Severe form (type I): death by late childhood Mild form (type II): survival into adulthood, variable degree of mental retardation
α-Mannosidosis	α-Mannosidosis	Deafness, mild Hurler phenotype, mental retardation. Onset in infancy or early childhood	++ to +++	Slow intellectual deterioration, eventual developmental level at 5–7 years. Survival into adulthood usual
Sialic acid storage disorder	Defective sialic acid transport out of lysosomes	Infantile form (infantile sialic acid storage disorder): psychomotor retardation, coarse facies, hepatosplenomegaly, cardiomyopathy, onset in first few months of life Late-onset form (Salla disease): ataxia, nystagmus, developmental delay in childhood; severe mental retardation in adulthood	Infantile form: ++ to +++ Late-onset form: –	Infantile form: rapid deterioration, death by 1–5 years Late-onset form: prolonged survival with slow neurodegeneration; average age at death 30–40 years

Mucopolysaccharidoses				
MPS I (Hurler or Scheie)	α-L-Iduronidase	Hurler: psychomotor retardation, coarse facial features, growth retardation, dysostosis multiplex, corneal clouding, visceromegaly. Milder features in Scheie syndrome	Hurler: ++ Scheie: – to +	Hurler: psychomotor retardation and death by 8–10 years Scheie: normal intellect and lifespan; orthopedic problems common. Enzyme replacement therapy available
MPS II (Hunter)	Iduronate 2-sulfatase	Symptoms similar to Hurler syndrome, no corneal clouding. Rare milder variant with no mental retardation	Severe: ++ Mild: – to +	Death by mid-teens Milder variant: normal life span. Enzyme replacement therapy available
MPS III (Sanfilippo) • Type A • Type B • Type C • Type D	*N*-sulfoglucosamine sulfohydrolase *N*-α-acetylglucosaminidase Heparan acetyl-CoA: α-glucosaminide *N*-acetyltransferase *N*-acetylglucosamine-6-sulfatase	Commonest MPS disorder in UK, identical phenotype in all types. Marked mental retardation, severe behavioral and sleep disturbance. Mild somatic involvement, mild coarse features, no corneal clouding	All forms: – to +	Survival into late teens or adulthood with severe mental retardation
MPS IV (Morquio) • Type A • Type B	Galactosamine-6-sulfate sulfatase β-galactosidase	Both types phenotypically similar. No mental retardation, severe skeletal deformities and growth retardation, cervical myelopathy a potentially fatal hazard, mild corneal clouding	Both forms: +	Survival into adulthood common if death does not occur earlier due to cervical myelopathy. Cardiopulmonary compromise later due to thoracic deformity
MPS VI (Maroteaux–Lamy)	Arylsulfatase B	Skeletal deformities similar to Hurler syndrome, but no mental retardation. Variable cardiac involvement, mild corneal clouding	+	Severe forms: survival into late teens Mild form: normal lifespan. Enzyme replacement therapy available
MPS VII (Sly)	β-glucuronidase	Variable phenotype ranging from hydrops fetalis to mild adult type similar to MPS I	++	Variable, depending on severity

MPS, mucopolysaccharidosis.
–, Absent; +, mild; ++, moderate; +++, marked.

The clinical spectrum of the storage disorders is wide, ranging from prenatal hydrops fetalis to mild disease in adulthood. Suggestive signs include coarsening of facial features, neurological deterioration, and hepatosplenomegaly. Patients with storage disorders often have a characteristic skeletal dysplasia (dysostosis multiplex), with a large skull, spinal deformities, and short, thick tubular bones. The liver and spleen are important sites for abnormal lysosomal storage, and hepatosplenomegaly is thus a frequent finding, but the clinical picture is dominated by neurodevelopmental regression. Nevertheless, Gaucher disease, Niemann–Pick disease, Wolman disease and cholesterol ester storage disease have important hepatic manifestations. There have been major developments in the treatment possibilities for LSD, especially enzyme replacement therapy (ERT).

Gaucher disease

Gaucher disease has a global frequency of 1 in 200,000. It is caused by deficiency of β-glucosidase (glucocerebrosidase), resulting in accumulation of glucosylceramide, a normal intermediate in the synthesis and catabolism of complex glycosphingolipids. Gaucher disease is classified according to the presence and severity of neurological manifestations:

- Type 1 (non-neuronopathic) Gaucher disease includes patients without neurological manifestations.
- Type 2 (acute neuronopathic) Gaucher disease includes patients with neurological involvement presenting in infancy.
- Type 3 (subacute neuronopathic) Gaucher disease presents in childhood and is associated with variable neurological manifestations.

Type 1 is the most common subtype; the relative frequencies of types 1, 2, and 3 Gaucher disease are 94%, 5%, and 1%, respectively The hallmark of this condition is the accumulation of characteristic tissue macrophages (Gaucher cells), which have a "crumpled-paper" appearance histologically, due to abnormal accumulation of phagocytosed glycosphingolipids. Gaucher cells are found in all tissues, and clinical manifestations reflect the sites and extent of abnormal glycosphingolipid storage. The main storage sites are the liver, spleen, and bone marrow, although significant storage also occurs in the central nervous system, lymph nodes, lungs, and glomerular mesangium. Some of the pathological consequences in these tissues may relate to vascular blockage by the Gaucher cells. The major accumulating lipids, glucosylceramide and glucosylsphingosine, also induce the tissue macrophages to secrete a large number of proinflammatory cytokines, which are believed to mediate the acute inflammatory responses and tissue damage that underlie the clinical and biochemical manifestations.

The gene has been localized to chromosome 1q21 and over 430 mutations have been described. Six common mutations (N370S, 84GG, IVS2(+1), V394L, R496H, and L444P) account for >90% of Jewish and 60–70% of non-Jewish alleles. Broad phenotype–genotype correlations exist, with the N370S allele generally associated with milder and non-neuronopathic disease, and the L444P allele strongly associated with neuronopathic disease.

Presentation

Clinical expression is heterogeneous, ranging from "congenital" Gaucher disease presenting with hydrops fetalis to asymptomatic glucocerebrosidase deficiency.

Type 1 Gaucher disease. The age at presentation varies from childhood to late adulthood. Presenting features are related to:

- Growth retardation.
- Bone pain.
- Hepatosplenomegaly.
- Abdominal pain from hepatic or splenic infarction.
- Hypersplenism.

Without treatment, progressive splenomegaly leads to transfusion dependency, and the enlarged spleen may rupture with trauma. Liver fibrosis has been reported, but cirrhosis is rare. Clinically, hepatic complications include portal hypertension, abnormal liver function, and liver infarction. The extent of bone involvement is a major determinant of long-term morbidity, and most adults with Gaucher disease develop complications including bone pain, osteoporosis, lytic lesions, pathological fractures, and avascular necrosis. Other significant complications include bone marrow failure, pulmonary hypertension, and an increased risk of lymphoproliferative malignancy.

Type 2 Gaucher disease. Type 2 differs from type 1 disease, as the presentation is in early infancy with:

- Marked hepatosplenomegaly.
- Severe neurological involvement characterized by paralytic squint, dysphagia, persistent head hyperextension, trismus, and generalized spasticity.

Death usually occurs by 2 years of age, associated with progressive psychomotor regression and brainstem dysfunction.

Exceptional patients have a prenatal onset with hydrops fetalis, or a later onset with similar but slower progression.

Type 3 Gaucher disease. Type 3 has intermediate severity between types 1 and 2. The main clinical features include:

- Severe visceromegaly, which can lead to death from liver disease and portal hypertension in the second to fourth decades.
- Characteristic oculomotor apraxia (horizontal supranuclear gaze palsy).
- Dementia, ataxia, spasticity, and epilepsy (myoclonic or complex partial seizures) develop over time and progress at variable rates.
- Other patients have mild systemic manifestations, but severe progressive neurological involvement leads to death in childhood from neurological complications.

Diagnosis

Supportive tests include:

- Demonstration of Gaucher cells in bone marrow aspirate or liver biopsy (Figure 19.4). Gaucher cells are not specific for Gaucher disease and have been described in leukemia, lymphoma, thalassemia, multiple myeloma, and acquired immunodeficiency syndrome (AIDS) complicated by tuberculosis.
- Marked elevation of plasma angiotensin-converting enzyme (ACE), tartrate-resistant acid phosphatase (TRAP), and/or chitotriosidase or the chemokine CCL18/PARC.

A number of *non-specific* hematological and biochemical abnormalities may be present:

- Anemia, leukopenia, thrombocytopenia.
- Abnormal hepatic transaminases.
- Abnormal coagulation and fibrinolytic tests.
- Increased ferritin and transcobalamin.
- Polyclonal hypergammaglobulinemia.

Specific diagnostic tests are:

- Glucocerebrosidase assay (leukocytes or cultured fibroblasts).
- Mutation analysis.

Management and outcome

Recombinant enzyme therapy is the main treatment for types 1 and 3 disease, allowing many individuals to live near-normal lives. No satisfactory treatment is available for type 2 disease.

ERT results in rapid improvement of liver, splenic, and bone marrow pathology, with corresponding clinical improvement, but skeletal disease is slow to respond and may be resistant to ERT. Neurological disease as well as advanced liver, splenic, and bone marrow disease are not reversible by ERT.

Substrate deprivation therapy is a recent development in the treatment of storage disorders. In lysosomal disorders, there is an imbalance between the rate of production of a particular substrate and its catabolism, leading to accumulation within the lysosome. Substrate deprivation therapy aims to restore this balance by reducing the rate of synthesis of the substrate. Miglustat (*N*-butyldeoxynojirimycin) inhibits glucosyltransferase and impairs the synthesis of glycosphingolipids, which accumulate in a number of storage disorders. This product has been used in the treatment of non-neuronopathic Gaucher disease with favorable results and few side effects.

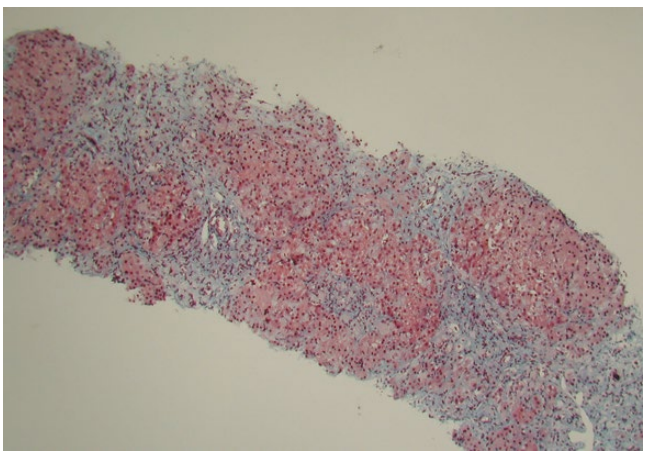


Figure 19.4 Gaucher disease affects reticuloendothelial cells in the liver, bone marrow, and lung. Liver histology shows fibrosis and Gaucher cells around the portal tract.

Supportive and symptomatic treatment such as pain relief, bisphosphonates, calcium, vitamin D, steroids, bone marrow transplantation, splenectomy, and liver transplantation are important in managing patients with Gaucher disease. Splenectomy is indicated only if severe hypersplenism is resistant to ERT or for splenic rupture, as acceleration of neurological, hepatic, and pulmonary disease has been reported following splenectomy.

Niemann–Pick disease

Niemann–Pick disease is a group of storage disorders that are associated with a particular storage cell with a morphological appearance resembling "foamy" histiocytes, as a result of sphingomyelin storage. Currently, three forms of Niemann–Pick disease are recognized: types A, B, and C.

Types A and B

Types A and B disease are autosomal recessive disorders caused by deficiency of the lysosomal enzyme sphingomyelinase. Niemann–Pick disease type A (NPA) is the infantile neurodegenerative phenotype, whereas type B (NPB) is defined by the absence of neurological involvement, with relatively late-onset hepatosplenomegaly and survival into adulthood. The sphingomyelinase gene has been localized to chromosome 11p15.4, and about 180 mutations are known. With NPA disease, three mutations (R496L, L302P, and fsP330) account for >95% of mutant alleles in the Ashkenazi Jewish population; ΔR608 appears to be a relatively common type B mutation. Several other "private" mutations have been identified in Jewish and non-Jewish families with NPA and NPB.

The spleen, lymph nodes, liver, bone marrow, kidneys, and lungs are the main sites of storage in both types A and B, while type A patients also accumulate sphingomyelin in the brain.

Presentation

Type A. The clinical presentation of type A is uniform and includes the following findings:

- A protuberant abdomen with massive liver and spleen enlargement becomes apparent in the first few months of life (usually by 3–4 months).
- Lymphadenopathy.
- Prolonged neonatal jaundice due to giant cell transformation in some cases.

- Early neurological features include feeding difficulties, hypotonia, and muscular weakness.
- Recurrent vomiting and constipation.
- Repeated chest infections and aspiration pneumonia; a chest radiograph often reveals alveolar infiltration with a diffuse reticular or finely nodular pattern of the lung fields.
- A cherry-red macular spot is seen on ophthalmological examination in about 50% of patients.
- Psychomotor retardation becomes evident after 6 months, with progressive loss of developmental milestones; eventually, all motor and intellectual abilities are lost.
- Failure to thrive, spasticity, and rigidity are prominent features in the later stages.
- Cirrhosis and multiple hepatocellular adenomas have been described.
- Death occurs by 2–3 years of age, usually from respiratory complications.

Type B. The clinical presentation of type B is more variable. Some individuals may not be diagnosed until adulthood, as there may be minimal clinical manifestations. The features include the following findings:

- Hepatosplenomegaly, which may become less apparent as the child grows older. Progressive liver disease with biliary cirrhosis, portal hypertension, and ascites has been reported. Hypersplenism may lead to pancytopenia or splenic rupture.
- Pulmonary involvement, in the form of diffuse reticular or finely nodular infiltration on chest radiography. Lung disease may be progressive and lead to chronic hypoxia, dyspnea, recurrent bronchopneumonia, and in extreme cases, cor pulmonale.
- The long-term natural history is characterized by hepatosplenomegaly with progressive hypersplenism, a worsening atherogenic profile, gradual deterioration in pulmonary function, and stable liver dysfunction.
- Neurodevelopmental complications are rare, but neurological involvement such as cherry-red maculae, ataxia, parkinsonism, and mental retardation have been recorded; these patients are thought to have an intermediate phenotype between types A and B.

Diagnosis

The diagnosis may be suspected when the characteristic Niemann–Pick "foam" cells are found on bone marrow aspirate or on a liver biopsy. Niemann–Pick cells are not diagnostic of sphingomyelinase deficiency, as these cells may also be seen in other storage disorders, including Niemann–Pick type C disease, cholesterol ester storage disease, Wolman disease, and G_{M1} gangliosidosis.

Specific diagnostic tests are:

- Sphingomyelinase assay (leukocytes, lymphocytes, or skin fibroblasts).
- Mutation analysis.

Antenatal diagnosis is possible by mutation analysis or when mutation is not known by assaying sphingomyelinase activity in chorionic villi or cultured amniocytes.

Management and outcome

Currently, no specific treatment is available for NPA and NPB, and management is entirely supportive. Liver transplantation in an infant with NPA and amniotic cell transplantation in NPB patients have been attempted, with little success. Early bone marrow transplantation failed to prevent progressive neurodegeneration in one infant; however, bone marrow transplant was successful in reducing the size of the liver and spleen as well as improving the radiographic appearance of the lungs in one child with severe type B disease.

ERT is potentially useful in type B disease, and clinical trials are in progress; if these are successful, ERT may become the treatment of choice for NPB disease in the near future. Developments with gene therapy are ongoing.

Type C

Niemann–Pick disease type C (NPC) originally referred to a group of patients who had the classical histopathological findings of "foamy" histiocytes and increased tissue sphingomyelin, along with a slowly progressive neurological illness. It is now known that NPC disease is clinically, biochemically, and genetically distinct form NPA and NPB; it is caused by a defect of intracellular lipid trafficking.

The estimated incidence of NPC is approximately 1 in 150,000, making it more common than NPA and NPB combined. It is inherited in an autosomal recessive manner. The first defective gene (*NPCI*) has been localized to chromosome 18q11-12 in >95% of patients. It codes for an endosomal membrane protein that plays an important role in intracellular cholesterol and glycosphingolipid trafficking, although the exact function of the protein is not known. Mutations in *NPC1* result in impaired cholesterol esterification and accumulation of cholesterol and sphingolipid species in the endolysosomal compartment of multiple cell types. Over 390 mutations have been described, and three mutations (I1061T, P1007A, and G992W/G992R) occur more commonly in some populations. Genotype–phenotype correlation may be possible on the basis of the type and location of the mutation within the NPC1 protein. Less than 5% of patients with NPC have a defect in another gene, *NPC2*, which has been localized to chromosome 14q24. The product of *NPC2* is a small (132 amino acid), soluble, ubiquitously expressed lysosomal protein that has a high affinity for unesterified cholesterol (Figure 19.5). In vitro studies suggest that NPC1 and NPC2 act in concert to facilitate the intracellular trafficking of lysosomal lipids. Although broad genotype–phenotype correlation is possible, especially with *NPC2* mutations, discrepancy in the phenotype has been described in some affected sibling pairs.

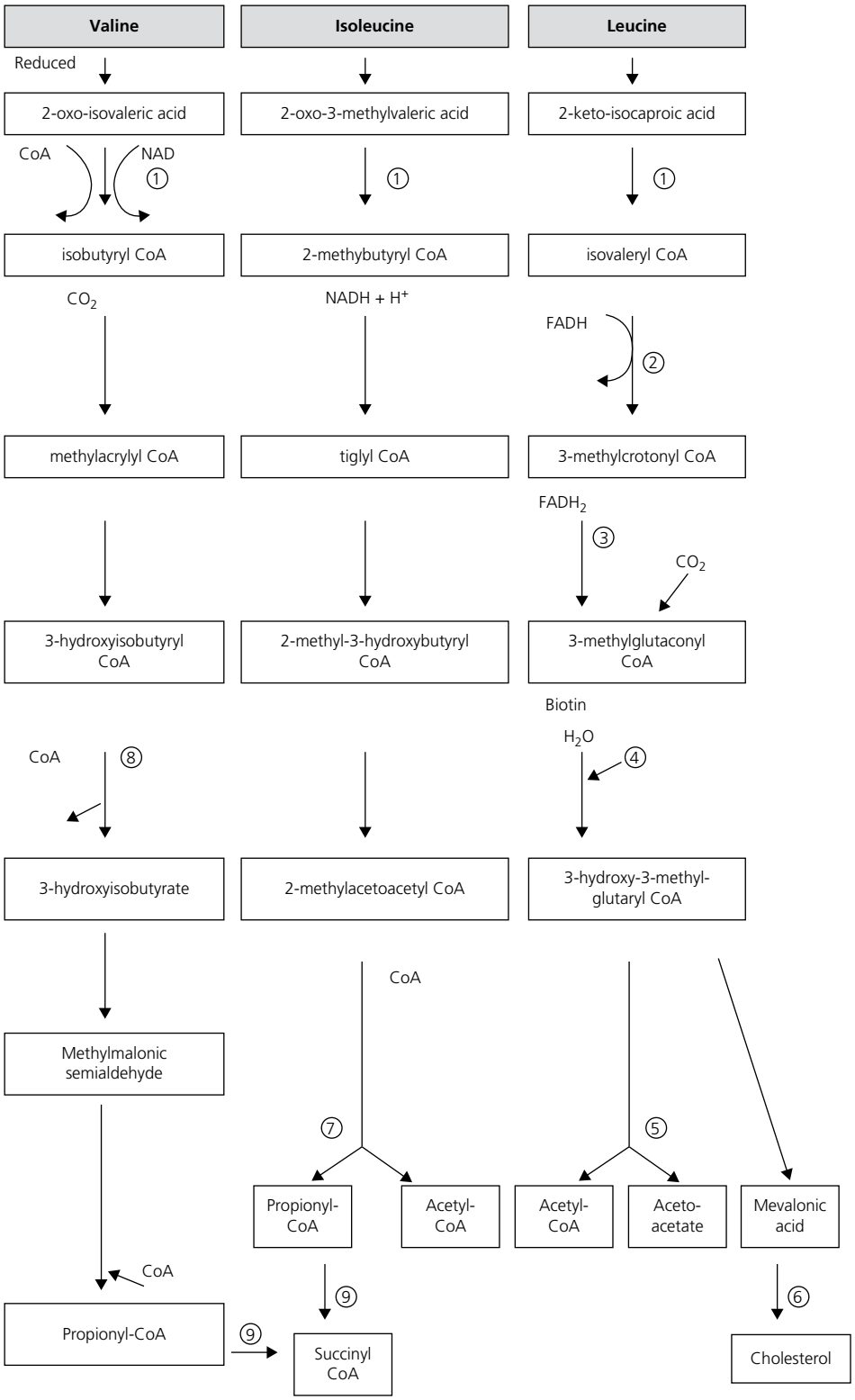
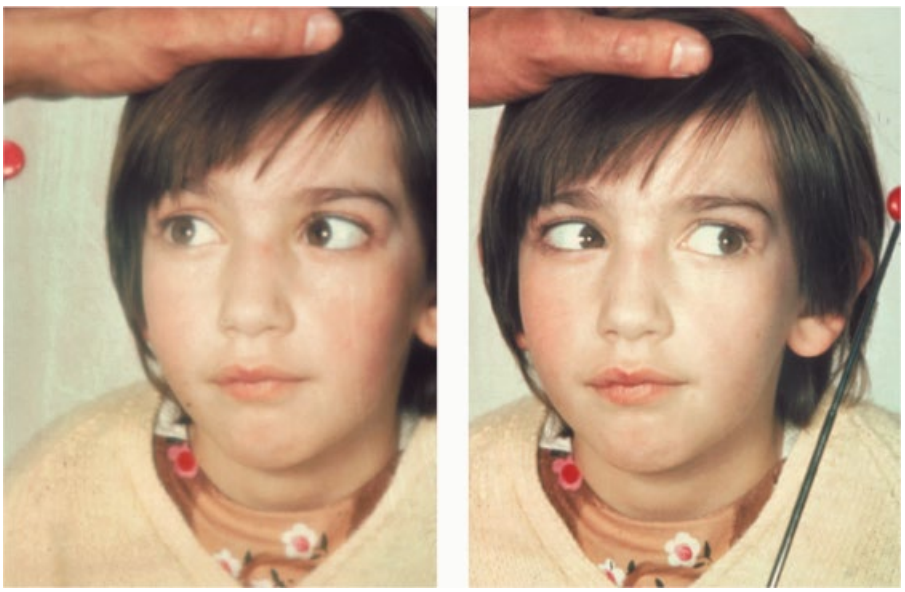


Figure 19.5 Disorders of branched amino acid metabolism. (1) Maple syrup urine disease; (2) isovaleric acidemia; (3) methylcrotonylglycinuria; (4) methylglutaconic aciduria; (5) 3-hydroxy-3-methylglutaric aciduria; (6) mevalonic aciduria; (7) β-ketothidase deficiency; (8) 3-hydroxyisobutyric aciduria; (9) propionic and methylmalonic acidemia.

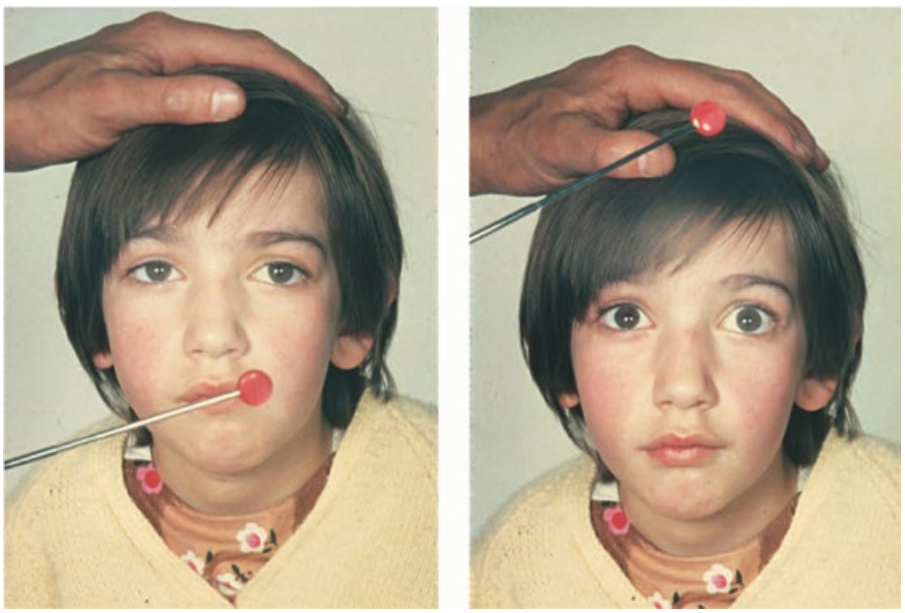
Presentation

The manifestations of NPC disease are extremely heterogeneous, and presentation can be at any time from intrauterine life to adulthood. The most common ("classic") phenotype presents in childhood with:

- Neonatal cholestasis, which is self-limiting (see Chapter 8).
- Hepatosplenomegaly is prominent in childhood, but becomes less apparent with advancing age, although portal hypertension has been reported.
- Clumsiness and ataxia.
- Early childhood development is usually normal, but behavioral problems may be noted as early as the preschool period.
- Supranuclear vertical gaze palsy, which is the neurological hallmark of this disorder and is found in virtually all childhood presenting cases by adolescence; it may manifest in early childhood as eye blinking and head thrusting on attempted vertical gaze and is due to a defect in initiating rapid saccades (Figure 19.6).
- Gelastic cataplexy (atonic seizures induced by emotional change).



(A)



(B)

Figure 19.6 Vertical supranuclear gaze palsy in Niemann–Pick disease type C. (A) The patient has a normal horizontal gaze. (B) Paralysis of upward gaze when the patient attempts to look upwards.

A number of publications divide the NPC patient cohort into three groups based on the age of onset of neurological symptoms. Approximately equal numbers of patients presented in each age group:

- *Neonatal-onset NPC*. This category is characterized by neonatal jaundice and a more aggressive clinical course, with developmental delay, spasticity, and progressive liver disease appearing in infancy. These infants do not survive beyond 5 years, and vertical supranuclear gaze palsy is rarely seen. In patients who survive the neonatal liver disease, characteristic progressive neurological signs, including vertical supranuclear gaze palsy, ataxia, dementia, and spasticity, appear over a variable time course over years to decades, similar to the childhood-onset form.
- *Childhood/juvenile-onset NPC*. The onset is typically with mild learning difficulties in early childhood (4–9 years), followed by a slowly progressive onset of supranuclear gaze palsy, ataxia, and spasticity. Gelastic seizures, cataplexy, and other seizure types commonly occur. Dementia usually appears in the teenage years. Death, commonly from respiratory complications, may occur from the teenage years to adulthood.
- *Adolescent/adult-onset NPC*. This presents with signs and symptoms similar to those of childhood-onset NPC, but in later life and with a more slowly progressive course.

A non-neuronopathic form of NPC has also been described in adults with isolated organomegaly.

In addition, very severe variants of NPC may present with hydrops fetalis and liver and respiratory failure, leading to death in early infancy. Severe pulmonary involvement leading to early death from respiratory failure may be associated with mutations in the *NPC2* gene.

Diagnosis

The diagnosis is suggested by the finding of liver dysfunction in the neonatal period, associated with foam cells and sea-blue histiocytes in liver or bone marrow histology (Figure 19.7).

Diagnostic tests are:

- Plasma chitotriosidase levels may be modestly elevated (20–30-fold) and can be a helpful clue to the diagnosis in a patient with a suggestive clinical picture.
- Accumulation of intracytoplasmic unesterified cholesterol in skin fibroblasts (by filipin staining).

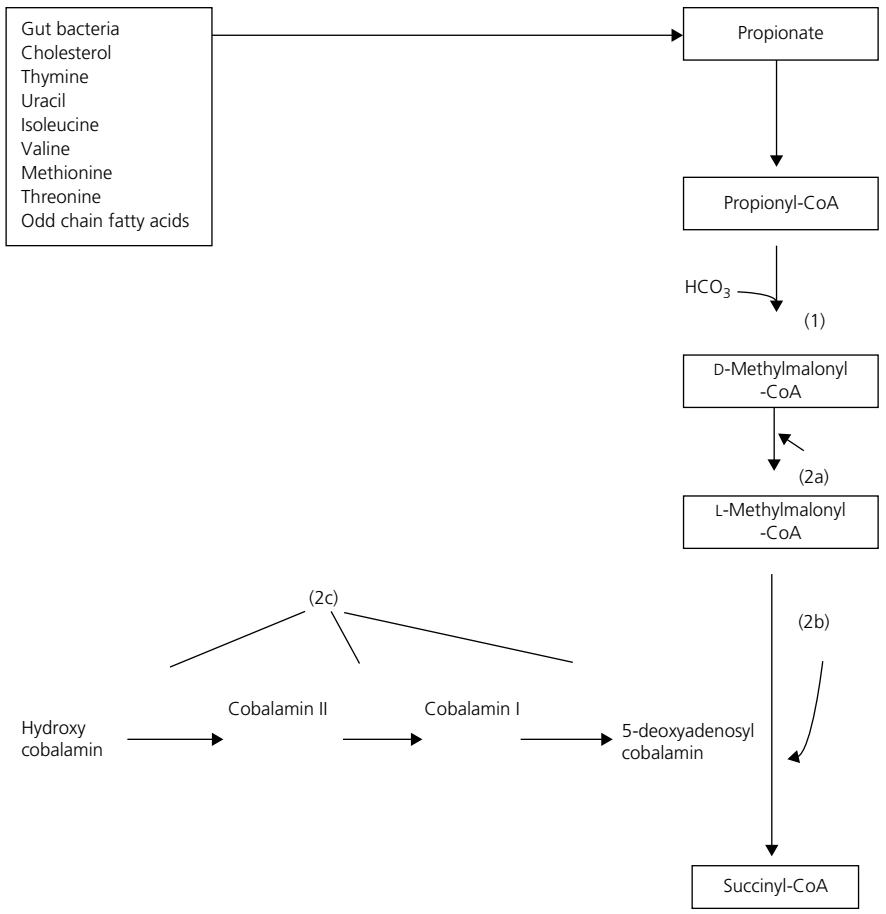


Figure 19.7 Disorders of propionate metabolism. (1) Propionic acidemia; (2a, 2b) methylmalonic acidemia due to mutase deficiency; (2c) methylmalonic acidemia due to defects in cobalamin metabolism.

- Defective cholesterol esterification (skin fibroblasts).
- Mutation analysis of the *NPC1* and *NPC2* genes.
- Novel diagnostic biomarkers such as plasma oxysterol and lysosphingomyelin analysis have been introduced recently and should make it easier to diagnose the cases earlier.

Antenatal diagnosis is possible by filipin staining, cholesterol esterification studies, and/or mutation analysis of cultured chorionic villous cells or cultured amniocytes.

Management and outcome

Currently, Miglustat (described above as treatment in type I Gaucher disease) is the only disease-specific treatment for NPC that is licenced for this indication in a number of countries outside the US. Glycosphingolipid accumulation in the brain is likely to contribute to the neuropathology of NPC disease. Miglustat which inhibits glucosyltransferase, impairs the synthesis of glycosphingolipids, and crosses the blood–brain barrier. In animal models of NPC, *N*-butyldeoxynojirimycin has been shown to delay the onset of neurological symptoms, reduce glycosphingolipid accumulation, and increase the average lifespan. The report from the therapeutic trial of miglustat in 29 juvenile and adult NPC patients indicated improvement in several neurological parameters – including horizontal saccadic eye movements, swallowing capacity, and auditory acuity – in comparison with standard care in an untreated control population. Long-term follow-up of patients suggests slowing of the neurodegeneration in patients on miglustat. Supportive and symptomatic management includes nutritional support for early liver disease, antiepileptic therapy, tricyclic antidepressants, or selective serotonine reuptake inhibitors for cataplexy and anticholinergics for dystonia and tremor. Various antispasmodics including botox injections may be offered for severe spasticity. Multidisciplinary support is essential in the later stages, when there is significant neurological and psychomotor disability.

A number of different therapeutic approaches have failed to halt neurological progression. Liver transplantation in a 7-year-old girl with NPC disease and hepatocellular carcinoma led to initial stabilization, but abnormal storage recurred in the transplanted liver and neurological deterioration continued. Similarly, bone marrow transplantation in a 2 year old resulted in regression of hepatosplenomegaly and decreased infiltration of foamy macrophages in the bone marrow and lung, but failed to prevent neurological deterioration. Combinations of cholesterol-lowering agents have reduced hepatic cholesterol stores, but have not altered the long-term outcome.

Although rare, NPC disease due to *NPC2* mutations may be treatable with bone marrow transplantation, as the NPC2 protein is a soluble molecule that is secreted by cells and is amenable to uptake into lysosomes via specific pathways. Most recently significant improvements in extending lifespan and halting neurodegeneration have been demonstrated in the animal models of NPC treated with 2-hydroxypropyl-β-cyclodextrin and the clinical trial of this compound in NPC patients are now in progress.

Wolman disease and cholesteryl ester storage disease (lysosomal acid lipase deficiency)

These two rare disorders are caused by a recessively inherited deficiency of lysosomal acid lipase (LAL-D) resulting in accumulation of cholesterol esters and triglycerides in most body tissues. The disorders are allelic conditions that represent extreme variants of the same enzyme deficiency, with some residual enzyme activity in cholesteryl ester storage disease (CESD). A number of secondary changes occur, including increased cholesterol synthesis, upregulation of low-density lipoprotein receptor gene expression, and increased lipoprotein production. These changes are more pronounced in Wolman disease, which is associated with more severe acid lipase deficiency than in CESD.

Wolman disease and CESD are recessively inherited. The gene has been located to chromosome 10q22.2-22.3, and over 50 mutations have been described. There is some genotype–phenotype correlation for CESD, with a common splice junction mutation in exon 8 (called $\Delta 254\text{-}277_1$). No common mutation has been described in Wolman disease.

Presentation

Wolman disease. Patients usually present in the first few weeks of life with vomiting and diarrhea, malabsorption, failure to thrive, and hepatosplenomegaly. Jaundice, low-grade pyrexia, anemia, abdominal distension, and leukopenia may be present initially. The most striking feature is adrenal calcification, which is demonstrable radiographically in most patients; other characteristic features are vacuolated lymphocytes in peripheral blood films and foam cells in bone marrow aspirates. Neurological signs and symptoms are not prominent, although lipid storage in neurons, microglia, and astrocytes, as well as delayed myelination may be found histologically. A rapid downhill course follows the initial presentation, and most patients die by 3–6 months of age.

CESD. The clinical manifestations are variable and less severe. The usual presenting feature is hepatomegaly in adult life, although liver enlargement is frequently detectable from early childhood. Liver dysfunction, splenomegaly, hyperlipidemia, and xanthelasma are often present. Malabsorption and adrenal calcification are rare. Hepatomegaly increases over time with fibrosis, and liver failure has been described. Although premature atherosclerosis and atheromas have been detected in autopsied patients, clinically significant coronary or systemic vascular atherosclerosis is rare.

Diagnosis

Suggestive abnormalities include:

- Adrenal calcification on abdominal radiography or ultrasound (decreased adrenal responsiveness may be found on provocative tests).

- Vacuolated lymphocytes on peripheral blood film.
- Sea-blue histiocytes in bone marrow aspirate.
- Liver dysfunction.
- Hypercholesterolemia and hypertriglyceridemia.

Specific diagnosis:

- Acid lipase assay (leukocytes or cultured fibroblasts) and/or DNA sequencing.

Liver histology in both conditions usually reveals enlarged and vacuolated hepatocytes and Kupffer cells, as well as large numbers of foamy histiocytes. Periportal fibrosis may be prominent and cirrhosis may also be evident. Foam cells may also be seen in bone marrow aspirates, spleen, and lymph nodes. In Wolman disease, small-intestinal biopsy usually reveals extensive infiltration of the lamina propria with foamy histiocytes.

Antenatal diagnosis is possible by direct enzyme assay in chorionic villus cells, or by mutation analysis.

Management and outcome

Lipid abnormalities in CESD can responds to 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, cholestyramine, a low-cholesterol diet, and fat-soluble vitamin supplements, with significant improvement in plasma lipoprotein abnormalities and possible improvement in organomegaly and adrenal dysfunction. Successful liver transplantation for chronic liver failure has been reported.

Promising results have been reported with ERT in mouse models of acid lipase deficiency which paved way for the human trials. A multicenter, randomized, double-blind, placebo-controlled study of the recombinant human ERT in 66 CESD patients, achieved normalization of the alanine aminotransferase level after 20 weeks of treatment. In addition improvements in lipid levels and reduction in hepatic fat content was seen with good tolerability of the drug.

Treatment of Wolman disease with intravenous alimentation, plasma infusion, corticosteroids, and dietary supplements has been of limited benefit. Bone marrow transplantation has led to significant clinical and biochemical improvement for up to 4 years in some patients. The ERT with the same product as in CESD are currently in progress.

Congenital disorders of glycosylation [12]

CDG is a group of metabolic disorders that arise from defective glycosylation of proteins. Almost all plasma proteins, many proteins of cellular membranes and connective tissues, blood group substances, immunoglobulins, and certain hormones are glycoproteins. The synthesis and function of glycoproteins are complex. The pathway involves at least 40 steps in the cytoplasm, ER and Golgi apparatus and is essential for many structural, transport, immunological, hormonal, cell–cell signaling, and enzymatic functions. There are numerous defects in this pathway with a range of multisystem clinical manifestations involving the central nervous system, connective tissue, musculoskeletal system and other organs. Some of these disorders have prominent hepatic manifestations.

Two main groups of CDG are traditionally recognized: disorders of *N*-glycosylation and disorders of *O*-glycosylation, but a growing group of disorders with combined defects of *N*-glycosylation and O-glycosylation have been described. Additionally, an increasing number of defects affecting lipid-linked glycosylation and glycophosphoinositol glycosylation (GPI anchor disorders) have been identified; the total number of CDG described is well over 50.

The currently known disorders of *O*-glycosylation are mainly associated with brain malformations and musculoskeletal manifestations and will not be discussed further. Over 40 *N*-glycosylation disorders are known; they are classified into two broad biochemical categories: CDG I (types a–r) is caused by defects of synthesis and transfer of the carbohydrate chain to the nascent protein molecule, whereas CDG II (types a-l) results from defective processing of the carbohydrate chains. The genes for most of these defects have been identified. The nomenclature of these disorders was changed in 2009 to include the gene symbol followed by "-CDG". Thus the most common condition, phosphomannomutase (PMM) deficiency (CDG Ia) is now known as PMM2-CDG. Over 700 affected individuals are known worldwide. Two other disorders, MPI-CDG (CGG Ib) and ALG6-CDG have been described in 20–30 individuals. Most other *N*-linked glycosylation defects and multiple pathway defects are only known in single or very few individuals.

Presentation

The most common disorder is PMM2-CDG or CDG Ia, which is caused by PMM deficiency. Although neurological symptoms dominate the clinical picture in most cases, liver and gastrointestinal function pathology is common and is the predominant feature in CDG Ib. CDG syndrome should be considered in any patient with unexplained liver dysfunction, especially if there is multisystem disease.

PMM2-CDG (CDG Ia). The clinical spectrum is broad. Neurological manifestations are seen in all patients and include hypotonia, ataxia, squint, cerebellar hypoplasia, hyporeflexia, and psychomotor retardation. Younger patients may have facial dysmorphism (almond-shaped, upslanting palpebral fissures, high forehead, and prominent maxilla), inverted nipples, and abnormal gluteal, perineal, or suprapubic pads of fat, and failure to thrive. Hepatic involvement may include hepatomegaly, abnormal transaminases, hypoalbuminemia, coagulopathy, steatosis, fibrosis, and cirrhosis. Some patients develop pericardial effusions and cardiomyopathy. Mortality is high in those with severe multiorgan involvement, and many patients do not survive beyond 2 years. Older children often have stroke-like episodes, retinitis pigmentosa, areflexia, spinal deformities, fixed flexion deformities, and in females, absent puberty.

They do not have progressive neurodegenerative disease, although the neurological signs become more prominent as children grow older. At the milder end of the spectrum, patients may have mild psychomotor disability, without any dysmorphism or multisystem involvement.

Over 90 mutations have been described in the *PMM2* gene and the R141H substitution is the most common in Europe.

MPI-CDG (CDG Ib) is caused by phosphomannose isomerase (PMI) deficiency, and is phenotypically different from CDG Ia. Prominent features include absence of neurological involvement, abdominal pain, protein-losing enteropathy, recurrent thromboses, gastrointestinal bleeding, and congenital hepatic fibrosis. Infants present with diarrhea, vomiting, failure to thrive, hepatomegaly, edema due to severe hypoalbuminemia, coagulopathy, a thrombotic tendency, and hyperinsulinemic hypoglycemia. Inverted nipples and abnormal fat pads have been reported, but not facial dysmorphism.

ALG6-CDG (CDG Ic) is associated with hypotonia, squint, seizures and mild dysmorphism and psychomotor delay. Liver involvement has not been described.

PGM1-CDG is a recently described disorder of glycosylation that secondarily results in GSD and defective glycolysis. Clinical features include cleft plate or uvula, short stature, hepatomegaly, liver dysfunction, endocrine abnormalities, cardiomyopathy, and coagulopathy.

Other forms of CDG are rare. These patients have variable combinations of dysmorphism, epilepsy, psychomotor retardation, bleeding and thrombotic manifestations, musculoskeletal abnormalities, and peripheral neuropathy Some have been described with cholestsis, hepatomegaly, and liver dysfunction. Not all patients with CDG match known subtypes (CDG X).

Diagnosis

Supportive findings are:

- Increased hepatic transaminases.
- Hypoalbuminemia.
- Coagulopathy.
- Hypoglycemia.

Histological examination of the liver usually reveals fibrosis, and jejunal biopsies may show villous atrophy and lymphangiectasis.

Separation of plasma transferrin isoforms usually reveals characteristic patterns, although not all cases are abnormal. Biochemical studies of other glycoproteins such as apolipoprotein C-III, α_1-antitrypsin, and α_1-antichymotrypsin may be helpful. Confirmation of the diagnosis requires specific enzyme analysis of PMM and/or PMI enzyme activities in skin fibroblasts and mutation analysis. NGS techniques are increasingly used in diagnosing CDG after the common disorders have been excluded.

Antenatal diagnosis is possible by a combination of enzyme analysis and mutation analysis.

Management

MPI-CDG (CDG Ib) is the only efficiently treatable disorder amongst the CDGs, where oral mannose supplementation results in improvement in gastrointestinal symptoms, hematological abnormalities, and growth. Heparin therapy has also been used in MPI-CDG successfully for protein-losing enteropathy. Cases resistant to mannose therapy have responded well to liver transplanataion. In PGM1-CDG, oral galactose therapy has been reported to improved hepatomegaly, improved liver dysfunction and resolution of hypoglycemia. There is no therapy available yet for other forms of CDG, and treatment is supportive.

Mitochondrial respiratory chain disorders [13, 14]

The mitochondria are intracellular organelles that are the main source of ATP, which is essential for all cellular processes. They are membrane bound and harbor the mitochondrial respiratory chain consisting on complexes I–V, which mediates ATP production via oxidative phosphorylation. The mitochondrial respiratory chain complexes are made up of proteins that are encoded in the both the maternally inherited mitochondrial DNA (mtDNA) and the nuclear DNA. In addition, nuclear DNA also codes for a large number of assembly factors and cofactors that are essential for the integrity of the respiratory chain and for the maintenance and repar of mtDNA. The number and clinical phenotypes of genetically defined mitochondrial disorders is constantly expanding.

As the mitochondrial are essential for the function of many different tissues, mitochondrial disorders typically lead to multiysystem involvement. The metabolically active liver has a high energy requirement and it is often involved in the phenotypic manifestation of mitochondrial disease, either as the primary affected organ or as part of multisystem disease.

Mitochondrial hepatopathies are classified as "primary" in which mitochondrial disease is the main cause of liver disease, or "secondary" in which injury to the mitochondria results from genetic defects of non-mitochondrial proteins or from acquired causes such as toxins. "Primary" mitochondrial liver disease is further classified into those casued by mutations affecting mtDNA (class Ia) and those affecting mutations in relevant nuclear genes (class Ib). Inheritance is maternal or autosomal, depending on whether the defects are due to mitochondrial or nuclear DNA.

Clinical presentation

The clinical presentation of mitochondrial liver disease is very wide, ranging from prenatal manifestation as hydrops fetalis, acute neonatal liver failure (see Chapter 9), lactic acidosis, cholestasis, or chronic liver disease. Most cases present in the neonatal period, infancy, or early childhood.

Multisystem involvement is characteristic of respiratory chain disorders. Liver disease or acute liver failure in the neonatal period (see Chapters 8 and 9) or early childhood is a common presentation. Extrahepatic features are common and may include lethargy, hypotonia, vomiting, poor neonatal reflexes, seizures, recurrent apnea, and cardiomyopathy. Intrauterine growth retardation, fetal hydrops, neonatal ascites, renal tubular disease, elevated α-fetoprotein, and hypoalbuminemia have also been described. Usually, progressive hepatic, neurological, and/or other systemic deterioration leads to death in infancy or early childhood. Occasionally, liver disease may be static or even resolve with time. A number of specific molecular defects have been described in mitochondrial liver disease.

Mutations in BCS1L (a complex III chaperonin) are associated with the GRACILE syndrome spectrum of phenotypes (growth restriction, aminoaciduria, cholestasis, iron overload, lactic acidosis).

Pearson syndrome is a multisystem disorder of infancy that is characterized by exocrine pancreatic insufficiency and sideroblastic anemia. Some patients develop progressive liver disease or renal Fanconi syndrome; those who survive may improve spontaneously but develop progressive external ophthalmoplegia (resembling Kearns–Sayre syndrome) in later life. Pearson syndrome is usually associated with mtDNA rearrangements.

mtDNA depletion syndrome presents as hepatic failure, hypotonia, renal dysfunction, and lactic acidosis in the first few weeks of life and is associated with multiple respiratory chain enzyme defects and is caused by a variety of nuclear DNA defects including POLG, DGUOK, SUCLG1, and MPV17; for a detailed discussion, see Chapter 9.

Alpers–Huttenlochler syndrome is a progressive neurological disorder that may be associated with liver failure caused by mutations in the *POLG1* gene that is important in mtDNA mainenance; it is discussed later.

A syndrome of severe anorexia, diarrhea, vomiting, villous atrophy, and liver dysfunction in infancy associated with mtDNA rearrangements has been reported. The diarrhea may improve or resolve by 5 years of age, but progressive neurological symptoms lead to rapid deterioration and death.

Reversible acute liver failure in infancy associated with lactic acidosis, multiple respiratory chain enzyme deficiencies in muscle and liver and absence of mtDNA depletion has been described in patients harboring mutations in the *TRMU* gene. Many of these patients recover spontaneously with no recurrence of liver failure, although some go on to develop stable cirrhosis.

Diagnosis [15, 16]

Investigations for respiratory chain disease include:

- Plasma lactate. Persistently elevated blood lactate concentrations are an important clue to respiratory chain disease, but are not specific and may be found in any sick neonate or infant, especially with significant liver disease.
- Increased cerebrospinal fluid lactate concentrations are more specific for neurological involvement and must be compared to plasma lactate.
- Hypoglycemia is often an early feature in aculte liver failure due to mitochondrial disease.
- Muscle biopsy for:
  - histology for evidence of steatosis, ragged red fibers (see Chapter 9)
  - histochemistry staining for cytochrome oxidase and succinate dehydrogenase
  - electron microscopy for the number and morphology of mitochondria
  - respiratory chain enzyme analysis (complexes I–V) usually confirms the diagnosis; defects are often expressed in muscle, even in the absence of myopathy; expression of results as ratios of activity improves discrimination.
- Liver histology characteristically reveals a combination of steatosis, fibrosis, cholestasis, and necrosis, and on electron microscopy there may be increased numbers of structurally abnormal mitochondria (see Chapter 9). Respiratory chain enzyme analysis is possible on liver biopsy specimens, but the results may be difficult to interpret in the presence of liver failure.
- Mutation analysis for known mitochondrial and nuclear DNA defects (see earlier).
- Urine organic acid analysis may reveal abnormal but nonspecific findings.
- Evidence of multisystem involvement (echocardiography, neuroimaging, and neurophysiology).

When causative nuclear DNA mutations that underlie mitochondrial disease are known, antenatal diagnosis is readily available. For disorders caused by mtDNA mutations, if the respiratory chain defect is expressed in skin fibroblasts from the index case, reliable biochemical diagnosis may be possible on chorionic villus cells.

In view of the number and diversity of potential genes involved (>1500) in the etiology of mitochondrial respiratory chain disorders, NGS techniques are increasingly used to identify causative mutations in patients with these disorders. Targeted exome sequencing or whole exome sequencing have been successful in identifying causative mutations in up to 60% of cases with respiratory chain abnormalities, and this approach can be applied to mitochondrial liver disease.

Management

No specific treatment is effective and management is mainly supportive. A number of different strategies to specifically treat mitochondrial disease have been attempted with variable results, including cofactor therapy with riboflavin, artificial electron acceptors such as menadione and vitamin C, a variety of free radical scavengers such as

coenzyme Q_{10} (ubiquinone), vitamin E, idebenone, carnitine, and methylene blue, induction of mitochondrial biogenesis and energy supply with creatine, bezafibrate, or ketogenic diet, and reduction of lactic acidosis with dichloroacetate. A recent Cochrane review identified several clinical trials and found no clear evidence to support the use of any intervention in mitochondrial disorders.

Consideration of liver transplantation is a difficult management issue in an infant with liver failure due to suspected mitochondrial liver disease. In general, multisystem respiratory chain disease is a contraindication to liver transplantation. In patients in whom the clinical disease appears to be confined to the liver, liver transplantation may be a therapeutic option. A few patients with isolated mitochondrial liver disease have undergone successful liver transplantation, with excellent long-term outcomes and no evidence of extrahepatic involvement. However, other patients have developed progressive neurological disease after transplantation, even though they had no neurological involvement prior to transplantation. The use of liver transplantation in treating progressive mitochondrial liver disease remains controversial (see also Chapter 9). In an acute situation, hepatocyte infusion may be considered in order to help control acute liver failure and to allow further evaluation of suitability for liver transplantation.

Alpers–Huttenlocher syndrome (progressive infantile poliodystrophy, progressive neuronal degeneration of childhood) [17]

Alpers–Huttenlocher or Alpers syndrome is a rapidly progressive early-childhood encephalopathy with intractable seizures and neuronal degeneration. It is caused in almost all cases by mutations in the mtDNA polymerase gamma (*POLG1*) gene, which plays an important role in mtDNA replication. Two mutations, A467T and W748S, are particularly common, and screening for these has been proposed as the most rapid and sensitive test for Alpers syndrome. Inheritence is autosomal recessive.

Presentation

Typically, the neonatal period is normal. Presentation is between 2 months and 8 years with physical and developmental delay, feeding difficulties, recurrent vomiting, and failure to thrive followed by the sudden onset of intractable epilepsy. Rapid neurological deterioration and blindness usually follow the onset of seizures. Overt hepatic disease presents later, with jaundice, hepatomegaly, coagulopathy, and rapidly progressive liver failure, although biochemical evidence of liver dysfunction may predate the seizures. The hepatic symptoms may be exacerbated by treatment with valproic acid. Most patients do not survive beyond 3 years, but some may follow a protracted course. A few patients may have typical neurological features of Alpers syndrome without liver disease; these infants follow an identical neurological course (see also Chapters 8 and 9).

Diagnosis

- Liver dysfunction may initially be mild, with elevation of transaminases and bilirubin, but later, synthetic function is impaired.
- Plasma carnitine concentrations may be low.
- Urinary organic acids are non-specific, consistent with liver dysfunction.
- Electroencephalography demonstrates high-amplitude polyspikes.
- Visual evoked responses are reduced or absent.
- Electroretinograms are normal.
- Magnetic resonance imaging (MRI) scans show progressive cerebral atrophy with low-density areas in the occipital and posterior temporal areas; the white matter is usually spared.
- Liver histology reveals microvesicular fatty change, bile duct proliferation, and focal necrosis, leading to bridging fibrosis and cirrhosis (see Chapter 9). Neuropathology reveals cortical involvement, with neuronal cell loss and gliosis.
- Some patients may have complex I deficiency in liver or muscle enzyme analysis.
- Definitive diagnosis relies on DNA analysis of the *POLG* gene.

Management

There is no effective treatment. The condition is fatal, with most children dying before 3 years or within a few months of developing overt liver disease. Liver transplantation is contraindicated, as neurological progression continues after transplantation.

MEGDEL syndrome (3-methylglutaconic aciduria, deafness, encephalopathy, Leigh-like syndrome) [18]

Phospholipids are involved in numerous cellular processes, as structural components of cell and intracellular membranes, organelle fission and fusion, mitochondrial function, and signal transduction and defects of the biochemical pathways involved in the biosynthesis and remodeling of these compounds underlie an emerging class of inherited metabolic disorders. The recently described MEGDEL (3-methylglutaconic aciduria, deafness, encephalopathy, Leigh-like) syndrome is a disorder of phospholipid remodeling which results in a severe neurodegenerative disorder that is often associated with transient infantile cholestasis and liver failure, caused by autosomal recessively inherited mutations in the *SERAC1* gene.

Presentation

Patients usually present in infancy with feeding difficulties, failure to thrive, and hypotonia. Neonatal liver disease is usually a prominent feature with cholestasis, elevated liver enzymes, and liver failure described in most patients. Liver disease tends to spontaneously improve after infancy, when neurological features such as developmental regression, deafness, seizures, spasticity, and dystonia become prominent, progressing at a variable rate to a fully dependent state by late childhood.

Diagnosis

Investigations include:

- Urinary organic acids show prominent excretion of 3-methylglutaconic acid and 3-methylglutaric acid; other causes of 3-methylglutaconic aciduria must be excluded.
- Lactic acidosis.
- MRI changes suggestive of Leigh syndrome.
- Liver biopsy may reveal micro- and macrovesicular steatosis, bridging fibrosis and mitochondrial ultrastructural changes on electron microscopy.
- Other features suggestive of mitochondrial disease, such as decreased respiratory chain complexes and mitochondrial DNA depletion, may be found in some patients.
- Mutation analysis of the *SERAC1* gene is confirmatory.

Management

There is no specific treatment and management is supportive. Survival is variable but limited by liver failure and infections; some patients succumb to neonatal/infantile liver disease, whereas others may survive into the second decade.

Peroxisomal disorders [19]

Peroxisomes are small, membrane-bound intracellular organelles that contain 40 different anabolic and catabolic enzymes. Their functions include:

- β-oxidation of very-long-chain fatty acids (VLCFAs).
- β-oxidation of phytanic acid, a dietary branched-chain fatty acid.
- β-oxidation of dihydroxycholestanoic and trihydroxycholestanoic acids to chenodeoxycholic acid and cholic acid, which are bile acid precursors.
- Conjugation of chenodeoxycholic acid and cholic acid with taurine and glycine to form the bile acids.
- The initial reactions of isoprenoid (cholesterol, dolichol, and ubiquinone) biosynthesis, plasmalogen synthesis.
- Lysine metabolism.
- Glyoxylate metabolism.
- Hydrogen peroxide metabolism.
- Eicosanoid (prostaglandins, leukotrienes, thromboxane, prostacyclin) degradation.

Peroxisomal disorders are classified into two main groups:

- Multiple-enzyme deficiencies (such as the "Zellweger spectrum" disorders and rhizomelic chondrodysplasia punctata) which arise from defective peroxisome synthesis, assembly, and enzyme import.
- Genetic deficiency of a single peroxisomal enzyme (such as adrenoleukodystrophy, classical Refsum disease, and hyperoxaluria type I). Over 20 different disorders have been described. The overall prevalence has been estimated to be 1 in 25,000.

Peroxisomal biogenesis is a complex process that involves the import of matrix proteins, fission of existing peroxisomes, and synthesis of new organelles; the coordinated function of 16 distinct proteins called peroxins is necessary. Each peroxin is encoded on a *PEX* gene and mutations in any of these can result in a peroxisomal biogenesis defect resulting in multiple enzyme deficiencies. Mutations in the genes responsible for peroxisomal assembly (*PEX* genes) are known to be associated with Zellweger syndrome, neonatal adrenoleukodystrophy, infantile Refsum disease, and rhizomelic chondrodysplasia punctata.

Presentation

Disorders with multiple-enzyme deficiencies. Patients who have a disorder of peroxisomal biogenesis lack normal peroxisomes. Four conditions are recognized: Zellweger syndrome (see also Chapter 8), neonatal adrenoleukodystrophy, infantile Refsum disease, and rhizomelic chondrodysplasia punctata. They are all associated with severely deranged peroxisomal assembly, loss of multiple-enzyme activities, and multisystem involvement. Peroxisomes are absent or greatly reduced in number in skin fibroblasts and liver biopsy specimens. Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease, respectively, represent the severe, intermediate, and mild forms of peroxisomal biogenesis defects and are part of the same spectrum of conditions called the Zellweger spectrum disorders.

Infants with Zellweger syndrome present in the neonatal period with characteristic dysmorphic features (prominent forehead, large anterior fontanelle, broad nasal bridge, epicanthal folds, high arched palate, micrognathia, redundant neck skin folds, clinodactyly, and talipes equinovarus). Neurological abnormalities are prominent, including severe hypotonia, areflexia, poor suck reflex, and seizures. Neuronal migration defects may be observed on neuroimaging. Other features such as corneal clouding, cataracts, pigmentary retinopathy, polycystic kidneys, cryptorchidism, dislocated hips, and stippled epiphyses on radiographs (chondrodysplasia punctata) may be present. Liver disease is common and includes hepatomegaly, conjugated hyperbilirubinemia, progression to cirrhosis, and liver failure in the first few months of life, but the hepatic involvement is overshadowed by the neurological symptoms. Occasionally, the presentation resembles malabsorption, with hepatomegaly,

prolonged jaundice, liver failure, anorexia, vomiting, and diarrhea leading to failure to thrive. Failure of psychomotor development is evident in early infancy, and survival beyond 1 year is rare. Patients with neonatal adrenoleukodystrophy and infantile Refsum disease have the same features as in Zellweger syndrome, but a milder phenotype. Infants often have hepatomegaly and neonatal cholestasis; progressive liver disease may significant in children who survive the first decade. A number of patients with normal intellect but presenting with ataxia and spastic paraparesis has been recently described.

There is no hepatic involvement in "classic" rhizomelic chondrodysplasia punctata, which represents a different group of peroxisomal biogenesis disorders (PBDs), and presents with rhizomelic dysmorphism and psychomotor retardation.

Disorders due to single peroxisomal enzyme deficiencies. There are at least 11 isolated peroxisomal enzyme deficiencies, some of which present clinically as Zellweger spectrum disorders, but others that present primarily with neurological problems without dysmorphism; hepatic involvement may be present in some (Table 19.4). Intact peroxisomes are found in liver biopsy and skin fibroblast specimens, and the biochemical and clinical abnormalities relate to the individual pathway.

Diagnosis

For peroxisome biogenesis (with multiple-enzyme defects), initial investigations on plasma include:

- VLCFAs.
- Dihydroxyacetone phosphate acyltransferase (DHPAT; blood).
- Phytanate/pristanate (plasma).
- Plasmalogens (plasma).
- Plasma and urine bile acids.

Morphological studies of liver/skin for fibroblasts may show a complete absence or a reduced or abnormal structure of peroxisomes. The diagnosis is confirmed by specific mutation analysis or by enzyme analysis in skin fibroblasts.

Antenatal diagnosis is possible by mutation analysis, or by measurement of the VLCFA concentration and/or plasmalogen synthesis in cultured chorionic villus samples or amniocytes.

Management

There is no effective treatment for multiple-enzyme dysfunction, but supportive care with anticonvulsants, dietary supplements for the liver disease, and muscle relaxants is essential. Bile acid supplements have reduced cholestasis in a child with Zellweger syndrome, while docosahexanoic acid (DHA) has led to some clinical improvement in the Zellweger spectrum disorders. Therapeutic options are available for some of the single-enzyme disorders.

Reye syndrome [20]

Reye syndrome is an acute childhood illness characterized by encephalopathy and fatty degeneration of the liver. The definition of this disorder is non-specific, and it is now recognized that a number of different conditions, especially some IEMs, can present as Reye syndrome. There has been a substantial decline in the number of cases of "classical" Reye syndrome, attributed to public health campaigns warning against the use of salicylates in children with influenza-like illnesses, the declining use of antiemetic medications in childhood illnesses, and an increasing recognition that many patients previously diagnosed as having Reye syndrome have an underlying metabolic disorder.

Table 19.4 Disorders due to single peroxisomal enzyme deficiencies associated with liver disease.

Disorder/enzyme defect	Genetics	Clinical features	Biochemical features	Hepatic involvement	Outcome
Peroxisomal acyl-CoA oxidase deficiency	*ACOX1* mutations; AR	Severe hypotonia, psychomotor retardation, seizures in infancy; no dysmorphism	Elevated plasma VLCFA	Hepatomegaly, fibrosis	Death by 2–4 years
Bifunctional protein deficiency (enoyl-CoA hydratase and hydroxyacyl-CoA dehydrogenase deficiency)	*HSD17B4* mutations; AR	Dysmorphism similar to Zellweger syndrome, severe hypotonia, intractable seizures, epiphyseal stippling	Elevated plasma VLCFA, DHCA, THCA, pristanic acid	Hepatomegaly, hepatic dysfunction, coagulopathy	Death by 6 months–2 years
2-Methylacyl CoA racemase deficiency	*ACAMR* mutations; AR	Liver failure, cholestasis; response to cholic acid therapy	Elevated DHCA, DHCA	Liver failure, coagulopathy, neuropathy	Unknown
Bile acid CoA: amino acid acyltransferase deficiency	*BAAT* mutations; AR	Familial hypercholanemia; fat malabsoprtion, itching, failure to thrive, coagulopathy	Elevated plasma unconjugated bile acids	Liver failure, coagulopathy	Unknown

AR, autosomal recessive; CoA, coenzyme A; DHCA, dihydroxycholestanoic acid; HIDS, hyperimmunoglobulin D syndrome; THCA, α-trihydroxy-5-β-cholestannic acid; VLCFA, very-long-chain fatty acid.

Presentation and definition

The classical definition of Reye syndrome is a child under 16 years of age with:

- Unexplained non-inflammatory encephalopathy *and* one or more of the following:
- Serum hepatic transaminases elevated three or more times upper limit of normal
  - *or* plasma ammonia levels elevated three or more times upper limit of normal
  - *or* characteristic fatty infiltration of the liver.

There are two major groups:

- *Classic* or *idiopathic* Reye syndrome typically occurs in children over 5 years of age, usually associated with an influenza or varicella-like prodrome with aspirin use in therapeutic dosage. There is a biphasic presentation
  - a viral prodrome (upper respiratory tract or gastrointestinal infection), followed several days later by the abrupt onset of encephalopathy heralded by profuse vomiting, personality changes, and altered consciousness. Raised intracranial pressure may result in death or permanent neurological sequelae. The etiology of this form of Reye syndrome remains unclear, but epidemiological studies have suggested an association with aspirin exposure; alternatively, the combination of a viral illness and the extrapyramidal reactions induced by antiemetics may result in a clinical syndrome indistinguishable from Reye syndrome.
- *Atypical* Reye syndrome or *Reye-like* illnesses present in a similar manner to classical Reye syndrome, but in children less than 5 years of age. Atypical Reye syndrome is often associated with inherited metabolic disorders of fatty acid oxidation (such as MCADD), disorders of organic acid and amino acid metabolism, as well as urea cycle defects. A number of patients previously diagnosed with Reye syndrome have had the diagnosis revised when a metabolic, toxic, or other cause has been identified on further investigation.

There is considerable overlap between these two groups, and all children with any form of Reye syndrome must undergo thorough investigation to rule out potential underlying causes.

Investigation of Reye syndrome and Reye-like illness

Typically, investigations demonstrate:

- Prolonged prothrombin time.
- Hepatic dysfunction with raised aminotransferases.
- Elevated ammonia.
- Hypoglycemia.
- CT scan may demonstrate cerebral edema.
- Electroencephalography demonstrates marked slowing.

Liver histology is not specific; the usual findings are a microvesicular steatosis with glycogen depletion and cytoplasmic swelling. Electron microscopy confirms a loss of glycogen and demonstrates proliferation of smooth ER and an increase in peroxisomes. Mitochondria may be pleomorphic. Skeletal muscle demonstrates glycogen deposition and fat deposition.

Etiological factors for treatable metabolic disorders include:

- Urine organic acids.
- Urine amino acids.
- Plasma amino acids.
- Plasma and/or blood spot acylcarnitine profiles.
- ScreeningforcommonMCADDandlong-chain3-hydroxyacyl-coenzyme A dehydrogenase deficiency (LCHAD) mutations.

It is also important to rule out other causes of coma associated with abnormal biochemical liver function tests, including toxins, severe hypoxia, and infections such as hepatitis and septicemia.

Management

The management of classic Reye syndrome is directed towards supportive treatment of acute cerebral edema, metabolic abnormalities, coagulopathy, and hepatic encephalopathy (see Chapter 18). It is important to treat cerebral edema adequately in intensive care units with full facilities for monitoring and controlling raised intracranial pressure (see Chapter 18), as the prognosis depends on the prevention of irreversible brain damage. If an underlying cause such as a metabolic disorder or infection is identified, appropriate specific therapy is indicated. Liver transplantation is usually not necessary and may be contraindicated if there is severe multisystem involvement.

Disorders of intermediary metabolism

Metabolic defects in intermediary metabolism involve catabolic pathways of amino acids, organic acids, fatty acid oxidation, and the urea cycle. Some of these disorders can present with liver dysfunction, a Reye-like encephalopathy, a mild "biochemical" liver dysfunction or, more rarely, as acute liver failure.

Tyrosinemia type I

Amino acid disorders generally do not present with liver disease, with the exception of tyrosinemia type I (Tyr I), which is described in Chapter 9.

Organic acidemias

Organic acids are carboxylic acids of low molecular weight and are metabolites of amino acids, carbohydrates, and fats. Organic acid disorders are due to defects in the catabolism of the branched-chain amino acids, isoleucine, isoleucine and valine, and metabolism of propionate. The accumulated organic acids exist as carnitine conjugates, e.g., propionyl carnitine and isovaleryl carnitine. Over 50 different disorders

have been described, and their combined incidence is in the order of 1 in 5000–10,000. The commonest disorders are: methylmalonic acidemia (MMA), propionic acidemia (PA; see Chapter 9), and isovaleric acidemia (IVA).

Presentation

All of these disorders have a neonatal presentation in first few days of life (see Chapter 5), but a less acute presentation in infancy or early childhood with developmental delay, failure to thrive, and metabolic acidoses secondary to episodic illness is common. A Reye-like encephalopathy also occurs. In these cases, there may be mild elevation of hepatic transaminases (Table 19.5).

Diagnosis

Characteristic features of these disorders include:

- Metabolic acidosis.
- Hypoglycemia.
- Hypocalcemia.
- Ketonuria.
- Neutropenia.
- Hyperlacticacidemia.
- Hyperuricemic.
- Increased plasma and urine glycine.

Diagnostic tests:

- Urine organic acids.
- Acylcarnitine species (blood).
- Enzymes/substrate incorporation studies (fibroblasts).

DNA analysis is not usually required, as metabolite profiles (organic acids and acylcarnitines) are usually diagnostic.

Management

Whenever possible, specimens – blood (e.g., heparinized plasma) and urine (random) – should be collected when the infant is acutely ill, i.e., before treatment with a low-protein diet is started (see Chapter 9). Liver transplantation has been reported in a few cases, but the outcome and long-term benefits are unclear.

Urea cycle disorders

There are six disorders of the urea cycle. Most present with hyperammonemia in the neonatal period. The later-onset forms have variable presentations, which include an encephalopathic (Reye-like) episode, anicteric hepatitis, and/or mild hepatomegaly (see Chapter 9). Liver transplantation or hepatocyte transplantation may be considered as a treatment option in selected cases [4].

Fatty acid oxidation defects (see Chapter 9)

Mitochondrial β-oxidation of fatty acids plays a major role in energy production, especially during periods of fasting. It is a complex process that involves the uptake of fatty acids into the cell, activation to acyl-CoA, and then transport into the mitochondria, which requires the carnitine transport cycle. Within the mitochondria, the β-oxidation spiral

Table 19.5 Organic acid disorders. CoA, coenzyme A (see also Figure 19.5).

Conditions	Presentation/clinical features	Hepatic involvement
Propionic acidemia Methylmalonic acidemia	*Neonatal presentation:* acute encephalopathy, hyperammonemia, acidosis	Hepatomegaly, hyperammonemia, elevated transaminases, fatty infiltration on biopsy; pancreatitis has been described
Isovaleric acidemia	*Acute intermittent late-onset form:* recurrent encephalopathy or Reye-like illness in infancy. *Chronic, progressive form:* anorexia, failure to thrive, gastrointestinal symptoms, psychomotor retardation	–
Isolated 3-methylcrotonyl-CoA carboxylase deficiency	Reye-like illness in infancy or early childhood; recurrent acidosis, hypoglycemia, coma; chronic presentation with developmental delay has been described	Biochemical and histological features resembling Reye syndrome
3-Methylglutaconyl-CoA hydratase deficiency	Variable presentation, including recurrent acidosis, hepatomegaly, Reye-like episodes, speech delay, hypotonia	Liver dysfunction and Reye-like features have been described
3-Hydroxy-3-methylglutaryl-CoA lyase deficiency	Neonatal, infantile, or childhood presentation with vomiting, lethargy, coma, hyperammonemia, and hypoketotic hypoglycemia; Reye-like illness beyond the neonatal period	Biochemical and histological features resembling Reye syndrome
Mevalonate kinase deficiency (mevalonic aciduria)	Variable presentation with dysmorphism, failure to thrive, psychomotor retardation, ataxia, recurrent fever with rash, diarrhea and vomiting; milder variant with periodic fever and hyperimmunoglobulinemia D	Hepatosplenomegaly and cholestatic liver disease have been described
Mitochondrial acetoacetyl-CoA thiolase deficiency (β-ketothiolase deficiency)	Infantile presentation with ketoacidosis during acute infections; hyperglycemia and hyperammonemia may occur; asymptomatic between attacks	Fatty infiltration of the liver has been described
3-Hydroxyisobutyric aciduria	Infantile presentation with dysmorphism, brain malformations, ketosis, acidosis, failure to thrive	Not described
Malonic aciduria	Presentation in infancy or early childhood with acidosis, hypoglycemia, developmental delay and cardiomyopathy	Not described

requires a series of enzymes with carbon chain length specificity.

These disorders can present as a Reye-like illness (e.g., MCADD) or with acute illness usually in early infancy, with hepatomegaly/liver dysfunction.

The porphyrias [21]

The porphyrias are disorders of heme biosynthesis, which result in neurovisceral/psychiatric symptoms and/or cutaneous photosensitivity. They do not usually present as liver disease, but several forms can exhibit liver dysfunction. Although porphyrias usually present in adulthood, symptoms can occasionally occur in childhood. Most are caused by genetic deficiencies of the enzymes involved in heme biosynthesis. Each form of porphyria results from mutations in one of these genes: *ALAD*, *ALAS2*, *CPOX*, *FECH*, *HMBS*, *PPOX*, *UROD*, or *UROS*. Porphyria cutanea tarda is thought to be an acquired condition, but 20% of cases have mutations in the *UROD* gene.

Heme is synthesized in the bone marrow for hemoglobin synthesis and in the liver for cytochrome P450 enzymes. The liver and bone marrow pathways are differently regulated, and drugs, hormones, and diet that can influence the pathway in the liver do not affect the bone marrow.

The porphyrias are classified according to the primary tissue affected (hepatic or erythropoietic porphyrias), the specific enzyme deficiency, or the clinical presentation (acute neurovisceral/psychiatric or chronic cutaneous photosensitivity) (Table 19.6).

Presentation

The symptoms may be non-specific. Liver disease is not usually a presenting feature, although secondary liver disease may occur in some of the defects. Neurovisceral features are common if there is an accumulation of porphyrin precursors, especially δ-aminolevulinic acid.

Acute intermittent porphyria is the commonest of the acute porphyrias, with an estimated prevalence of 5 per 100,000 in northern European populations. There is a reduction in the enzyme hydroxymethylbilane synthase (HMBS). Symptoms rarely occur in childhood, and many adults with a genetic mutation *HMBS* remain asymptomatic.

Acute attacks are precipitated by certain drugs, steroid hormones, and poor nutrition. Typical manifestations during an acute attack include abdominal pain, nausea, vomiting, limb and chest pain, muscle weakness, peripheral neuropathy, tachycardia, hypertension, tremors, and hypertension. Electrolyte imbalance, seizures, motor neuropathy, and death may occur if the porphyria is not recognized and treated. Attacks may last several days, and complete recovery follows appropriate treatment. There is an increased risk of hepatocellular carcinoma in acute intermittent porphyria, as well as in porphyria cutanea tarda (see later). Similar neurovisceral symptoms occur in other acute porphyrias (Table 19.7).

Porphyria cutanea tarda is the most common cutaneous porphyria [21] and presents with chronic blistering skin lesions on sun-exposed parts of the skin, such as the hands, neck, face, and back. Hypertrichosis, hyperpigmentation, thickening, scarring, and calcification of affected skin may occur. Precipitating factors include alcohol intake, hepatitis C infection, carriage of the *HFE* gene for hemochromatosis, and estrogen use.

Table 19.6 Classification and genetics of porphyrias.

Condition	Deficient enzyme	Genetics	Porphyria types			
			Acute neurovisceral	Cutaneous	Hepatic	Erythropoietic
δ-ALA dehydratase deficiency	ALA dehydratase	AR	+		+	
Acute intermittent porphyria	PBG deaminase (hydoxymethylbilane synthase)	AD	+		+	
Congenital erythropoietic porphyria	Uroporphyrinogen cosynthase	AR		+		+
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase	80% acquired 20% AD		+	+	
Hepatoerythropoietic porphyria	Uroporphyrinogen decarboxylase	*		+	+	+
Hereditary coproporphyria	Coproporphyrinogen oxidase	AD	+	+	+	
Variegate porphyria	Protoporphyrinogen oxidase	AD	+	+	+	
Erythropoietic protoporphyria	Ferrochelatase	Mainly AD		+		+

AD, autosomal dominant; ALA, aminolevulinic acid; AR, autosomal recessive; PBG, porphobilinogen.
* Considered to be the homozygous variant of porphyria cutanea tarda.

Table 19.7 Diagnosis, clinical features, and treatment of the porphyrias.

Condition	Diagnostic tests	Clinical features	Treatment
δ-ALA dehydratase deficiency	Increased urine δ-aminolevulinic acid and coproporphyrin, normal urine porphobilinogen; enzyme and DNA studies	Acute neurovisceral symptoms; anemia, failure to thrive; onset may be in childhood	Similar to acute intermittent porphyria (see later)
Acute intermittent porphyria	Increased urinary porphobilinogen, δ-aminolevulinic acid, and anduroporphyrin, normal fecal porphyrins; RBC enzyme assay and DNA studies	Acute neurovisceral symptoms; increased risk of hepatocellular carcinoma	Avoidance of precipitating factors; acute attacks: analgesia, i.v. heme infusion, oral or i.v. carbohydrate loading
Congenital erythropoietic porphyria	Increased urinary, fecal, and plasma porphyrins, especially uroporphyrin I; increased RBC zinc and free protoporphyrin enzyme and DNA studies	Severe cutaneous symptoms; onset in utero (fetal hydrops) or in neonatal period	Skin protection from sunlight; blood transfusion to suppress erythropoiesis; BMT
Porphyria cutanea tarda	Increased urinary, fecal, and plasma porphyrins with characteristic fecal porphyrin present	Cutaneous symptoms; onset in adulthood; risk of hepatocellular carcinoma	Avoidance of precipitating factors; skin protection; repeated phlebotomy; chloroquine
Hepatoerythropoietic porphyria	Increased urine, fecal, and plasma and urine porphyrins; increased RBC zinc and free protoporphyrin; enzyme assay and DNA studies	Cutaneous symptoms; onset variable: neonatal period to adulthood	Skin protection from sunlight
Hereditary coproporphyria and variegate porphyria	Increased urine δ-aminolevulinic acid, porphobilinogen, and coproporphyrin; increased plasma porphyrins with characteristic fluorescence spectra; VP: fecal porphyrin with characteristic pattern HCP: enzyme assay and DNA studies	Cutaneous and/or acute neurovisceral symptoms Onset after puberty	Combination of strategies used in acute intermittent porphyria and skin protection from sunlight
Erythropoietic protoporphyria	Increased plasma porphyrins and free erythrocyte protoporphyrin; increased fecal protoporphyrin	Cutaneous symptoms; onset usually early childhood; liver dysfunction and liver failure may occur	Skin protection from sunlight; oral β-carotene, cholestyramine; transfusion and heme therapy; monitoring of liver function; liver transplantation for liver failure

ALA, aminolevulinic acid; BMT, bone marrow transplant; HCP, hereditary coproporphyria; RBC, red blood cell.

Diagnosis is based on the biochemical finding of elevated porphyrins in the urine (predominantly uroporphyrin and heptacarboxylporphyrin) and confirmed by the presence of a heterozygous *UROD* disease-causing mutation.

Other forms. Patients with *congenital erythropoietic porphyria* may also have hemolytic anemia and discoloration of the teeth. The diagnosis is by detection of *UROS* mutations or rarely the identification of a hemi-zygous mutation in the X-linked gene *GATA*.

Erythropoietic protoporphyria may be complicated by liver disease, gallstone, and rapidly progressive hepatic failure, which may be related to protoporphyrin accumulation in the liver. Pathogenic variants in *FECH*, encoding ferrochelatase, are reported.

If liver dysfunction is associated with skin abnormalities or acute neurovisceral symptoms, then porphyria should be considered.

Diagnosis and genetics

The clinical presentation determines the relevant diagnostic tests. With acute neurovisceral symptoms, urinary δ-aminolevulinic acid, porphobilinogen, and total porphyrins are the most useful first-line tests, whereas with cutaneous symptoms, a plasma porphyrin fluorescence emission screen should be measured initially. Detailed fecal and erythrocyte porphyrins, specific enzyme assays, and DNA analysis are necessary for confirmation of the specific diagnosis (see Table 19.7).

Management

The management is complex and varies for different disorders and specific patients (see Table 19.7). Liver function should be monitored where appropriate. In erythropoietic porphyria, blood transfusions or heme arginate therapy may be indicated in acute situations in addition to symptomatic treatment. Bone marrow transplantation and/or liver transplantation may be beneficial.

Abetalipoproteinemia [22]

Lipids are transported in plasma as soluble lipoproteins and are classified according to their density and electrophoretic mobility. The typical lipoprotein consists of a lipid core (cholesterol and triglycerides) surrounded by a layer of phospholipid and cholesterol molecules and protein moieties called apoproteins. There are two major lipoprotein transport pathways. The exogenous pathway involves the transfer of dietary lipids from the intestines to the liver as chylomicrons, whereas the endogenous pathway transports lipids from the

liver to peripheral tissues as very-low-density lipoproteins (VLDLs). Apoprotein B is the major component apoprotein of chylomicrons and VLDL, and abnormalities of this protein result in significant disruption of the major lipid transport pathways, with potentially serious clinical consequences.

Abetalipoproteinemia is a rare, autosomal recessive disorder that is associated with an absence of plasma β-lipoprotein and undetectable plasma chylomicrons, low-density lipoprotein (LDL) levels, and VLDL levels. It results in severe fat malabsorption and secondary deficiency of fat-soluble vitamins.

There is defective processing of B apoproteins or defective assembly and/or secretion of VLDLs and chylomicrons. Microsomal triglyceride transfer protein (MTP) permits the transfer of lipid to apoprotein B, and several mutations in the *MTTP* gene controlling this protein have been reported in patients with abetalipoproteinemia.

Presentation

The main clinical features include:

- Presentation in early infancy with diarrhea, vomiting, and failure to thrive. The intestinal symptoms relate to the amount of fat in the diet, and many patients develop a striking aversion to dietary fat.
- Fat malabsorption with fat-soluble vitamin deficiency.
- Acanthocytosis occurs as a result of altered lipid composition of erythrocyte membranes and results in shortened erythrocyte survival, hyperbilirubinemia, erythroid hyperplasia, and reticulocytosis.
- Spinocerebellar degeneration begins in adolescence and consists of ataxia, dysmetria, dysarthria, and peripheral neuropathy.
- Pigmentary retinal degeneration develops in late childhood and may lead to progressive blindness.
- Anemia secondary to nutritional deficiency and/or hemolytic may be present.
- Fatty infiltration of the liver is common, and cirrhosis has been reported in a number of individuals, especially after medium-chain triglyceride (MCT) supplementation.

Diagnosis

The diagnosis is suspected from:

- Acanthocytosis.
- Low plasma vitamin concentration.
- Absence of B lipoprotein on electrophoresis.
- Undetectable apolipoprotein B.

Hypobetalipoproteinemia is a distinct group of conditions associated with mutations of the apoprotein B. Over 30 mutations are currently known. The condition is dominantly inherited. Heterozygotes are asymptomatic. In the homozygous state, the clinical symptoms are indistinguishable from those of abetalipoproteinemia, and these patients can only be differentiated by demonstrating hypolipidemia in their parents. The approach to treating homozygotes is the same as for abetalipoproteinemia.

Management

The gastrointestinal symptoms respond to a low-fat diet (total fat intake >15 g/day), with clinical improvement and accelerated growth. Essential fatty acid supplementation is important. MCTs release fatty acids without the formation of chylomicrons for absorption, and could be used as a dietary energy source in abetalipoproteinemia, but there is a risk of hepatic fibrosis. Nevertheless, short-term use of MCT feeds may be helpful for extremely malnourished individuals.

Fat-soluble vitamin supplementation, especially vitamin A and vitamin K, is necessary. Tocopherol (vitamin E) supplementation (150–200 mg/kg/day) inhibits the progression of neurological and retinal disease and may ameliorate these symptoms if started early.

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CHAPTER 20

Disorders of Copper Metabolism

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Key points

- Any unexplained liver disease needs a diagnostic work-up for Wilson disease.
- The diagnosis is based on identification of a low serum copper and ceruloplasmin, an increased urinary or liver copper, and *ATP7B* mutation analysis.
- Knowledge of the limitations of current diagnostic tools is crucial to enable a diagnosis.
- If Wilson disease cannot be definitely excluded, repeat all biochemical tests including liver biopsy with hepatic copper quantitation.
- Treatment options include penicillamine, trientine, and zinc.
- Acute liver failure with encephalopathy requires urgent liver transplant.
- Non-wilsonian hepatic copper toxicosis is distinct from Wilson disease by normal serum ceruloplasmin, absence of a Kayser–Fleischer corneal ring, negative *ATP7B* mutation analysis and typical liver histology including abundance of Mallory–Denk bodies and strongly positive orcein staining.

The concentration of copper in the normal liver is <55 μg/g dry weight. An increased concentration associated with liver disease is found in the genetic disorder Wilson disease, a defect in biliary copper excretion, and occurred historically in the environmental infantile copper-related infantile cirrhoses, Indian and Tyrolean childhood cirrhosis. Cholestasis causes a secondary rise in liver copper concentration. Animal models for these situations exist (Table 20.1).

Copper – essential but toxic

Copper-containing enzymes are essential to life (Table 20.2). Copper deficiency, whilst well-recognized in sheep ("sway-back"), is rare in childhood as copper is freely available in the diet and drinking water. It has been associated with anemia, neutropenia, and bone changes in malnourished infants. Copper deficiency myelopathy after bariatric surgery in adults is a recently described disorder. It is difficult to distinguish true deficiency from excess of competing minerals such as molybdenum or zinc. The effects of copper deficiency are demonstrated by Menkes syndrome, in which *ATP7A* deficiency prevents egress of copper from intestinal cells.

Copper is toxic, but questions remain concerning the mechanism of copper-induced cell death. In the Fenton reaction, Cu^{1+} causes production of the highly reactive hydroxyl radical which causes oxidative damage to macromolecules. Given that free copper is almost non-existent in the cell, the adequacy of this simple explanation is disputable. An alternative suggested pathway implicates the X-linked inhibitor of apoptosis, XIAP. XIAP inhibits caspases 3 and 7 and is a potent inhibitor of cell death. Cu binds to XIAP leading to a conformational change which impairs its caspase-binding affinity and reduces its half-life. XIAP also regulates the levels of COMMD1, a protein which binds to the aminoterminus of ATP7B and presumably regulates its function by a mechanism yet to be identified. Another proposal is that Cu stimulates acid sphingomyelinase which release proapoptotic ceramide raising the possibility of pharmacological inhibition of acid sphingomyelinase by amitryptiline as a potential new approach to treatment.

The earliest events in Cu-induced damage to hepatocytes in culture, before morphological changes appear, include Cu accumulation in the nucleus in the absence of protein oxidation, with specific and limited changes in the mRNA profile suggesting that remodeling of RNA processing machinery is an important component in cells' response to elevated copper.

Diseases of the Liver and Biliary System in Children, Fourth Edition. Edited by Deirdre A. Kelly.

Table 20.1 Hepatic copper overload states in humans.

Clinical scenario	Cause	Animal model
Neonate	Physiological	Most mammals
Prolonged cholestasis (e.g., biliary atresia)	Impaired biliary Cu excretion	Bile duct ligation
Wilson disease	Absent *trans*-Golgi Cu exporter	LEC rat Toxic milk mouse ATP7B knockout mice
Infantile copper toxicosis (e.g., ICC, Tyrolean childhood cirrhosis)	Increased Cu ingestion, ? + other genetic or toxic factors	North Ronaldsay sheep
Sporadic copper-related cirrhosis	Unknown	
Human analogue not known	*MURR1* (*COMMD1*) mutations Unknown	Bedlington terrier disease Physiologically and apparently harmless in some species (mute swan; white perch) [3]

ICC, Indian childhood cirrhosis; LEC, Long–Evans Cinnamon.

Table 20.2 Copper-containing enzymes.

Enzyme	Action	Function
Cytochrome *c* oxidase	Transfers 4 electrons to O_2 $O_2 + 4e^- + 4H^+ \rightarrow 2\ H_2O$	Cellular respiration
Superoxide dismutase	$2O_2^- \rightarrow H_2O_2 + O_2$	Free radical scavenging Antioxidant defence Dysfunction associated with amyotrophic lateral sclerosis
Lysyl oxidase	Oxidative deamination of lysine in newly formed collagen and tropoelastin	Connective tissue synthesis
Tyrosinase	Monophenol monooxygenase	Melanin synthesis
Dopamine β-monooxygenase	Dopamine → norepinephrine	Catecholamine synthesis
Ceruloplasmin	Ferroxidase	Oxidizes Fe^{2+} to Fe^{3+}
Hephaestin	Ferroxidase in basolateral membrane of enterocyte	Fe egress from enterocyte
Clotting factors V and VIII	"A" domains homologous to ceruloplasmin	Blood clotting
Peptidylglycine monooxygenase	Neuropeptide processing	Neural function
Prion protein	$PrP^{(C)}$ binds Cu	Cell signaling

Copper metabolism

Body copper status is largely regulated by biliary excretion, whereas iron status is regulated by intestinal absorption. At every stage from intestinal absorption to excretion, copper is bound to a series of transporters and chaperones. There is less than one free Cu ion per cell. Hepatocyte copper metabolism is shown in Figure 20.1. The proteins concerned with uptake (Ctr1), intracellular chaperoning (atox1, CCS, cox17), serum transport (ceruloplasmin), and the Wilson disease protein ATP7B and its associated proteins are considered later. Within the enterocyte, copper is bound to metallothionein, a 10-kDa cytosolic cysteine-rich protein. Metallothionein synthesis is induced by zinc, causing copper to be bound in the enterocyte and lost as it is desquamated at the villous tip – a "mucosal block" to absorption. Copper is exported from the enterocyte to portal blood by the Menkes protein, ATP7A. Copper is carried in portal blood loosely bound to albumin and histidine.

Ctr1 is responsible for high-affinity Cu^{1+} uptake into human cells. It is associated with a membrane metalloreductase, is demonstrable in many tissues, and shows changes with age and copper status. A Ctr1 knockout mouse is not viable, whilst an intestinal epithelial cell-specific Ctr1 knockout mouse exhibits striking neonatal defects in Cu accumulation in peripheral tissues, hepatic iron overload, cardiac hypertrophy, and severe growth and viability defects and kinky whiskers, reminiscent of Menkes syndrome. Ctr1 is involved in the uptake of platinum-containing drugs. Theoretically, *CTR1* mutations might influence severity in Wilson disease.

Copper is carried to its various intracellular destinations by chaperones. Atox1, or HAH1 (human atx homolog-1), is a 68-amino-acid protein abundantly and ubiquitously expressed, which carries Cu to the six Cu-binding sequences at the N-terminal end of ATP7A and ATP7B. These, like atox1, contain the common highly conserved metal-binding motif MXCXXC. Atox1 preferentially transfers Cu to metal binding sites 2 and 4 in ATP7B. Atox1 knockout mice pups

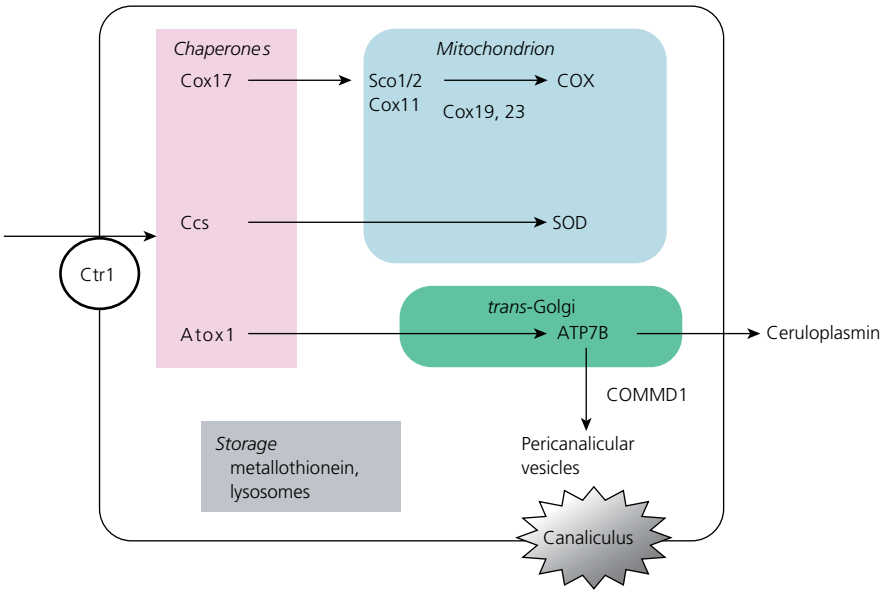


Figure 20.1 Pathways of copper through the hepatocyte. After uptake by Ctr1, copper is chaperoned to its sites of action: by cox17 to the mitochondrion for the synthesis of cytochrome oxidase (COX), with the participation of assembly proteins Sco1/2, cox11, cox19, cox23; by CCS (copper chaperone for superoxide dismutase) to superoxide dismutase; and by atox1 to the Wilson disease protein ATP7B in the *trans*-Golgi. Copper loading of ATP7B induces trafficking to pericanalicular vesicles, with the participation of *COMMD1*. ATP7B passes copper to apoceruloplasmin for export. The tripeptide glutathione, the 10 K protein metallothionein, and insoluble deposits in lysosomes store excess copper.

usually die before weaning, and survivors show growth failure, skin laxity, hypopigmentation, and seizures because of perinatal copper deficiency. Atox1-deficient cells accumulate high levels of intracellular copper due to impaired cellular copper efflux. Atox1 mutations have been sought unsuccessfully in Wilson disease patients without ATP7B mutations. Defects in atox1 do not have a known role in copper storage disease in humans.

The copper chaperone for superoxide dismutase (CCS) carries copper to Cu,Zn-SOD, an important protector against cell injury by reactive oxygen species. Amyotrophic lateral sclerosis is a progressive lethal disorder of large motor neurons which is due to a defect in Cu,Zn-SOD1 in 20% of familial cases. CCS interacts with and attempts to deliver copper to the defective SOD1, even when the enzyme cannot readily accept it, resulting in Cu-induced toxicity.

Cox17p chaperones copper to the mitochondrion, where it is essential for the assembly of cytochrome *c* oxidase (COX), the terminal enzyme of the energy-transducing respiratory chain. COX has 13 subunits, and an additional 30 proteins are required for COX assembly. CoxI and CoxII contain copper. Five proteins (Cox11, Cox17, Cox19, Cox23, and Sco1/2) have been implicated in their synthesis and copper incorporation. Cox17 (locus 3q) is a soluble metallochaperone localized both in the cytosol and in the mitochondrial intermembranous space. Cox17-null mouse embryos died between embryonic days 8.5 and 10. Sco1/2 and Cox11 function downstream as membrane proteins in copper insertion into Cu_A and Cu_B, respectively. *SCO2* mutations are associated with neonatal encephalocardiomyopathy, and *SCO1* mutations with neonatal hepatic failure and ketoacidotic coma. Cox19, also involved in yeast Cu insertion, has a human homolog, but its function and that of Cox23 are less clear. A patient with features suggestive of mitochondrial cytopathy and repeatedly low copper and ceruloplasmin levels in whom no mutations were found, but in whom remarkable clinical and biochemical improvement followed copper histidinate supplementation, shows that there are other copper transport proteins yet to be discovered [1].

The Wilson disease protein, ATP7B

The Wilson disease gene on chromosome 13 codes for a copper-transporting P-type ATPase, *ATP7B*. This is one of a family of transmembrane proteins that mediate the translocation of cations across cellular membranes, often against concentration gradients. Mammals have two copper-translocating P-type ATPases, ATP7B and the Menkes disease protein ATP7A (Table 20.3). Common features are an ATP-binding site (GDGIND), a phosphorylation domain (DKTGT), a phosphatase domain (TGE), eight transmembrane regions, six Cu-binding sites in the N-terminal region, and a conserved Cys-Pro-X motif (X = Cys, His, or Ser) Cu-binding domain in the cation transduction channel in the sixth transmembrane domain (Figure 20.2). ATP7B is expressed predominantly in hepatocytes, but also in regions of the brain, breast, and placenta, while ATP7A is expressed in most extrahepatic tissues, but not the liver.

Table 20.3 Wilson disease and Menkes disease.

	Wilson disease	Menkes disease
Synonym	Hepatolenticular degeneration	Kinky hair, steely hair
Inheritance	Autosomal recessive	X-linked
Gene locus	13q14.3	Xq13.3
Gene	*ATP7B*	*ATP7A*
Tissue expression	Liver, kidney, placenta, brain	All tissues except liver
Cellular location of P-type ATPase	*trans*-Golgi, trafficking toward bile canalicular membrane	*trans*-Golgi, trafficking to plasma membrane
Biochemistry	Cu trapped in hepatocytes Impaired ceruloplasmin synthesis Basal ganglia Cu deposition Cu deposition in eye	Cu trapped in enterocytes Systemic Cu deficiency Impaired copper enzyme synthesis
Pathology	Hepatic damage Basal ganglia dysfunction Kayser–Fleischer rings	Abnormal connective tissue, and cerebral vasculature
Phenotypic variability	Extreme variability in severity of hepatic and neurological disease	*Severe:* Menkes unresponsive to Cu-histidine treatment *Mild:* Occipital horn syndrome
Animal models	LEC rat Toxic milk mouse Knockout mouse	Mouse mutants (mottled, brindled)

LEC, Long–Evans Cinnamon.

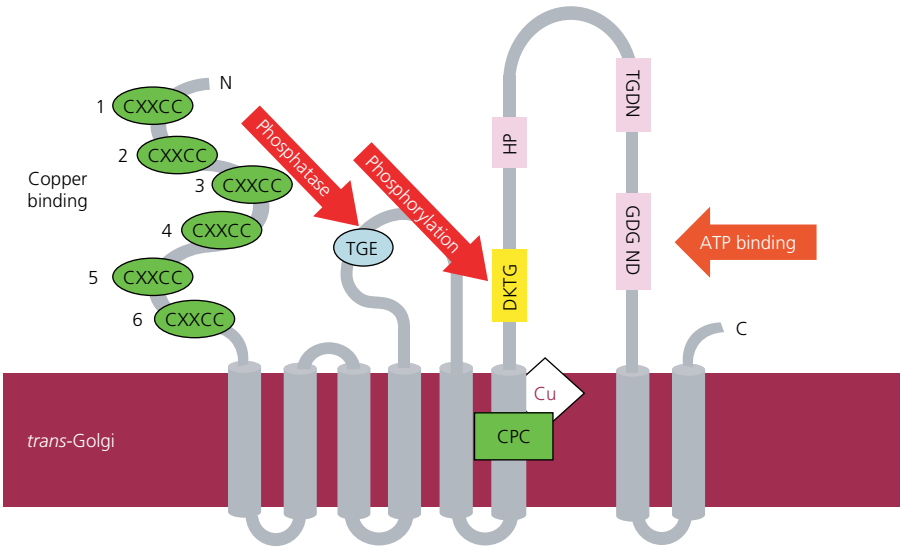


Figure 20.2 ATP7B has eight transmembrane domains that secure the protein in the *trans*-Golgi membrane. The $CPC_{983–985}$ motif in the sixth transmembrane domain reversibly binds copper to facilitate its translocation through the membrane. The ATP binding site G_{1266} DGVND, the phosphorylation domain D_{1027} KTG, and the phosphatase domain $TGE_{858–860}$ are invariant features of P-type ATPases. Six copper-binding sites (CXXC) lie in the N-terminal region.

Function of ATP7B

ATP7B has two functions: to translocate Cu into the *trans*-Golgi for the synthesis of ceruloplasmin; and to export copper from the cell. Copper binding stimulates ATP binding, phosphorylation of an aspartic acid at position 1027, copper translocation, and trafficking. ATP binds via an adenosine buried in the cleft near residues H1069, R1151, and D1164 of ATP7B, which is brought into the vicinity of D1027 (asp 1027) by relative domain motions. ATP binding and phosphorylation of the aspartic acid drives protein conformational changes, leading to the translocation of the bound cations across the lipid bilayer. The reaction cycle is completed with dephosphorylation by an intrinsic phosphatase activity (the TGE motif),

returning the pump to its original conformation to allow further cation binding and translocation. In situations of low copper concentration, ATP7B resides in the membrane of the *trans*-Golgi. Copper loading causes it to migrate toward the canalicular membrane. In the hepatocyte, ATP7B moves from the Golgi to lysosomes and imports copper into their lumen thereby enabling lysosomes to undergo exocytosis through the interaction with p62 subunit of dynactin that allows lysosome translocation toward the canalicular pole of hepatocytes [2]. By contrast, the Menkes protein ATP7A traffics to the basolateral membrane.

Ceruloplasmin

The blue copper-containing enzyme ceruloplasmin (Cp) is a ferroxidase, the function of which is best demonstrated by the effects of its deficiency in aceruloplasminemia (Table 20.4). The low plasma ceruloplasmin concentrations seen in Wilson disease are not associated with an obvious disturbance of iron metabolism. In its synthesis, Cp is produced first as a precursor, which is glycosylated in the secretory compartment to the apoprotein, into which copper is introduced. Apo-Cp has a short plasma half-life in humans, although it is readily detectable in the ATP7B knockout mouse. Cp is an "acute-phase reactant," in that its serum concentration rises in inflammatory states. The mechanism of this is not known, but it might be important in increasing the delivery of copper to macrophages, where copper may participate in the generation of reactive oxygen species in phagosomes.

Most serum copper is in Cp. Non-Cp copper includes a fraction bound to albumin, and to a putative high-molecular-weight carrier, transcuprein. Canine albumin lacks a copper binding site, and this may contribute to the copper-related liver diseases in a number of breeds.

COMMD1

A mutation in the gene *COMMD1* (previously called *MURR1*) is responsible for the copper toxicosis of Bedlington terriers. *COMMD1* interacts with ATP7B and may participate in the cellular copper excretion pathway. Mutations in *COMMD1* have been sought but not found in Wilson disease or non-wilsonian hepatic copper toxicosis.

The cellular prion protein, $PrP^{(C)}$

The cellular prion protein, $PrP^{(C)}$, is a normal cell surface glycoprotein, which in the prion diseases becomes an infectious, conformationally altered isoform ($PrP^{(Sc)}$). The physiological functions of $PrP^{(C)}$ include cellular uptake or binding of copper ions. $PrP^{(C)}$ is strongly expressed in the placenta in the first trimester, together with Ctr1, ATP7A, ATP7B, and COMMD1. There is controversy as to whether copper-bound $PrP^{(C)}$ is an antioxidant supporting neuronal function or a pro-oxidant leading to neural damage. A $PrP^{(C)}$ polymorphism at codon 129 results in either methionine or valine. In one study of Wilson disease patients, homozygosity for the 129 M allele was associated with later onset of neurological symptoms, and in another with significantly more severe neurological symptoms in elderly patients.

Normal values

Normal values for copper-related parameters are shown in Table 20.5. The neonate differs from these values in having low plasma ceruloplasmin and copper, and a raised hepatic copper concentration, with values of up to 450 µg/g dry weight at birth, falling to adult values (<55 µg/g) by 6 months. The newborn thus biochemically resembles the patient with Wilson disease. This state presumably allows the fetus to store liver copper during gestation. It also makes a biochemical diagnosis of Wilson disease impossible before 3 months of age.

Table 20.4 Aceruloplasminemia.

Inheritance	Autosomal recessive
Clinical features	Dementia, dysarthria, dystonia, diabetes mellitus, onset age 40–60 years
Pathology	Iron deposition in liver, pancreas, and brain Neuronal loss, gliosis
Biochemistry	Plasma ceruloplasmin and Cu very low: hepatic Cu normal Plasma Fe low; hepatic Fe raised Basal ganglia Fe deposition
Cause	Failure of ceruloplasmin synthesis
Gene locus	3q25
Described mutations	5-bp insertion in exon 7 nt2389delG in exon 13
Treatment	None

Table 20.5 Biochemical diagnosis of Wilson disease.

	Normal	Wilson disease
Plasma ceruloplasmin (mg/L)	>200	<200 in 85–90% of cases
Urine Cu pre-penicillamine		
• µmol/24 h	<0.5	>1.25
• µg/24 h	<40	>100, but > 40 in asymptomatic patients
Urine Cu after penicillamine		
• µmol/24 h	<25	>25
• µg/24 h	<1600	>1600*
Liver copper (µg/g dry weight)	15–50	>250
Serum copper (µM)	11–24	Low, normal, or high
Free copper (µM)	<1.6	>7

* See text for discussion of the penicillamine challenge test.

Wilson disease (OMIM277900)

History [3]

Clinical descriptions of Wilson disease by Gowers, Strumpell, and Ormerod between 1888 and 1890 preceded Wilson 1911 description of four patients with dysarthria, tremor, and progressive movement disorder who at postmortem had lenticular nucleus cavitation and cirrhosis. Kayser and Fleischer independently described a pigmented corneal ring in neurological patients in 1902–3. Excess hepatic copper was found by Rumpel in 1913, and Hall coined the term "hepatolenticular degeneration" in 1921 and described autosomal recessive inheritance. In 1952, Scheinberg and Gitlin demonstrated low plasma levels of ceruloplasmin, but attempts to treat the disease with ceruloplasmin proved fruitless. Dimercaprol ("British anti-Lewisite," BAL) was shown in the early 1950s to produce clinical improvement. In 1953, Walshe found dimethylcysteine (penicillamine) in the urine of a penicillin-treated liver patient, and 2 years later gave it to a patient with Wilson disease to increase copper excretion after trying it on himself. Sheep were noted to be protected against copper toxicity by a high dietary molybdenum content, shown later to be due to the formation of ammonium tetrathiomolybdate (TM) in the rumen. Schouwink in 1961 noted evidence that zinc sulfate could induce copper deficiency in sheep and showed negative copper balance and clinical improvement in patients. Hoogenraad studied zinc treatment intensively, and in 1979 described resolution of Kayser–Fleischer (KF) rings with zinc. In 1969, Walshe gave triethylenetetramine hydrochloride to patients intolerant of penicillamine. In 1986, Walshe suggested the use of ammonium TM in patients, and Brewer's subsequent studies have described its effect.

The Wilson disease gene locus was shown to be linked to esterase D on chromosome 13 by a study of three kindreds in the Middle East. January 1993 saw publication of the gene for Menkes disease, *ATP7A*, and later in the same year three groups published a homologous gene for Wilson disease (*ATP7B*). Subsequent research has clarified the functions of ATP7B, intracellular copper chaperones, ceruloplasmin, and COMMD1. Animal models – both naturally occurring, such as the LEC rat and the toxic milk mouse, or created by gene knockout – have been exploited. Genetic epidemiology has included identification of many mutations in large populations, and the behavior of particular mutations in isolated communities such as Sardinia.

Epidemiology

Widely quoted prevalence figures vary between 1 in 30,000 and 1 in 100,000. In the only prospective study of incidence, 415 new cases were submitted to the EuroWilson [4] database in 4 years. Of these, 219 were less than 18 years of age and sex incidence was approximately equal. Allowing for incomplete ascertainment, this nevertheless demonstrates that Wilson disease is a rare disease, but that longevity causes relatively high prevalence. There is evidence that genotypic prevalence may be much higher than phenotypic, indicating incomplete penetrance.

Incidence was higher in central and eastern Europe, where the H1069Q mutation is more common than in western Europe, where many patients are compound heterozygotes. Age at presentation varied from infancy to the seventh decade (Figure 20.3). Most infants were detected through investigation of serendipitously discovered abnormal liver function tests rather than through family screening.

Clinical features

Wilson disease may present in many different ways (Table 20.6). Adding together various series, the relative proportions of the four major presentations in 400 patients were: hepatic 20%; hepatic and neurological 20%; neurological or psychiatric 50%; and 10% other causes. However, since these surveys were from adult neurological units, they almost

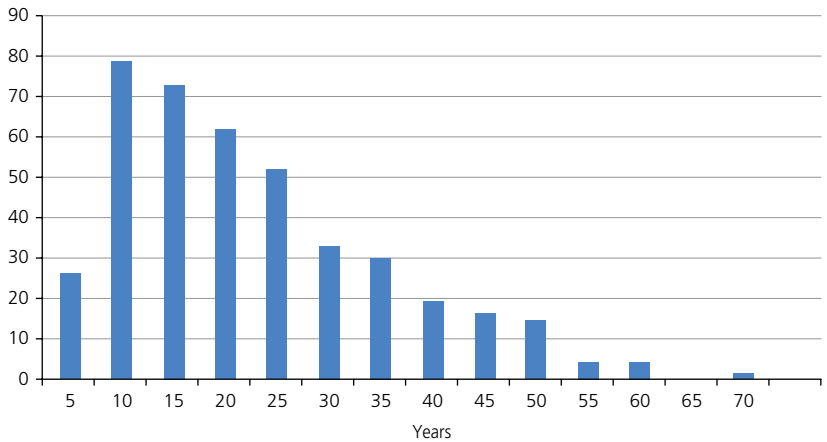


Figure 20.3 Age at diagnosis of Wilson disease 2005–09 taken from the EuroWilson database [4]. (www.eurowilson.org last accessed July 2016. Reproduced with permission of EUROWILSON.)

Table 20.6 Modes of presentation of Wilson disease.

Asymptomatic	Detected during screening of family members
Hepatic (usually age 4–12 years)	Abnormal liver function tests, even as early as the first year of life Incidental finding of hepatomegaly Hepatomegaly or abnormal liver function tests identified during examination of a neurologically affected patient Insidious onset of vague symptoms followed by jaundice Acute hepatitis Chronic hepatitis Acute hepatic failure with hemolysis, with or without encephalopathy Portal hypertension: bleeding varices Decompensated cirrhosis
Neurological and psychiatric (in adolescents and adults)	Abnormalities of speech Mood/behavior changes Incoordination (handwriting deteriorates) Deteriorating school work Resting and intention tremors Dysarthria, excessive salivation Dysphagia, mask-like facies
Hematological	Acute hemolytic anemia
Renal	Renal tubular dysfunction (Fanconi, renal tubular acidosis, aminoaciduria) Renal calculi
Skeletal	Rickets/osteomalacia Arthropathy

certainly underestimate the frequency of childhood hepatic cases – in particular failing to recognize cases of fulminant Wilson disease in childhood. Of the 219 new cases <18 years in the EuroWilson database [4], 5% had liver failure with encephalopathy, 7% coagulopathy, 21% jaundice, ascites, or hematemesis, 16% hepato(spleno)megaly, 39% abnormal liver function tests, and 12% no hepatic features.

Hepatic manifestations

The hepatic manifestations of Wilson disease may be of any variety and severity (see Table 20.6). The progression may be slow or very rapid. The important practical message therefore is: *suspect Wilson disease in any child with undiagnosed liver disease*.

Acute liver failure in Wilson disease

The first presentation in a previously apparently well child may be the appearance of:

- Jaundice and hepatitis.
- Followed rapidly by coagulopathy (international normalized ratio (INR) ≥2).
- Encephalopathy.

There may be a history of previous episodes of jaundice that resolved, or of a previous episode of hemolytic anemia.

There are some difficulties concerning the definition of acute liver failure (see Chapter 18). Children with Wilson disease and liver failure often have cirrhosis in the explanted or postmortem liver. They have therefore had an acute deterioration in an already diseased liver ("acute on chronic"), but because their compensated cirrhosis was asymptomatic, they may fulfil the "adult" criteria for diagnosing fulminant liver failure: acute liver failure with coagulopathy and encephalopathy without pre-existing liver disease and within 8 weeks of the onset of clinical liver disease.

Encephalopathy may be difficult to diagnose or exclude in young children at an early stage. These difficulties are reflected in the slightly different definitions of acute liver failure used in different series. In the Birmingham series [5] the criteria were prothrombin time (PT) ≥24 s prolonged or INR ≥ 2.0 and hepatic encephalopathy without pre-existing liver disease and within 8 weeks of the onset of clinical liver disease. Wilson disease was responsible for two of 97 cases.

In the Pediatric Acute Liver Failure Group (PALF) registry, it was defined as biochemical evidence of acute liver injury with no known evidence of chronic liver disease and a coagulopathy of PT ≥15 s prolonged (INR ≥1.5) and encephalopathy, or a more severe coagulopathy (PT ≥20 s) without encephalopathy. Metabolic causes, mainly Wilson disease and mitochondrial disease, accounted for 22 of 348 children [6].

There are reports of acute liver failure in Wilson disease being apparently precipitated by hepatitis E, hepatitis A, or measles, but in the majority no precipitant is recognized. Whilst adult series show a female preponderance, pediatric series do not, probably because most cases are prepubertal.

Pointers to the diagnosis of Wilson disease in acute liver failure are:

- KF rings, which if present on slit-lamp examination make the diagnosis almost certain, but if absent do not exclude it; they are rare in childhood.
- A family history of Wilson disease, or parental consanguinity; but this is unusual.
- Neurological features of Wilson disease are rare in childhood, although slurred or slow speech may be a feature.
- Jaundice.
- Coombs negative hemolytic anemia.
- Acute renal failure with rapid progression.
- A high bilirubin (>300 μmol/L) and relatively low transaminases (100–500 IU/L), and alkaline phosphatase (<600 IU/L) [7].

A markedly subnormal alkaline phosphatase has been a frequent finding in adult and pediatric series of acute Wilson disease. A ratio of alkaline phosphatase (IU/L) to total bilirubin (mg/dL) <2 showed good discriminative power in some series, although not all. The poorer performance in pediatric series may be due to the contribution of the bone isoenzyme. The low alkaline phosphatase is unexplained.

Without transplantation, survival from acute liver failure in Wilson disease with encephalopathy is extremely unlikely. Thus, the clinical scenario of acute liver failure, encephalopathy, hemolytic anemia, rapidly progressive renal failure in context with the laboratory parameters listed above *always* requires urgent liver transplantation. The diagnosis of Wilson disease may not be concluded before transplantation.

Chronic hepatitis and Wilson disease

Children with a more insidious onset of liver disease may be difficult to distinguish from those with autoimmune hepatitis. The presence of low-titer autoantibodies, presumed to be secondary to exposure of antigens by hepatocyte necrosis, may cause confusion. Cutaneous features of autoimmunity are usually absent, and plasma immunoglobulins are usually not raised. However, patients have been described in whom more convincing features of autoimmune hepatitis were present and an initial diagnosis of autoimmune disease led to treatment with steroids, with or without azathioprine, with initial improvement. In patients with autoimmune hepatitis, thorough screening for Wilson disease is therefore necessary.

Acute hepatitis

Patients diagnosed with Wilson disease may give a history of an "acute hepatitis" – an episode of jaundice and malaise from which they recovered. Whilst some of these may have been episodes of hemolysis or viral hepatitis, Wilson disease should always be excluded in the child with seronegative acute hepatitis.

Neurological presentation

In older children, the first symptoms may be neurological or psychiatric or both. They may develop insidiously or precipitously. Difficulty with speech is often reported. Movement disorders include tremors, poor coordination, and loss of fine motor control, chorea, or choreoathetosis. The intention tremor may be initially unilateral, then becomes coarse, generalized, and incapacitating ("wing-beat" tremor). Spastic dystonia presents with a parkinsonian, mask-like facies, rigidity, and gait disturbance. Pseudobulbar involvement causes drooling and dysphagia.

Features that are not usually present in Wilson disease are corticospinal or cerebellar signs, and abnormalities of the peripheral nerves, skeletal muscle, or cranial nerves.

Neurological Wilson disease patients have been classified into three groups:

- Pseudoparkinsonian, where bradykinesia predominates and cognitive impairment may occur.
- Pseudo-sclerotic, i.e., like multiple sclerosis, with prominent tremor.
- Dyskinetic.

The age of onset and the speed of progression are very variable, with some patients rapidly deteriorating to a chair-bound life with severe movement disorder while others continue with relatively mild symptoms.

The psychiatric disorders are also highly variable. Depression is common. Neurotic behavior includes phobias, compulsive behaviors, aggression, or antisocial behavior. Cognitive deterioration may also occur, with worsening school performance, poor memory, difficulty in abstract thinking, and shortened attention span. Pure psychotic disorders are uncommon.

The differential diagnosis of neuropsychiatric disease in the presence of liver disease includes (see also Chapter 19):

- Late presentation of Niemann–Pick disease type C, particularly if splenomegaly is a feature.
- Lafora disease.
- Congenital disorders of glycosylation.

Investigations

CT scan or MRI way demonstrate copper accumulation in the basal ganglia. MRI can reveal slight morphological changes in patients with no obvious neurological signs or those with a hepatic presentation. More marked findings in neurological Wilson patients become evident in T1- and T2-weighted MRI. T1-weighted MRI predominantly detects atrophic changes, whereas T2-weighted MRI regularly records signal changes in the putamen. Adequate decoppering therapy prevents progression and may improve symptoms.

Hemolysis

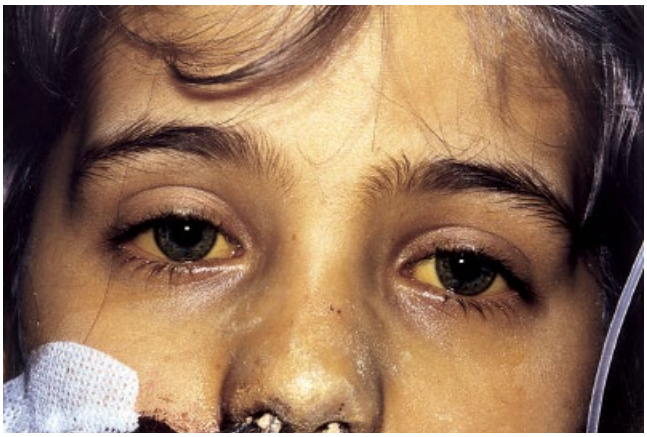
Coombs negative hemolysis may be the initial presentation, sometimes apparently precipitated by infection or drugs. There may be a history of a previous undiagnosed hemolytic episode in patients presenting with hepatic or neurological features, and hemolysis may be prominent in fulminant Wilson disease.

Ophthalmic abnormalities

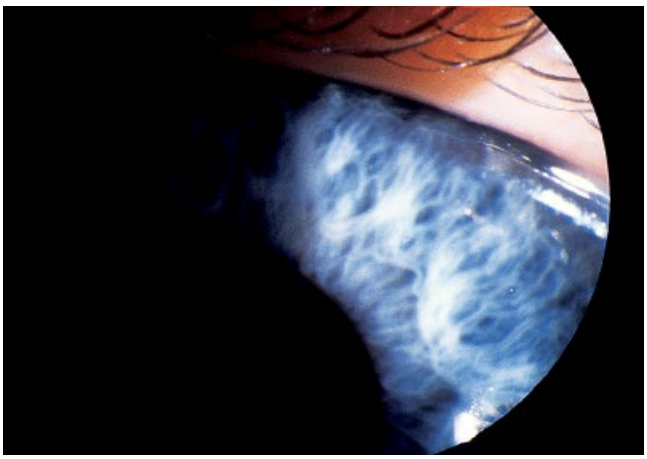
The KF ring is a gold or gray-brown opacity in the peripheral cornea (Figure 20.4). It first develops superiorly in the cornea (at the 12 o'clock position), then inferiorly, and finally in the horizontal meridian. It represents a deposit of copper and sulfur-rich granules in the Descemet membrane, and is reversible with treatment. Additional later ocular findings in Wilson disease include sunflower cataracts, saccadic pursuit movements, loss of accommodation response, and apraxia of opening the eyelid.

Renal abnormalities

Renal tubular abnormalities are frequent and include glycosuria, aminoaciduria, renal tubular acidosis, impaired phosphate reabsorption, or a full-blown renal Fanconi syndrome. They are the presumed consequence of tubular

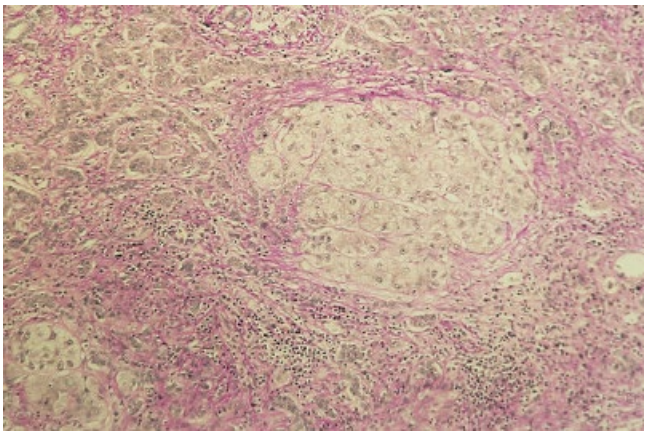


(A)

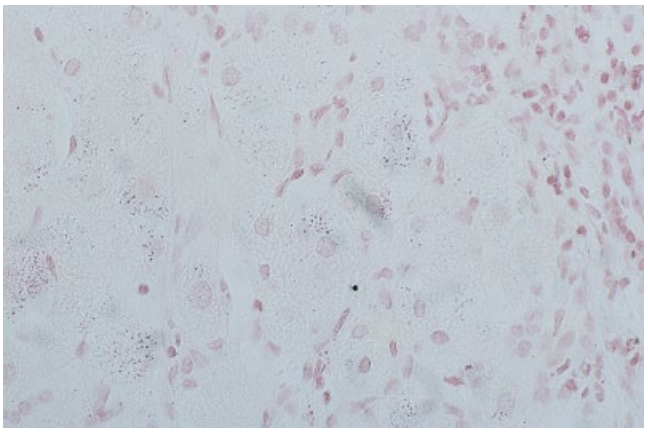


(B)

Figure 20.4 Wilson disease may present with fulminant hepatitis (A), hemolysis, and low alkaline phosphatase. This young girl, who underwent successful transplantation, was found to have Kayser–Fleischer rings on slit-lamp examination (B).



(A)



(B)

Figure 20.5 Liver biopsy demonstrated severe hepatitis and underlying cirrhosis (A). Copper storage was demonstrated using orcein staining (B).

copper deposition. Glomerular dysfunction is less frequent, but proteinuria may be exacerbated by penicillamine. Recurrent hypokalemic muscle weakness, hyperoxaluria, renal calculi, and nephrocalcinosis are uncommon features.

Skeletal manifestations

Copper-mediated oxidative damage to collagen probably underlies the arthritis that occurs in a small number of patients with Wilson disease. The secondary effects of renal tubular phosphate leak and hepatic osteodystrophy are likely to be the cause of the radiological abnormalities, such as rickets or osteoporosis, which occur in more patients. Skeletal complications appear to be more frequent in Asian/Indian patients.

Pathology

The earliest ultrastructural abnormalities are seen in mitochondria, which are pleomorphic and show increased matrix density, separation of the normally apposed inner and outer membranes, and widening of intercristal spaces. These changes are sufficiently specific to be of diagnostic value.

The earliest histological changes comprise microvesicular and macrovesicular fatty deposition, glycogen-containing vacuoles in the nuclei of periportal hepatocytes, dense and enlarged peroxisomes. With progression, portal fibrosis and inflammation are seen. Children presenting with clinical liver disease may show a histological picture indistinguishable from autoimmune hepatitis with interportal fibrous bridging or frank cirrhosis (Figure 20.5). Features suggesting Wilson disease are:

- Fatty change.
- Mallory–Denk bodies.
- Glycogen-containing vacuoles in the nuclei.
- Lipofuscin.
- Copper staining.
- Iron deposition in Kupffer cells in patients who have had hemolysis.

Mallory–Denk bodies are irregularly shaped cytoplasmic inclusions comprising aggregated keratin 8, ubiquitin,

heat-shock proteins, and the stress protein p62, stabilized by transglutaminase cross-linking. Well-circumscribed homogeneous cytoplasmic eosinophilic globules lacking keratins also occur [8].

In well-established liver disease, copper may be demonstrable by rhodanine or rubeanic acid staining. The elastin stains orcein and aldol fuchsin will then usually show granular staining, thought to represent lysosomal copper–protein polymer. These methods may be negative in early cases, presumably because at that stage the copper is cytosolic and in low-molecular-weight complexes. The absence of histochemically demonstrable copper *never* excludes a diagnosis of Wilson disease.

Liver copper in pre-symptomatic children is higher than in older symptomatic children. This contradicts the common view that in Wilson disease, copper builds up in the liver to a level that causes damage, and suggests that some other factor initiates damage in the copper-laden liver. The relationship between copper and organelle damage remains unclear. In particular, it is unknown why some patients develop severe hepatic necrosis and others only minimal damage.

In contrast to hemochromatosis, Wilson disease is rarely associated with hepatoma.

Wilson disease heterozygotes may have a hepatic Cu of the order of 100–200 μg/g dry weight and mild histological portal tract changes, but there is no evidence of progression of liver disease.

Diagnosis

The first essential in making the diagnosis is to think of it.

KF rings, if present, are highly suggestive, but:

- Are absent in 50% children with Wilson disease below 10 years of age.
- At an early stage, will only be detected by slit-lamp examination (see Figure 20.4B).
- Are difficult to see in brown or green eyes.
- Are not entirely pathognomonic; they may rarely occur in copper overload due to chronic cholestasis.

Biochemical diagnosis

The biochemical diagnosis (see Table 20.5) depends upon finding:

- A low plasma ceruloplasmin.
- A raised basal urine copper.
- A raised liver copper concentration.

However, there are numerous pitfalls in these laboratory parameters, as outlined here.

Serum copper

Most plasma copper is within ceruloplasmin. In Wilson disease, serum copper may be low because ceruloplasmin is low, or raised because hepatic necrosis releases "free" copper into the plasma, or somewhere in between. A normal plasma copper does not exclude a diagnosis of Wilson disease. In the patient treated with TM, serum copper remains high because complexed copper is retained.

Free (non-ceruloplasmin) serum copper reflects the proportion of copper that is non-protein bound, presumably released from the damaged liver, and available to cause toxicity. It is calculated (in μmol/L) on the basis that 1 mg ceruloplasmin contains 3 μg or 3/63.5 μmol copper, and is approximately total Cu μM–(0.047 × ceruloplasmin in mg/L).

There are major problems in measuring free copper as it is calculated from two measured parameters (holo- and apoceruloplasmin) and is thus subject to error.

A more direct measurement of non-ceruloplasmin copper is the "relative exchangeable copper" [9]. Early results suggest high sensitivity and specificity but more work is needed to evaluate this promising method in larger groups.

Ceruloplasmin

Various factors may affect plasma ceruloplasmin. Ceruloplasmin is a glycoprotein and plasma levels may be low in some disorders of glycosylation. It is also an acute-phase reactant, and will be elevated by hepatic or other inflammation which might explain why plasma ceruloplasmin exceeds 200 mg/L in approximately 10% of Wilson disease cases.

Plasma ceruloplasmin may be low if overall hepatic protein synthesis is low, either acutely as in fulminant failure, or in decompensated cirrhosis, or hypoproteinemia from protein losing enteropathy or severe malnutrition. A significant number of Wilson disease heterozygotes will have a plasma ceruloplasmin <200 mg/L.

Aceruloplasminemia may cause diagnostic confusion in neurological cases (see Table 20.4), and an unknown percentage of normal individuals will be heterozygotes for aceruloplasminemia.

Urine copper

Urine copper may be increased in acute hepatitis, but is usually much higher in children with Wilson disease (Tables 20.5 and 20.7). It is important to ensure an accurate 24-h urine collection of an uncontaminated sample. If the investigation is designed as a screen for Wilson disease, or as a basis for further testing, then >40 μg/24 h suggests Wilson disease, while a value of 100 μg/24 h, whilst less sensitive, carries a higher specificity [10].

A penicillamine challenge test may be performed in symptomatic children if a diagnosis of Wilson disease is suspected but basal urinary copper excretion is normal. In the original description of this test in children with liver disease, penicillamine 0.5 g was given 12-hourly two times. Urine copper exceeded 25 μmol/24 h in 15 of 17 patients with Wilson disease and one of 58 with other liver disorders [11]. Recent re-evaluation studies confirm its usefulness in Wilson disease children with active liver disease [12], but it has poor sensitivity in pre-symptomatic siblings [10, 12], in whom

Table 20.7 Diagnostic score in Wilson disease, agreed at a consensus meeting [13]. In the EuroWilson database [4], patients scoring ≥4 are accepted as having Wilson disease.

Score	–1	0	1	2	4
Kayser–Fleischer rings	–	Absent	–	Present	–
Neuropsychiatric symptoms suggestive of Wilson disease (or typical brain MRI)	–	Absent	–	Present	–
Coombs negative hemolytic anemia + high serum Cu	–	Absent	Present	–	–
Urinary copper (in the absence of acute hepatitis)	–	Normal	1–2 × ULN	>2 × ULN, or >5 × ULN 1 day after 2 × 0.5 g D-penicillamine	–
Liver copper quantitative	–	Normal	<5 × ULN	>5 × ULN	–
Rhodanine-positive hepatocytes (only if quantitative Cu measurement is not available)	–	Absent	Present	–	–
Serum ceruloplasmin	–	>0.2 g/L	0.1–0.2 g/L	<0.1 g/L	–
Disease-causing mutations detected	–	None	1	–	2
Assessment of the Wilson disease diagnostic score:					–
• 0–1: unlikely	–	–	–	–	–
• 2–3: probable	–	–	–	–	–
• 4 or more: highly likely	–	–	–	–	–

MRI, magnetic resonance imaging; ULN, upper limit of normal: >40 µg/24 h in asymptomatic and >100 µg/24 h in symptomatic patients.

this test should not be performed. In the diagnostic scoring developed by an expert group [13], a postpenicillamine urine copper greater than five times the laboratory's normal upper limit (>40 µg/24 h in asymptomatic and >100 µg/24 h in symptomatic patients) is the stated cut-off (see Table 20.7). In 24-h collections of urine during a second and third day of penicillamine treatment, the copper concentration may rise further, which is suggestive of the diagnosis, but this has not been formally evaluated.

The penicillamine challenge has not been evaluated in neurologic cases and heterozygotes. The difficulties in reliably collecting 24-h urines and the fact that the same dose is recommended for all ages are limitations to this test.

Liver copper

Copper may or may not be histochemically demonstrable in the liver. A liver copper >250 µg/g dry weight (normal <55 µg/g) has been described as the gold standard diagnostic test, but it has limitations. Higher values are found in the newborn, settling to adult levels by 6 months. Prolonged cholestasis raises hepatic copper by inhibiting its biliary excretion, and this may cause confusion in cases of autoimmune hepatitis with sclerosing cholangitis. In a study of adult liver biopsies, a value >250 was found in only 95 of 114 patients with Wilson disease [14]. This may result from sampling error, particularly in the cirrhotic liver. Errors are reduced by providing a good-sized sample and scrupulously preventing contamination. Thus, two passes of liver biopsy should be obtained and an entire biopsy core (>1 cm) should be used for copper quantitation in an experienced laboratory [15].

Isotopic copper

Due to the limited availability of this test, described in previous editions, this is now rarely performed.

Genetics

The Wilson disease gene, the *ATP7B* gene, comprises 80,000 base pairs on 21 protein-coding exons and 20 non-coding introns, plus an incompletely characterized promoter. It is transcribed and processed into a 7500-base mRNA, which is translated into the 1411 amino acid, 159 kDa ATPase. More than 500 mutations were reported to the University of Alberta database before 2009 (http://www.wilsondisease.med.ualberta.ca/database.asp last accessed July 2016) [16] and new mutations continue to be reported. H1069Q (Hist1069Glu) is the commonest worldwide. Mutations in *ATP7B* have various effects altering protein expression levels, catalytic and transport activity, as well as intracellular localization [17].

The H1069Q mutation (exon 14)

This is the most common mutation in patients from central, eastern, and northern Europe. Approximately 50–80% of Wilson disease patients from these countries carry at least one allele with this mutation with an allele frequency of 30–70%. Homozygote and heterozygote H1069Q frequencies are 39% and 48% in eastern Germany, and 17% and 43% in Austria. The H1069Q allele frequency is reported to be 57% in a Czech and Slovakian cohort, 49% in Yugoslavia, 35% in a largely neurological series from Poland, 38% in the US, and approximately 30% in the UK. This distribution is consistent with the hypothesis that this mutation arose in Eastern Europe. H1069Q is rare in Asian, Japanese, and Chinese patients.

The effect of the H1069Q sequence change is to prevent tight binding of ATP to the N domain of ATP7B. It does not alter the conformation of ATP7B. Structural studies of this domain show that residues H1069, G1099, G1101, I1102, G1149, and N1150 contribute to ATP binding, and this area is the site of at least 30 known Wilson disease mutations affecting ATP binding or protein folding.

Other mutations

Some are common in particular populations, such as Met645Arg in Spanish patients, R778L in those from the Far East (China, South Korea, Japan, Taiwan), and asp1279ser mutation in Costa Rican patients. Many others are found at a low frequency.

Genotype–phenotype correlation

Although it is common to find patients with the same genotype with different phenotypes, there is statistical evidence that H1069Q homozygotes are more likely to present at a later age and with neurological rather than hepatic disease. Amongst 70 Dutch patients, those who were homozygous or heterozygous for the H1069Q mutation presented more frequently with neurological disease (63% and 43% versus 15%), and at a later age (20.9 and 15.9 versus 12.6 years) than patients without the H1069Q mutation. In a meta-analysis of 577 published patients, the odds ratio for neurological presentation in homozygous or heterozygous H1069Q versus non-H1069Q patients was 3.50 (95% CI, 2.01–6.09) and 2.13 (95% CI, 1.18–3.83), respectively, and the ages at presentation were 21.1, 19.2, and 16.5 years, respectively. Some, but not all, other series support this association.

Conversely, an increased frequency of severe truncating mutations was associated with an earlier age at onset and acute liver failure when compared to missense mutations. However, no clear phenotype–genotype correlation in Wilson disease has been established yet.

Penetrance

There is cumulating evidence of incomplete penetrance which has a number of practical implications by making the prognosis and hence management of an individual diagnosed pre-symptomatically more difficult to decide. Wilson disease has an approximate phenotypic incidence of 1 in 7000 live births in the Sardinian population. Molecular analysis of the Wilson disease chromosomes containing the most common haplotype showed a 15-nucleotide deletion in the promoter region [18]. This -441/-427del mutation was found in 122 of 5290 neonatal blood spots in Sardinia. This suggests a carrier frequency of 3.8%, which from the Hardy–Weinberg equation indicates a disease frequency of approximately 1 in 3000 – at least twice the frequency of phenotypic cases. Because of wide confidence intervals, this must be interpreted cautiously, but it raises the important possibility of incomplete penetrance, i.e., that not all genotypically affected patients present with disease. This has been re-examined in a UK study of neonatal blood spots. At the most conservative estimate, the calculated frequency of individuals predicted to carry two mutant pathogenic *ATP7B* alleles is 1 : 7026 [19]. It may thus be that Wilson disease continues to be underdiagnosed, or it may be that many individuals with two disease-causing Wilson disease mutations never clinically manifest. This would be a similar situation to the incomplete penetrance of other monogenic liver disorders such as genetic hemochromatosis or α1-antitrypsin deficiency.

Genetic testing strategy

A testing strategy must be developed for the population served. If one mutation is frequent, then direct testing is rapid and allows primary diagnosis. If there is a spectrum of mutations, then diagnosis is more challenging. Currently, most laboratories adopt a strategy of first sequencing the "hot-spot" exons for their population, which in the UK means exons 2, 8, 13–15, and 18–19.

Unlike Menkes syndrome, in which 22% of the mutations are sizable deletions, deletions are uncommon in Wilson disease. However, the possibility of a deletion must always be considered in apparent homozygotes; family studies should be performed to confirm that both parents are carriers.

Novel sequence changes may be disease-causing mutations or harmless variants. In silico prediction analysis is helpful to distinguish pathogenetic mutations from such variants.

The overall mutation detection frequency may be increased from 80 to 98% if direct sequence analysis of the entire *ATP7B* coding region and adjacent splice sites is undertaken. In addition, it is crucial to genotype both parents to detect rare genetic phenomena:

- Deletions.
- Unusual genetic mechanisms such as three *ATP7B* mutations in a single patient (two in *cis* on the same chromosome).
- Uniparental disomy (both homologues of a chromosomal region originating from only one parent).
- Apparently dominant inheritance due to the unaffected parent turning out to be a carrier of another Wilson disease mutation emphasizing the need to consider the diagnosis of Wilson disease in the children of affected parents.

However, negative mutation analysis does not exclude Wilson disease. Instead, all available biochemical tests including liver biopsy with hepatic copper quantitation should be repeated if suspicion of Wilson disease is still high (see Table 20.7: diagnostic score 2–3).

Diagnosis in practice

The diagnosis of Wilson disease is generally straightforward in patients with KF rings and low serum ceruloplasmin. Otherwise, correct diagnosis depends on the clinical presentation and expertise of the pediatric hepatologist to interpret the biochemical tests in context with the clinical presentation.

- In pre-symptomatic children, or neurological patients with mild abnormalities of liver function tests, serum ceruloplasmin will be generally low and basal urine copper >40 μg/24 h.

- In active liver disease, serum ceruloplasmin is less reliable, but basal urine copper should be >100 μg/24 h and the penicillamine challenge test positive.
- In acute liver failure, ceruloplasmin will be low, baseline urine copper, and postpenicillamine copper should be above the cut-off values given in Table 20.5. In all these situations, if a rapid test for H1069Q or the locally common mutation is available, a positive result supports the diagnosis and justifies starting treatment.

Treatment

Once the diagnosis has been made, treatment needs to be lifelong without any interruption.

Five drugs are available to treat the copper overload of Wilson disease: D-penicillamine, triethylenetetramine hydrochloride (trientine), zinc, ammonium TM, and BAL (Table 20.8). There are few randomized controlled clinical trials comparing agents.

Penicillamine

Although "decoppering" was the rationale for initially using penicillamine, penicillamine treatment does not cause liver copper levels to fall to normal. It is therefore thought to "detoxify" the liver copper, by induction of metallothionein, favoring lysosomal sequestration (like zinc). Since the patient is not "decoppered," they remain at high risk of deterioration if treatment is discontinued. Numerous reports of rapid decline in hepatic function within 18–24 months of stopping penicillamine emphasize the need to maintain compliance.

DL-Penicillamine was associated with a high incidence of nephrotic syndrome and pyridoxine deficiency. D-Penicillamine is much less toxic, but nevertheless causes significant side effects in 5–10% of treated patients. These include:

- Skin rash, usually urticarial, occurring soon after commencing treatment, which usually responds to cessation of treatment and reintroduction gradually under steroid cover.
- Proteinuria, which is mild in most cases and does not require cessation of treatment. In a small number of patients, there may be an immune complex nephropathy leading to nephrotic syndrome.
- Marrow depression, particularly affecting platelet count.
- Systemic lupus erythematosus (SLE).
- Pyridoxine deficiency, particularly during growth or pregnancy.
- Effects on cutaneous collagen usually occurring after prolonged therapy, namely elastosis perforans serpiginosa and cutis laxa.
- Appearance of, or deterioration in, neurological dysfunction on starting treatment in patients with neurological features, but also in cases without neurological signs.

Blood counts and urine testing for protein should be performed fortnightly for the first 2 months then monthly for 6 months. Pyridoxine 50 mg/week should be given.

Although there is considerable clinical experience with penicillamine, it is being replaced by trientine which is less toxic [20]. The advantages and disadvantages of penicillamine and trientine should be discussed with the patient and parents and a decision made on the basis of individual needs and the respective side effects profile.

Table 20.8 Drugs used in the treatment of Wilson disease.

Drug	Dose
D-Penicillamine	20–35 mg/kg/day*
Triethylenetetramine dihydrochloride (trientine)†	2–12 years: 300 mg b.i.d. 12–18 years: 300–600 mg b.i.d.
Zinc acetate†	<5 years: 25 mg b.i.d. 6–15 years: 25 mg t.d.s. >16 years or if >57 kg: 50 mg t.d.s.
Ammonium thiomolybdate	30 mg b.i.d.

* Should also have pyridoxine 50 mg/week.
† Should be administered 6 h apart to prevent chelation of zinc by trientine.

Trientine

Trientine was initially introduced as a second-line drug for patients intolerant of D-penicillamine. It is less toxic and the only significant side effects are SLE and sideroblastic anemia, particularly when given with zinc. Colitis, resolving on cessation of trientine, is also reported. It is now a logical first-line treatment.

Zinc

The rationale for using zinc is that induction of metallothionein in intestinal cells will bind copper in the enterocyte and reduce absorption. Since zinc, like penicillamine, also induces hepatic metallothionein, it is an attractive drug because it is more physiological than penicillamine or trientine, has lower toxicity, and zinc sulfate is cheap.

In high doses in animals it may cause pancreatic atrophy, but this has not been reported in humans. It may impair iron absorption. The principal practical problems are its unpalatability, and dyspeptic symptoms due to gastric irritation.

Zinc acetate (Wilzin®), is less likely to cause these side effects than zinc sulfate, but is more expensive. Serum levels of amylase and lipase may rise because they are zinc-containing enzymes. Compliance can be monitored by measuring the urinary zinc (>2 mg/24 h), while overtreatment is identified by monitoring urine copper and reducing the dose of zinc if it drops below 50 μg/24 h. Long-term follow-up in 17 symptomatic children with exclusive zinc monotherapy demonstrated a good outcome for neurological disease, but it was less satisfactory in hepatic disease [21].

Tetrathiomolybdate

TM is a powerful copper chelator and became an effective veterinary therapy for ovine copper poisoning. Unlike the above drugs, it is able rapidly to bind copper in tissues in an inert complex. Its clinical use is limited by toxicity – namely, bone marrow depression and, in growing animals, epiphyseal abnormalities. It may have a role in the initial treatment of neurological cases.

BAL

BAL (dimercaprol) was used for Wilson disease prior to the introduction of penicillamine. Given by intramuscular injection, it is painful and has many reported toxic side effects. Some authorities recommend its use in neurological cases refractory to other therapy.

Treatment regimens differ for the varying clinical scenarios described earlier.

Acute liver failure with encephalopathy

A child with acute liver failure and encephalopathy should be listed for urgent transplantation, and routine management of acute liver failure should be instituted (see Chapter 18). A chelator (trientine or penicillamine) and zinc should be started, even if the diagnosis of Wilson disease is not certain, and be given 6 h apart to prevent chelation of zinc. If there is renal failure, excretion of the copper–drug complex will be impaired unless the child is dialyzed.

Removal of copper from the acute liver failure in Wilson disease patient may be successful using plasmapheresis, plasma exchange with continuous hemodiafiltration, and albumen hemodialysis with continuous venovenous hemodiafiltration, based on the hypothesis that non-ceruloplasmin copper is largely albumin bound. The Molecular Adsorbent Recirculating System (MARS®) theoretically should remove albumin-bound copper by adsorption onto the MARS-flux membrane and the toxins responsible for hepatic encephalopathy. There are anecdotal cases in which MARS alone or MARS with albumin–continuous venovenous hemofiltration (CVVH) have provided a successful bridge to transplantation, but this technique is no longer available for young children [22].

Liver failure without encephalopathy

The decision to list for transplant is more difficult if the child does not have encephalopathy, and it is necessary to balance the risk of rapid deterioration and encephalopathy, with the risk of removing a native liver which may recover with chelation therapy. The new Wilson Predictive Index developed at King's College Hospital, London, is reported to be 93% sensitive and 98% specific, with a positive predictive value of 93% (Table 20.9) [23]. If the score deteriorates, or is >11, then the child should be listed urgently. A stable or improving score is an indication to continue with medical therapy.

A chelator and zinc should be started as above.

Chronic hepatitis, acute hepatitis, cirrhosis with or without portal hypertension

Treatment regimens are based on single-center series rather than randomized controlled clinical trials or agreed evidence-based guidelines. It is logical to use a combination of a chelator and zinc acetate (see Table 20.8). Once remission has been obtained, maintenance treatment with zinc acetate alone is possible.

Monitoring the effectiveness of and compliance with chelation therapy is difficult. Urine copper levels will rise to high values in the first 3 months, declining after 1 year of continued treatment. After this time, urine copper should be measured 6-monthly. A falling value suggests that patients may have discontinued the drug, whilst an unexpectedly very high value may suggest that they have restarted it recently in anticipation of the clinic visit.

Effectiveness of therapy is monitored by biochemical liver function tests, which should show a steady improvement over the first months of treatment, and by serial liver biopsy. It is difficult to interpret liver copper levels in follow-up biopsies, since two effects are operative:

1. Chelators remove that fraction of liver copper that is mobilizable, thus reducing liver copper.
2. Both penicillamine and zinc induce metallothionein, which binds copper and may increase liver copper concentration.

In interpreting serial liver copper levels, it is important to remember that the right lobe tends to have higher values. For this reason, changes in hepatic inflammation are of more significance than changes in liver copper. Likewise,

Table 20.9 Prognostic index in acute liver failure in Wilson disease.

Score	Bilirubin (μmol/L)	INR	AST (IU/L)	WCC ($\times 10^9$/L)	Albumin (g/L)
0	0–100	0–1.29	0–100	0–6.7	>45
1	101–150	1.3–1.6	101–150	6.8–8.3	34–44
2	151–200	1.7–1.9	151–300	8.4–10.3	25–33
3	201–300	2.0–2.4	301–400	10.4–15.3	21–24
4	>301	>2.5	>401	>15.4	<20

Sensitivity and specificity rates of 93% and 97%, and positive predictive value and negative predictive values of 92% and 97%, respectively, have been reported [23].
A score >11 indicates a need to list the patient for urgent liver transplantation. AST, aminotransferase; INR, international normalized ratio; WCC, white cell count.

liver histology may deteriorate despite no significant change in the hepatic copper concentration in the patient who discontinues treatment.

In contrast to hemochromatosis malignancy is extremely rare in hepatic Wilson disease.

Neurological presentation

Neurological deterioration occurs in some patients at the start of treatment with penicillamine, trientine, or zinc. This may be prevented by using a regimen of 8 weeks of TM followed by zinc, but in practice most patients will receive zinc or a chelator. The practice of starting with a small dose of chelator and increasing slowly has logic, but there are no data to prove its benefit. There is no clear association with the development of neurological symptoms and the type of anti-copper therapy.

Pre-symptomatic cases

All genotypically affected patients are thought to be at risk of developing clinical disease. The risk of disease is difficult to quantify, because Wilson disease is so phenotypically variable, and some cases do not develop until late in adult life. Even within sibships, there is phenotypic variability.

Despite these caveats, treatment is recommended. A logical but not evidence-based approach is to measure plasma transaminases at 1 year of age and 6-monthly thereafter, and to start zinc if transaminases rise or at the age of 3 years. Pre-symptomatic treatment should prevent liver and neurological damage, but patients should be monitored for evidence of disease, for copper deficiency, and pancreatic dysfunction.

Pregnancy

Treatment should not be discontinued in pregnancy. There are numerous reports of successful pregnancy in women treated with penicillamine, but reports of unusual connective tissue changes in babies born to women who were receiving penicillamine for cystinuria and rheumatoid arthritis suggest that zinc is a safer option during pregnancy.

Assuming no consanguinity, the fetus will be an obligate heterozygote. The risk that the baby will have Wilson disease is of the order of 1 in 300 for a population with a disease frequency of 1 in 100,000. It is recommended that the baby is breastfed, but the full blood count and ceruloplasmin are monitored to exclude hematological evidence of copper deficiency and confirm the physiological rise in ceruloplasmin.

Liver transplantation

Liver transplantation is indicated for those children who do not respond to therapy, or who have fulminant or advanced liver failure and/or portal hypertension (see Chapter 31). Following liver transplantation, the recipient has the donor's normal plasma copper and ceruloplasmin. If a live-related donor is a parent, i.e., an obligate heterozygote, this will be reflected in post-transplantation ceruloplasmin. Since there is no evidence of morbidity in the heterozygote, this is not a concern.

If the neurological abnormalities in Wilson disease are due to intrinsic abnormalities of copper metabolism in the brain, then liver transplantation does not improve them or alter the likelihood of their appearance. If they are due to overspill of copper from a copper-laden liver, then liver transplantation should benefit the brain also. In some cases, transplantation has arrested neurological deterioration or achieved improvement, but may have the opposite effect in others. This may be because patients with liver failure also have hepatic encephalopathy, whilst peritransplant events and subsequent immunosuppression may contribute to ongoing neurological abnormalities. In addition, patients with Wilson disease may be more susceptible to the neurological side effects of tacrolimus.

Although still controversial, there is growing evidence that neuropsychiatric symptoms improve after transplantation and that Wilson disease patients with predominantly neurological manifestations, but stable liver function might benefit from liver transplant [24]. The overall results of liver transplantation for Wilson disease are good (see Chapter 31).

The future

Human hepatocyte transplantation (HTx) is increasingly used as treatment for liver-based metabolic defects. HTx may benefit Wilson disease patients with ALF, either as transient support until chelation treatment is effective or as a cure through liver repopulation by healthy donor cells, as shown in animal models of Wilson disease. Although clinical trials of HTx have provided a "bridge to transplant" in ALF, it has not been used for Wilson disease. Gene editing offers an exciting though currently remote possibility for a "cure."

More immediate measures to improve patient outcomes include increasing awareness of Wilson disease, encouraging investigation for Wilson disease in patients with early symptoms possibly caused by Wilson disease, ensuring sibling screening is performed, mounting randomized treatment trials, and addressing compliance with therapy.

Non-wilsonian copper-related cirrhosis in childhood

Infantile copper toxicoses, in which infants and young children developed rapidly progressive and fatal disorders caused by excessive copper ingestion, are now largely of historical interest, as the feeding patterns that produced them now rarely occur. They are therefore only described here briefly. These were:

- Indian childhood cirrhosis (ICC), in which copper was acquired from milk that had been heated in brass utensils [25].

- Tyrolean childhood cirrhosis, in which copper was acquired from diluted sweetened milk that had been boiled in copper utensils, and for which a strong genetic susceptibility was demonstrated [26].
- Sporadic childhood copper-related cirrhosis, in which copper was acquired from water used to make up infant feeds, that water having taken up copper from plumbing. Characteristic of this group was the use of a private well for water [27].

Indian childhood cirrhosis

In the 1980s, ICC had an incidence of 1 in 4000 rural live births in Maharashtra, presenting at a mean age of 18 months. It affected boys more than girls, rural families more frequently than urban ones, middle-income families more frequently than very poor, and Hindus more frequently than Muslims.

The onset was insidious, with abdominal distension, malaise, and irritability, progressing to jaundice, ascites, edema and respiratory distress, and death. The liver was large and very hard. The histology was characteristic, showing necrosis of hepatocytes with ballooning and Mallory hyaline; pericellular intralobular fibrosis; an inflammatory infiltrate; poor regenerative activity, to the extent that there was often little nodular change; absence of fatty change; absence of cholestasis until an advanced stage; and granular orcein staining. There was severe ultrastructural damage, with prominent end-stage copper-rich and sulfur-rich lysosomes, and severe morphological abnormalities of the mitochondria.

Careful household epidemiological studies provided evidence that the etiology was an early introduction of cow or buffalo milk feeds contaminated with copper from untinned brass utensils. A recent review casts doubt on this conclusion [28]. It describes a study undertaken between 1983 and 1987, but curiously not first reported until 2006, of 885 children from six centers, 227 having "definite ICC". The authors concluded that whilst heavy orcein staining was a feature of ICC, exposure to dietary copper was not. To what extent confusion between ICC and other cirrhoses, or between the specific feeding practice of heating milk in untinned brass vessels versus use of copper water containers, led to this conclusion remains arguable. What is not in doubt is that changes in infant feeding practice have been temporally associated with a virtual disappearance of ICC as defined above.

Penicillamine given early at a dosage of 20 mg/kg/day reduced the mortality from 92% to 53%. Clinical improvement was accompanied by a change in histology to an inactive micronodular cirrhosis, with subsequent resolution – a rare example of reversal of cirrhosis. Liver copper concentrations fell to near normal levels. ICC was preventable by a change in infant feeding practice, and it has now virtually disappeared [25].

Tyrolean childhood cirrhosis

Between 1900 and 1974, 138 infants died in an area of the Austrian Tyrol from an illness similar to ICC in its age of presentation, clinical features, short survival, and high mortality. Unlike ICC, the sexes were affected equally. These infants came from isolated farming households where the practice was to make up an infant feed from cows' milk, diluted and sweetened with sugar, and heated in a copper vessel. Siblings were often affected, parental consanguinity was common, and the segregation ratio was 0.2159. Since some infants fed in the same way escaped the disease, it was hypothesized that both genetic and environmental factors were involved [26].

Sporadic infantile copper toxicosis related to well water

Individual cases in Australia, Germany, and the UK have resembled ICC. All of the patients affected have died or required transplantation, and all have been born in a rural household and have received milk made up with well water that has a low pH and has acquired high copper concentrations from copper plumbing or water heaters. In the well-documented cases, the water copper concentration has been high. No cases occurred in houses receiving a regulated water supply [27].

Childhood copper toxicosis without excess copper ingestion

Histological features resembling ICC, and raised hepatic copper, were seen in four siblings aged 4.5–6 years who died with progressive liver disease. Although these children were older than those with ICC, they had a similar clinical course and liver copper as high as 2083 mg/g dry weight. In these and in other reports of an ICC-like disorder, there was no identifiable cause of excess copper ingestion [27]. Amongst the small number of infants with ICC now being seen in India, are some with no history of exposure to copper-contaminated feeds.

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SECTION 9

Management of Chronic Liver Disease

CHAPTER 21

Complications and Management of Chronic Liver Disease

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Key points

- Chronic liver disease is defined as ongoing inflammation in the liver for at least 6 months with the potential to progress to cirrhosis and end-stage liver disease.
- Recommendations extrapolated from adult data regarding the management of portal hypertension and variceal bleeding should be treated with caution, particularly in young children.
- All eligible children with chronic liver disease should receive antipneumococcal vaccination for the prevention of spontaneous bacterial peritonitis and antibiotic prophylaxis is recommended after the first episode of spontaneous bacterial peritonitis.
- Conditions that decrease the effective circulating volume such as gastrointestinal bleeding and infection, specifically spontaneous bacterial peritonitis, may trigger hepatorenal syndrome.
- Hepatic encephalopathy can be precipitated by infection, gastrointestinal bleeding, diuretic overdose, electrolyte disorders, and constipation all of which require active evaluation and treatment.
- Pulse oximetry, is not an accurate diagnostic tool for early screening for hepatopulmonary syndrome as most children with mild to moderate hepatopulmonary syndrome will have normal pulse oximetry values (>98%).
- Given the subtlety of presentation of cirrhotic cardiomyopathy, periodic cardiac examinations are warranted in all children with cirrhosis.

Etiology and pathophysiology of chronic liver disease

Chronic liver disease is defined as ongoing inflammation in the liver for at least 6 months with the potential to progress to cirrhosis and end-stage liver disease. As described in earlier chapters, the etiologies of chronic liver disease are vast (Box 21.1). Progressive cholestatic liver diseases including biliary atresia and progressive familial intrahepatic cholestasis collectively are the primary indication for liver transplantation in children. In these conditions, high concentrations of retained bile acids (>100 μmol/L) lead to hepatocyte oncotic swelling and ultimately necrosis [1]. Locally injured hepatocytes then further propagate an inflammatory response mediated by released cytokines that lead to fibrosis and ultimately cirrhosis. In conditions with lower concentrations of retained bile acids (25–100 μmol/L), cell apoptosis may play a primary role in liver injury.

Genetic metabolic disorders, including hereditary tyrosinemia, fatty acid oxidation defects, mitochondrial disorders, cystic fibrosis, and Wilson disease, all have hepatic steatosis in addition to cholestasis in the liver (Figure 21.1). The exact contribution of steatosis to liver injury in these conditions is still under investigation.

Other mechanisms of chronic liver injury in specific conditions such as viral hepatitis and autoimmune liver disease are described in previous chapters with a common pathway of progressive liver fibrosis (see Chapters 11 and 13). Hepatic stellate cells and portal myofibroblasts play a primary role in hepatic fibrosis, which progresses to bridging fibrosis and ultimately to cirrhosis (Figure 21.2). Cirrhosis is the common end pathway for the majority of conditions that lead to chronic liver disease.

Diseases of the Liver and Biliary System in Children, Fourth Edition. Edited by Deirdre A. Kelly.

Clinical presentation and diagnosis of chronic liver disease

Physical examination may show the following (Figure 21.3A–C):

- No signs or symptoms of chronic liver disease.
- Jaundice, ascites, splenomegaly, growth failure, xanthomas, spider nevi, cyanosis, palmar erythema, and clubbing.
- Either hepatosplenomegaly or a shrunken hard nodular liver with splenomegaly may be present.
- Patients with cholestatic liver disease may also present with pruritus, dark urine, and acholic stools in addition to jaundice.
- Signs of hepatic encephalopathy may be subtle in children and are described later.
- Non-specific clinical symptoms may include anorexia, fatigue, nausea, vomiting, or abdominal pain.

Box 21.1 Etiologies of chronic liver disease in children.

Biliary

- Extra-hepatic biliary atresia
- Biliary obstruction: choledochal cyst, tumors
- Alagille syndrome
- Primary sclerosing cholangitis
- Graft-versus-host disease
- Caroli disease and fibropolycystic disease
- Histiocytosis X
- Cystic fibrosis
- Drugs

Hepatic

- Infectious
  - neonatal hepatitis
  - hepatitis B ± D
  - hepatitis C
- Immune
  - autoimmune hepatitis types 1 and 2
  - autoimmune sclerosing cholangitis ± inflammatory bowel disease
- Nutritional
  - non-alcoholic fatty liver disease
  - total parenteral nutrition induced cholestasis
- Drugs/toxins
- Genetic/metabolic
  - α1-antitrypsin deficiency
  - progressive familial intra-hepatic cholestasis: FIC-1 deficiency (ATP8B1), BSEP deficiency (ABCB11)
  - Carbohydrate defects
    - galactosemia
    - hereditary fructose intolerance
    - glycogen storage disease types III and IV
  - Amino acid defects
    - tyrosinemia type 1
  - Lipid storage diseases
    - Gaucher disease
    - Niemann–Pick disease type C
  - Metal storage defects
    - primary hemochromatosis
    - Wilson disease

Vascular

- Cardiac: congestive heart failure, congenital cardiomyopathy, constrictive pericarditis
- Sinusoidal obstruction syndrome
- Budd–Chiari syndrome

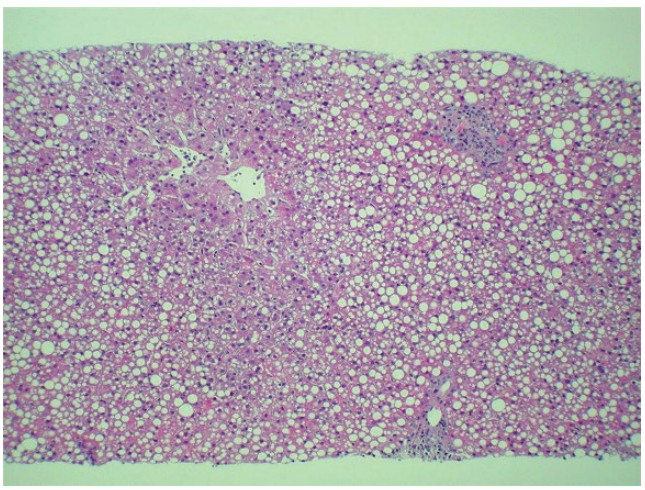


Figure 21.1 Liver histology with hepatic steatosis in a patient with progressive familial intra-hepatic cholestasis type 1.

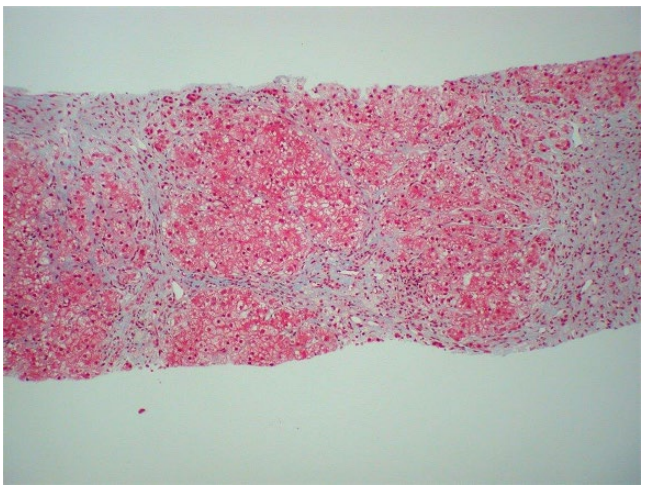


Figure 21.2 Liver histology with bridging fibrosis linking the portal tracts.

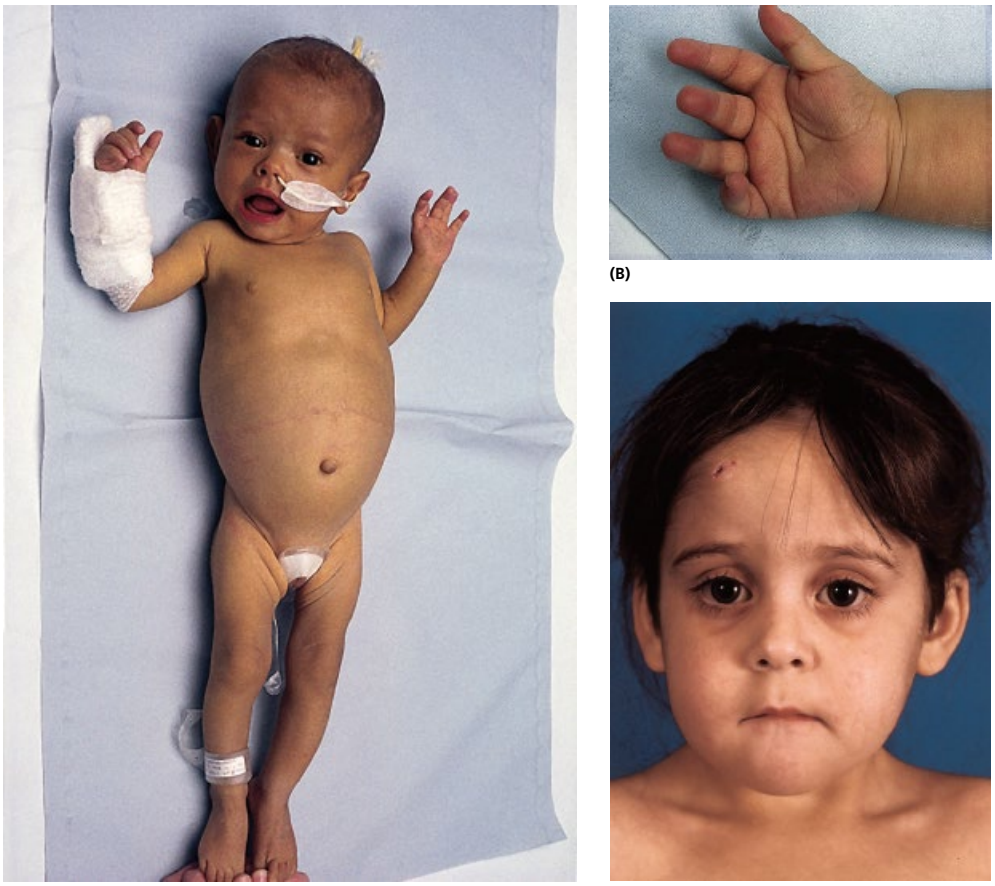


Figure 21.3 Clinical features of biliary cirrhosis in infancy. Jaundice is severe, with wasting, ascites, prominent abdominal veins, clubbing, and hepatosplenomegaly. The abdominal scar is from failed portoenterostomy for biliary atresia (A). Both plantar and palmar (B) erythema develops early. In older children facial telangiectasia (C) and spider nevi may be obvious.

- The dramatic presentation of new-onset hematemesis secondary to variceal bleeding may also be the first sign of chronic liver disease in a previously undiagnosed patient.

Laboratory studies may reveal:

- Elevated transaminases, bilirubin, γ-glutamyltransferase (GGT), and ammonia.
- Hypersplenism may be suggested by thrombocytopenia, anemia, and leukopenia.
- Evidence of renal dysfunction may also be noted and is described later in the section on hepatorenal syndrome (HRS).
- In decompensated chronic liver disease, abnormal hepatic synthetic function is denoted by hypoalbuminemia and a prolonged international normalized ratio (INR).
- An abdominal ultrasound may reveal splenomegaly and ascites. An abdominal ultrasound with Doppler will assess patency of the portal vein and identify hepatofugal flow patterns.
- Contrast-enhanced cross-sectional imaging with a computed tomography (CT) or magnetic resonance imaging (MRI) may identify portal vein thrombosis or Budd–Chiari syndrome.
- Magnetic resonance cholangiopancreatography (MRCP) may additionally identify anatomic abnormalities of the biliary tree.
- Echocardiogram can reveal cardiac abnormalities that lead to chronic liver disease (see Box 21.1).
- A liver biopsy is necessary to confirm the presence of fibrosis and cirrhosis and may clarify the underlying etiology of chronic liver disease. However, with progression to cirrhosis, distinguishing histopathologic features characteristic of the underlying diagnosis may be masked. Pathology findings in chronic liver disease are described in Chapter 2.

With few exceptions, such as in Wilson disease, chronic hepatitis B and C, and autoimmune hepatitis, there are no medications or interventions that halt the progression to uncompensated cirrhosis. The underlying principle of treatment in patients with chronic liver disease is to anticipate, prevent, identify, and ultimately manage complications in these patients (Box 21.2).

Box 21.2 Complications of chronic liver disease.

- Malnutrition, growth failure
- Fat-soluble vitamin deficiency
- Cutaneous manifestations: pruritus, xanthomas, palmar erythema, spider nevi
- Osteodystrophy
- Variceal bleeding
- Portal hypertensive enteropathy
- Hypersplenism
- Ascites
- Spontaneous bacterial peritonitis
- Hepatorenal syndrome
- Hepatic encephalopathy
- Hepatopulmonary syndrome
- Portopulmonary hypertension
- Cirrhotic cardiomyopathy
- Developmental delay
- Poor quality of life
- Hepatocellular carcinoma

Complications of chronic liver disease

Portal hypertension

Pathophysiology

Portal pressure rises with either increased portal blood flow or increased portal resistance. The causes of portal hypertension fall into three main groups: pre-hepatic, intra-hepatic, and post-hepatic etiologies.

- Pre-hepatic causes of portal hypertension are due to obstruction of the portal vein usually due to temporally distant thrombosis, classically from instrumentation of the umbilical vein in the neonatal period, but in most cases such a history is lacking.
- Intra-hepatic etiologies of portal hypertension are most common in the pediatric population and can be further divided into pre-sinusoidal, sinusoidal, or post-sinusoidal obstruction.
- Post-hepatic causes of portal hypertension include luminal obstruction of the hepatic vein secondary to thrombosis or an anatomic defect or external compression such as vascular congestion from primary cardiac disease such as right heart failure, pulmonary hypertension, or pericarditis (see Chapter 22).

Diagnosis and clinical features

Normal portal pressures are typically between 5 and 10 mmHg and normal hepatic venous pressure gradients (HVPG) are between 1 and 4 mmHg [2]. The measurement of portal pressure is invasive with insertion of a catheter through the jugular or femoral vein advanced to the hepatic vein where free hepatic vein pressure (FHVP) is measured and subtracted from wedge hepatic vein pressure (WHVP) to obtain the hepatic venous pressure gradient (HVPG = WHVP – FHVP). Portal hypertension is defined as a portal pressure >10 mmHg or a hepatic venous pressure gradient >4 mmHg.

Clinically significant portal hypertension typically occurs at a HVPG >10 mmHg with the sequelae of ascites, splenomegaly, and portosystemic collaterals. These findings can be demonstrated on Doppler ultrasound or CT.

To evaluate the progression of liver fibrosis in patients with chronic liver disease, liver elastography is being explored as a less invasive technique than liver biopsy. Transient elastography may be limited in children with high body masses indexes or in those with the presence of ascites. Currently, there are not enough data to recommend transient elastography to assess the progression of liver fibrosis in the pediatric population.

Complications

Variceal bleeding

As per expert guidelines, esophageal varices are described as:

- Grade I flattened by insufflation.
- Grade II not flattened by insufflation.
- Grade III not flattened by insufflation and confluent around the circumference of the esophagus [3].

In children with cirrhosis, up to two-thirds will have varices, with variceal bleeding occurring typically at a HVPG >12 mmHg. There are multiple factors that contribute to the initial risk of variceal bleeding including the underlying cause of portal hypertension and time from initial diagnosis.

The mortality from variceal bleeding is lower in children than in adults: 0–8% compared to 14%, respectively [4]. This may be explained by extra-hepatic portal vein obstruction (EHPVO) being a common cause of portal hypertension in children with typically preserved hepatic function compared to adults who more often have cirrhosis with other comorbidities.

Pre-primary prophylaxis. The goal of pre-primary prophylaxis is to prevent the formation of varices in patients with portal hypertension. Few animal and human adult studies have considered the use of β-blockers for pre-primary prophylaxis. There is not currently sufficient evidence to recommend the use of β-blockers in pediatric patients for pre-primary prophylaxis [3].

Primary prophylaxis. Primary prophylaxis may be considered in select populations to prevent the first episode of variceal bleeding. Surveillance endoscopy should be considered in patients at high risk of mortality from their first variceal bleed, which may include patients without prompt access to a medical center that can provide therapeutic endoscopy [2, 3]. Surveillance endoscopy should be performed in expert centers with the facilities to initiate primary prophylaxis if findings on endoscopy convey a high risk of bleeding.

In a study of patients with biliary atresia, endoscopic findings that are independently associated with a risk of bleeding included the presence of gastric varices in the cardia and red marks (cherry-red spots) on the varices. In patients with biliary atresia, primary prophylaxis has also been recommended in patients with grade I, II, or III esophageal varices who also had gastric varices along the cardia, as well as patients with grade II esophageal varices with red markings and grade III esophageal varices; although this recommendation has not

generally been accepted by published review of expert opinion [5]. Similar studies have not been undertaken to assess the value of surveillance endoscopy in predicting the risk for variceal bleeding in pediatric patients with non-cirrhotic etiologies of portal hypertension.

For patients who have a high risk of variceal bleeding, primary prophylaxis may be considered:

- Pharmacological primary prophylaxis consists of non-selective β-blockers. Propranolol (0.6–0.8 mg/kg/day divided b.i.d. or q.i.d.) decreases portal pressures by causing the constriction of splanchnic vessels and decreasing portal blood flow [2, 4]. The endpoint for dosage titration in research studies is a hepatic venous pressure gradient <12 mmHg in adults but less invasive clinical endpoints have included a 25% reduction in heart rate [4]. However, decreases in heart rate do not always correlate with a decrease in the hepatic venous pressure gradient and side effects can include dizziness and bradycardia. Similar studies in pediatric age groups have not been conducted and extrapolation from adult data is to be treated with caution.
- Endoscopic variceal ligation should only be performed in expert centers. In a non-controlled study of patients older than 4 years of age with portal hypertension secondary to cirrhosis or portal vein thrombosis, endoscopic variceal ligation was performed if there were high-risk endoscopic signs of bleeding or an increase by at least one grade in esophageal varices [6]. In this study, there were no episodes of variceal bleeding over 16 months of follow-up. Risks of primary prophylaxis with endoscopic variceal ligation include re-bleeding from incomplete control of varices and portal hypertensive gastropathy; though this risk is lower than previously described in patients undergoing prophylactic sclerotherapy.

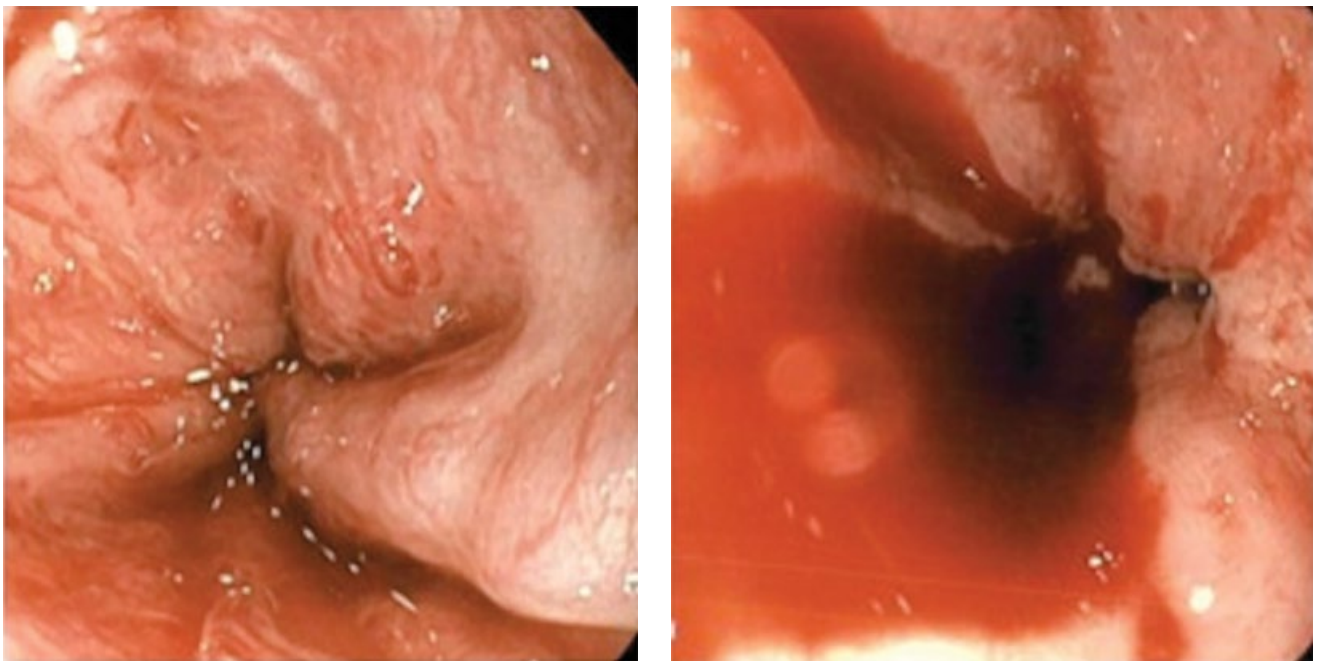
Expert guidelines recommend primary prophylaxis only for select patients considered at high risk of mortality after a sentinel variceal bleed [3]. In these patients, endoscopic variceal ligation is preferred over non-selective β-blockers.

- Endoscopic sclerotherapy is not recommended for primary prophylaxis because of the side effects of esophageal ulceration, perforation, stricture formation, and portal hypertensive gastropathy.
- Meso-Rex shunt may be considered for primary prophylaxis in patients with EHPVO.

Management of variceal bleed. In patients who present with their first variceal bleed, endoscopic variceal ligation should be performed as soon as the patient is hemodynamically stable and within 24 h of admission (Figure 21.4). For infants and children in whom endoscopic variceal ligation is technically not feasible due to the size of currently available banding devices, sclerotherapy is an alternative.

Management consists of:

- Hemodynamic resuscitation with a conservative target hemoglobin of 7–8 g/dL is recommended as more liberal transfusions can lead to higher variceal pressures, increasing the risk for re-bleeding. Vitamin K deficiency should be corrected and pre-procedural platelets and plasma/clotting factor support may be required.



(A) (B)

Figure 21.4 Esophageal varices (A) and variceal bleeding (B).

- Octreotide, administered initially as a bolus dose of 1–2 μg/kg one time followed by a continuous infusion of 1–2 μg/kg/h should be initiated immediately prior to therapeutic endoscopy [3]. Patients should have no oral intake while on octreotide given decreased splanchnic blood flow and this medication should not be continued for longer than 5 days (Figure 21.5). Weaning of octreotide should begin 24 h after variceal bleeding ceases with a decrease by 50% every 12 h for 24 h and then can be discontinued.
- Intravenous proton-pump inhibitor (PPI) such as omeprazole (1 mg/kg daily) may decrease the size of post-banding ulcers or the risk of post-banding hemorrhage.
- Broad-spectrum antibiotics: patients with cirrhotic liver disease are at higher risk for bacterial infections secondary to complement deficiencies. Endoscopic instrumentation may further increase the risk for infection and patients with acute bleeds should be started on a broad-spectrum antibiotic such as piperacillin-tazobactam, which can be discontinued if blood cultures remain negative for 48 h.

There is no role for β-blockers in the setting of an acute variceal bleed. In children, β -blockers blunt the circulatory systems compensatory ability to increase cardiac output by increasing the heart rate in the setting of a large variceal bleed, conveying added risk to these patients.

In patients with gastric varices, transjugular intra-hepatic portosystemic shunt (TIPS) should be considered if liver transplantation is not imminent.

Secondary prophylaxis. Following the first episode of variceal bleeding, endoscopic variceal ligation should be performed every 2–4 weeks thereafter for up to five sessions until varices are eradicated [3]. Thereafter, surveillance endoscopy should occur every 3 months for the first 6 months, then every 6 months with repeat endoscopic variceal ligation if there is recurrence of varices. If there is no recurrence of varices by 1 year, annual surveillance endoscopy is recommended. New or worsening fundal varices and portal hypertensive gastropathy may occur after secondary prophylaxis.

In patients for whom endoscopic variceal ligation is technically difficult, sclerotherapy may be used as secondary prophylaxis. Endoscopic variceal ligation, however, is superior requiring fewer procedures for variceal eradication with a lower rate of re-bleeding.

For patients with an intrahepatic etiology of their portal hypertension who have continued episodes of variceal bleeding despite endoscopic intervention or have compensated cirrhosis, surgical portosystemic shunting should be considered to decompress the portal system. If surgical portosystemic shunting is not feasible then TIPS should be considered, particularly in patients awaiting liver transplant (Figure 21.6).

In patients with EHPVO, meso-Rex bypass is recommended for secondary prophylaxis. A distal splenorenal shunt is recommended only if meso-Rex bypass is not technically possible.

Emergency therapy. In an emergency situation, balloon tamponade may be a bridge to more definitive endoscopic, interventional radiology or surgical management for uncontrolled variceal bleeding. Given the high risk for aspiration with balloon tamponade, intubation is recommended. A Linton tube is a single-balloon tube and may be preferable to a Minnesota tube or Sengstaken–Blakemore tube, which utilizes a two-balloon (esophageal and gastric) system. The Minnesota and Sengstaken–Blakemore tubes are also limited to patients weighing over 40 kg. These three tubes should not be left in place for more than 24 h given the high risk of morbidity and mortality from mucosal ischemia and esophageal perforation. Furthermore, the risk of complications and mortality increase in the hands of inexperienced providers and placement should only be performed by an individual trained in the procedure.

When variceal bleeding secondary to an intra-hepatic etiology cannot be controlled endoscopically, TIPS is required that connects the intra-hepatic portion of the portal vein and hepatic vein. Post-procedural complications include encephalopathy, shunt thrombosis, or stenosis.

TIPS is not recommended in patients with an extra-hepatic etiology of their portal hypertension. Rather, emergency portosystemic shunting should be considered in patients with poorly controlled variceal bleeding with EHPVO. Portosystemic shunts are classified as being either selective, including distal splenorenal and mesenteric-left portal shunts, or non-selective, which includes mesocaval and portocaval shunts where there is a higher risk of hepatic encephalopathy (see Chapter 27).

Emergency liver transplant should be considered in patients with uncontrolled variceal bleeding with other indications for liver transplant including HRS, hepatopulmonary syndrome, and decompensated liver disease [3].

Portal hypertensive enteropathy

Portal gastropathy was thought to occur more frequently in patients with cirrhotic portal hypertension, though additional studies have demonstrated equal incidence in patients with pre-hepatic, intra-hepatic, and post-hepatic etiologies of portal hypertension.

In patients with portal hypertension, portal gastropathy ranges between 40 and 64% and portal duodenopathy around 9%. Endoscopically, portal gastropathy is defined by a mosaic mucosal pattern in more mild disease and cherry-red spots, black-brown spots, and gastric antral vascular ectasia (though is a rare finding) in more severe disease. Clinically, portal gastropathy and duodenopathy is associated with acute bleeding and chronic anemia. There are conflicting studies on the severity of liver disease and findings of esophageal and/or gastric varices with presence of portal gastropathy and/or duodenopathy.

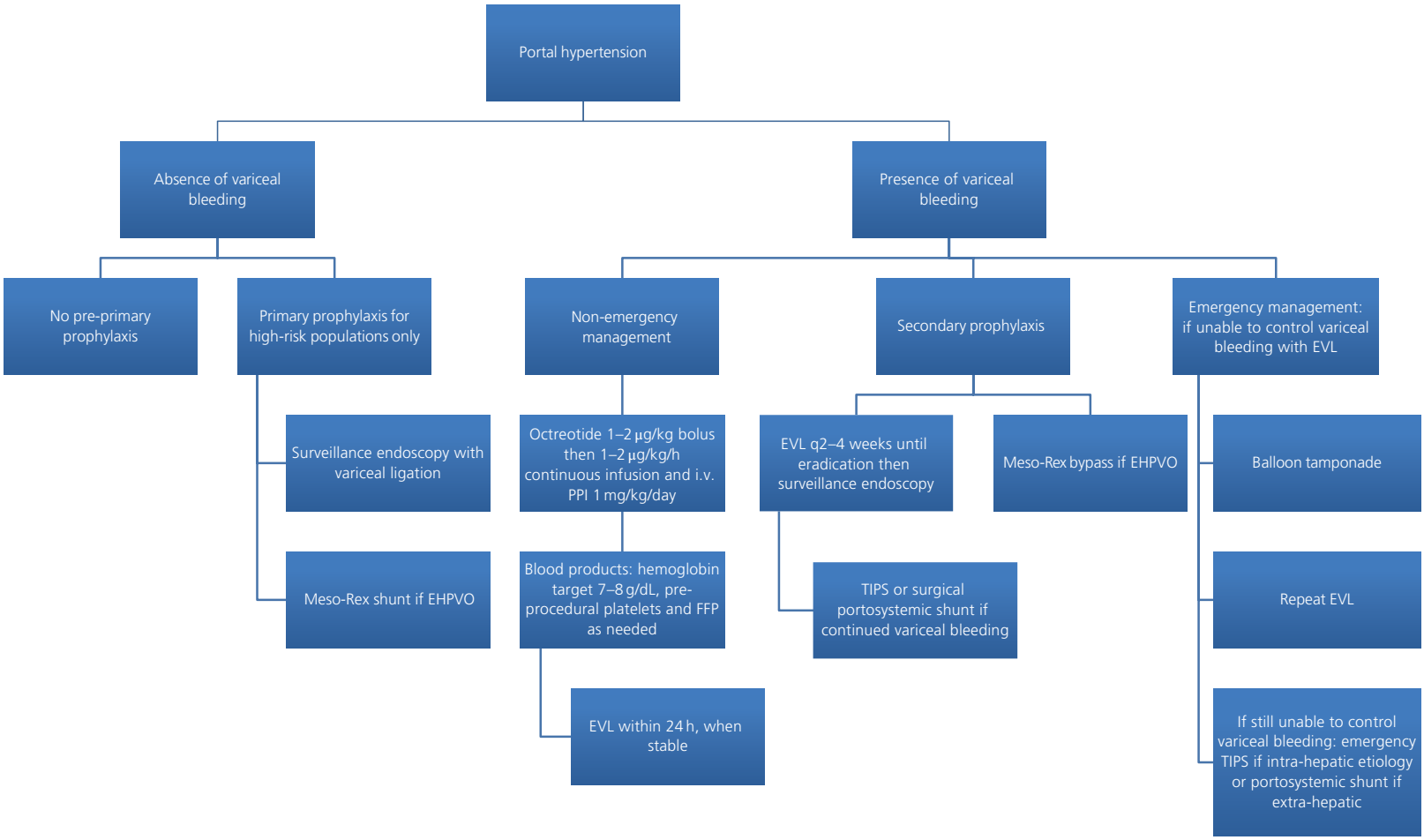


Figure 21.5 Prophylaxis and treatment of variceal bleeding. EHPVO, extrahepatic portal vein obstruction; EVL, endoscopic variceal ligation; FFP, fresh frozen plasma; PPI, proton-pump inhibitor; TIPS, transjugular intra-hepatic portosystemic shunt.

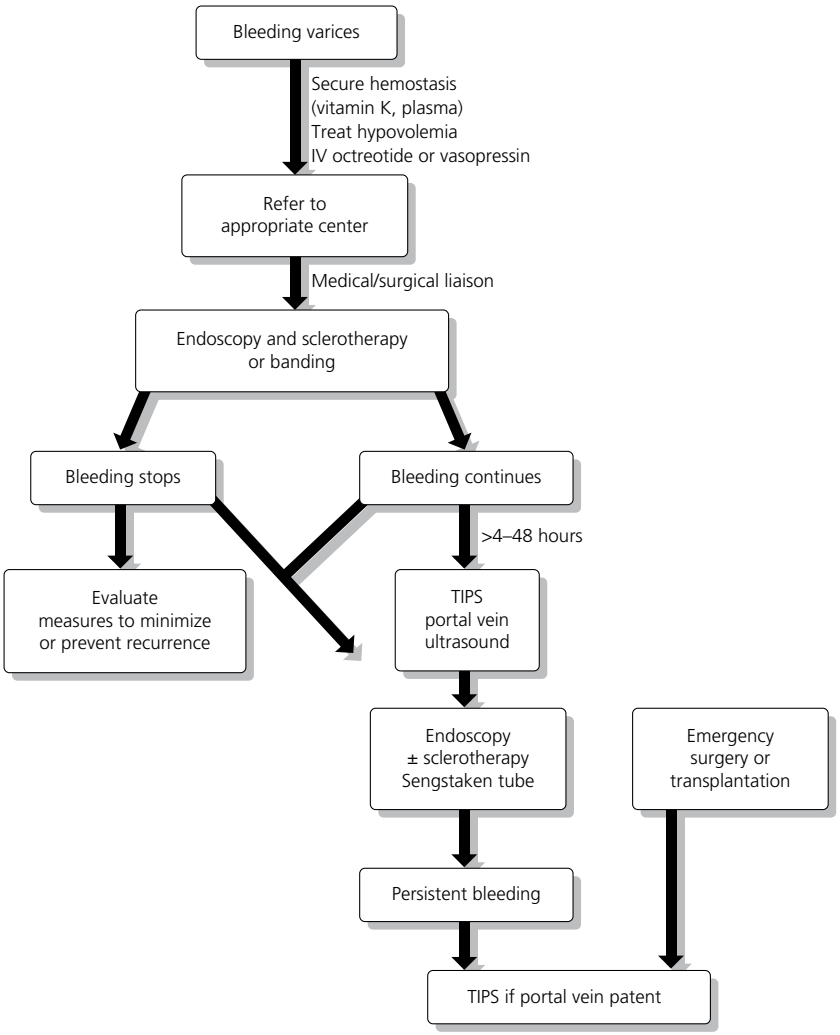


Figure 21.6 Transjugular intrahepatic portosystemic shunt (TIPS).

Portal hypertensive enteropathy also results in congestion of the small intestine with subsequent malabsorption, protein-losing enteropathy and an increased risk of translocation of enteral organisms leading to spontaneous bacterial peritonitis (SBP) and systemic sepsis.

Hypersplenism

Hypersplenism occurs in patients with both intra- and extrahepatic portal hypertension. Portal hypertension leads to splenic congestion and subsequently sequestration of blood components with resultant thrombocytopenia, anemia, leukopenia, or a combination of the three. Thrombocytopenia defined as platelets <50,000, significantly increases the risk for bleeding and has been used as an indication for meso-Rex bypass [3]. Splenectomy is not recommended as a treatment for hypersplenism and has been associated with the development of portal vein thrombosis.

Ascites

Pathophysiology

In a healthy patient, hepatic lymph is formed after filtration of sinusoidal plasma in the space of Disse, which ultimately drains into the thoracic duct. Hepatic sinusoids are highly permeable therefore albumin levels in hepatic lymph are approximately equivalent to that of plasma. Conversely, intestinal lymph is formed from filtration at the mesenteric capillaries, which is also drained into the thoracic duct though with a much lower concentration of albumin such that an osmotic gradient promotes the return of lymphatic fluid to the systemic circulation.

In patients with portal hypertension, through a mechanism that has not been fully described, increased synthesis of nitric oxide leads to splanchnic vasodilation causing arterial under filling, which is interpreted by renal baroreceptors as a decreased effective circulating volume. This in turn activates

the renin–angiotensin–aldosterone system leading to maximal retention of sodium and free water. However, as retained sodium and water do not appear to alter the stimulus for nitric oxide production at least in part due to the sequestration of retained fluid within the peritoneum, a decreased effective circulating volume continues to be perceived by the kidneys.

The mechanism of ascites differs between cirrhotic and non-cirrhotic patients. In children with cirrhosis, there is increased hydrostatic pressure in the liver. As hepatic sinusoids are highly permeable to albumin, there is no appreciable osmotic gradient and hepatic lymph formation increases exceeding the capacity of lymphatic drainage via the thoracic duct leading to worsening ascites. Furthermore, in decompensated cirrhosis lower albumin levels also decrease the osmotic gradient that draws interstitial fluid back into the intravascular space leading to higher intestinal lymph production.

Children with non-cirrhotic ascites include those with post-hepatic etiologies of portal hypertension including Budd–Chiari syndrome and right-sided heart failure. In these patients, hepatic congestion leads to increased sinusoidal and splanchnic hydrostatic pressures increasing hepatic and intestinal lymph production, respectively, again overwhelming the capacity of lymphatic drainage via the thoracic duct [7].

Diagnosis

On physical examination, the most sensitive test for children with ascites is the puddle sign (shifting dullness). When supine, percussion over the umbilicus is resonant, then when switched to the prone position becomes dull to percussion over the umbilicus when ascitic fluid pools in the dependent position. Other physical examination findings that suggest ascitic fluid accumulation includes increasing weight gain. Serial abdominal girths are not reliable to document progression of ascites.

Abdominal ultrasound is often used for the evaluation of ascites but is not sensitive for ascites, but may be suggestive if centralized bowel loops are noted with an otherwise diffuse fluid density throughout the abdomen. Cross-sectional imaging is rarely indicated for the diagnosis of ascites, but MRI is preferable over CT for the detection of ascites given its lack of ionizing radiation in a pediatric population though it often requires the additional need for anesthesia in younger patients.

Paracentesis is not indicated to determine the cause of ascites in patients with portal hypertension in whom ascites is an expected sequelae, but a diagnostic tap should be performed to exclude SBP.

Complications

Respiratory complications of ascites include decreased lung volumes with radiographic findings of elevated hemi-diaphragms and atelectasis.

Risk of SBP is discussed later in the section on bacterial infections.

Patients with massive ascites who are critically ill, are also at risk for abdominal compartment syndrome, which may present with hypoxemia, decreased renal perfusion, oliguria, and bowel hypoperfusion increasing the risk of bacterial translocation and sepsis. However, this is a rare occurrence when ascites is actively managed in patients with chronic liver disease.

Management

The main indication for the treatment of ascites is to improve patient comfort and minimize infectious complications. The primary principle of non-invasive management of ascites is to create a negative sodium balance through dietary sodium restriction and diuretics, which allows mobilization of fluid for urinary excretion (Figure 21.7).

- Dietary sodium should be restricted to no more than 2 mmol/kg/day but is rarely sufficient for monotherapy of ascites and diuretics are often required.
- Spironolactone (2–3 mg/kg/day given as a single morning dose or divided b.i.d.; maximum daily dose of 100 mg) is the most effective diuretic in the treatment of ascites because it competes with aldosterone at the distal renal tubules, inhibiting sodium and water reabsorption. Dose titration should not occur more frequently than every 5–7 days and can be increased by 2 mg/kg/day (maximum dose increase of 100 mg) to a maximum dose of 4–6 mg/kg/day (maximum daily dose of 400 mg) [7]. Adverse effects of spironolactone include hyperkalemia and renal insufficiency.
- Furosemide (1 mg/kg/day; maximum daily dose of 40 mg) is useful in patients without significant response to spironolactone monotherapy. It should be titrated every 5–7 days by 1 mg/kg/day (maximum dose increase of 40 mg) to a maximum dose of 2–4 mg/kg/day (maximum daily dose of 160 mg). Adverse effects of furosemide include hypokalemia that can be tempered by dual therapy with spironolactone as well as hypomagnesemia and hypocalcemia. Furosemide should not be used as monotherapy for the treatment of ascites as it acutely decreases the effective circulating volume and can precipitate renal failure.

Efficacy of treatment with spironolactone can be monitored with a random urinary sodium level with the goal being >50 mmol/L. With both spironolactone and furosemide, the overall goal for diuresis is prevention of further accumulation of ascites as well as reduction in body weight by 0.5–1% per day until ascites has resolved.

- Albumin infusion may be considered in hospitalized patients with portal hypertension and ascites with a serum albumin <2.5 g/dL. An albumin infusion can temporarily improve oncotic pressure drawing interstitial fluid back into the intravascular space for more effective diuresis in addition to being a volume expander with improvement of

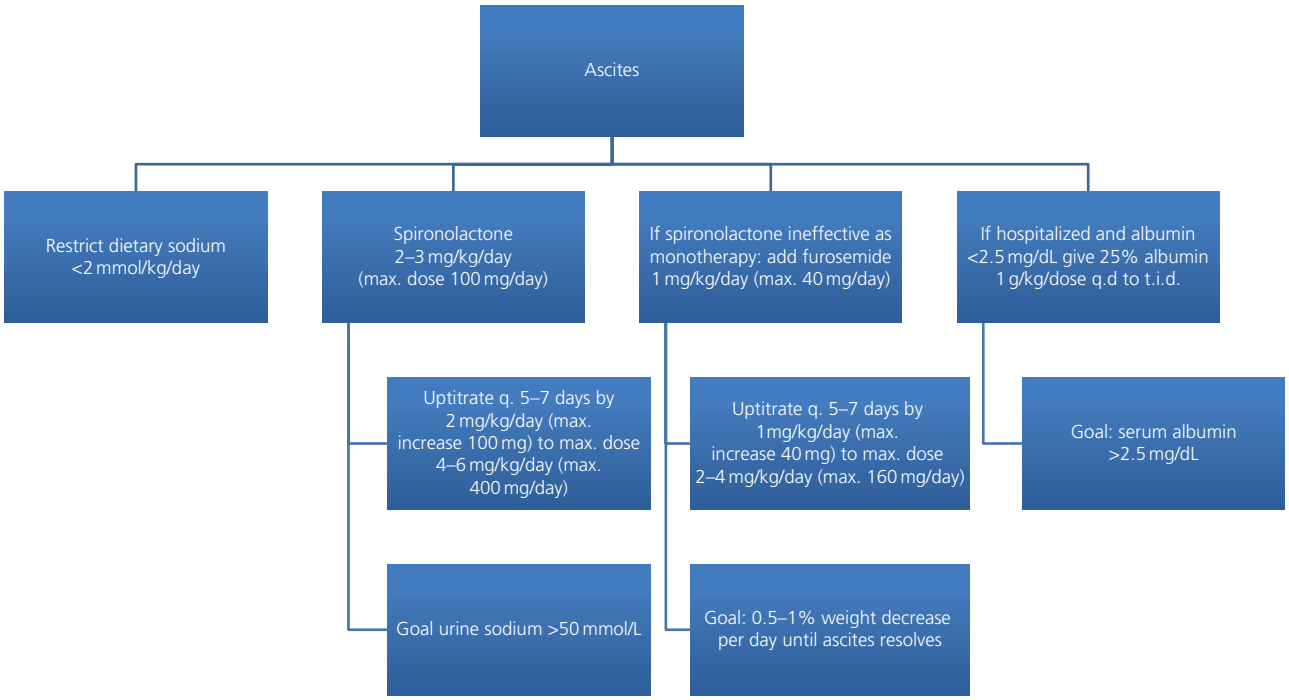


Figure 21.7 Management of ascites.

effective circulating volume. Appropriate dosing is with 25% albumin at 1 g/kg i.v. up to 3 times/day until serum albumin is >2.5 g/dL in addition to furosemide (0.5–1 mg/kg) to prevent excess fluid overload.

- Paracentesis is rarely performed except in patients with rapid accumulation of ascites with respiratory impairment or in patients whose ascites is refractory to maximal diuretic management. The technique for paracentesis is described later in the section on SBP. Careful replacement of albumin losses in ascitic fluid is required using 25% albumin infusion at 1 g/kg during or immediately following the procedure to maintain circulating volume. Continued drainage may lead to complement, coagulation factor, and other protein deficiencies. Intractable ascites is an indication for liver transplantation.

Bacterial infections

Immune deficits

Three factors put patients with chronic liver disease at higher risk for infection: alterations in immune defense mechanisms, bacterial overgrowth and increased bacterial translocation, increasing the risk for SBP and bacteremia.

Complement factors are synthesized by the liver and are necessary for opsonization of bacteria before phagocytosis by leukocytes. A pediatric study found low serum levels of complement factors C3 and C4 in more than 89% of patients at the time of their diagnosis of SBP suggesting a possible mechanism of increased infection in these patients [8]. Defects have also been shown in neutrophil, monocyte, and T-cell function contributing to immune deficits in patients with chronic liver disease leading to impaired clearance of translocated organisms. Additionally, impairment of the reticuloendothelial system phagocytic activity is thought to be secondary to intra-hepatic shunting.

Bacterial overgrowth in the small bowel is secondary to delayed intestinal transit and decreased bile acids in the small bowel in patients with liver cirrhosis. Differences in the microbiota are noted between these patients and healthy controls with a higher abundance of Gram-negative bacteria (Enterobacteriaceae and Bacteroidaceae) in patients with liver cirrhosis.

Intestinal bacterial translocation is higher in adult patients based on Child-Pugh class with a rate of 30% in Child C patients, 8% in Child B patients, and 3% in Child A patients, reflecting a higher immunodeficiency state in patients with more significant liver disease. The etiology of increased bacterial translocation in this group is likely multifactorial and related to portal hypertension, ongoing inflammation and oxidative stress as potential contributors to intestinal barrier dysfunction.

Spontaneous bacterial peritonitis

Pathophysiology

SBP is the infection of ascitic fluid without a known intra-abdominal cause such as an intestinal perforation. The proposed mechanism is bacterial translocation in an immunocompromised patient. Its early detection is crucial because of the high mortality rate if untreated. Clinical

symptoms are often subtle requiring a high degree of suspicion for diagnosis. Infections are typically monomicrobial; polymicrobial infections suggest perforation of the bowel lumen. An early pediatric study examining causative organisms of SBP found a higher incidence of Gram-positive organisms of respiratory origin, specifically *Streptococcus pneumoniae* [9]. However a more recent pediatric study found that Gram-negative bacteria were more prevalent in SBP consistent with the adult literature and may reflect current vaccine protocols [10].

Diagnosis

Clinical signs and symptoms of SBP are subtle and should be suspected in any patient with ascites with fever, new onset or worsening (tense) ascites, abdominal tenderness or rebound, worsening transaminitis, jaundice or reduced liver synthetic function, encephalopathy, renal insufficiency, or renal failure. The diagnosis of SBP requires a high degree of clinical suspicion; a prospective pediatric study found no difference in the presenting symptoms of fever, worsening ascites, or encephalopathy between patients with infected and non-infected ascites [10].

In all patients, investigations should include:

- Complete blood count with differential white cell count.
- Conjugated and unconjugated serum bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), GGT, and albumin.
- INR and prothrombin time.
- Blood urea nitrogen (BUN) and creatinine.
- Blood culture.
- Serum ammonia.
- Abdominal ultrasound for patency of vessels and detection of reversed flow in the portal vein.

Prior to diagnostic paracentesis, blood products should be administered if platelets are <50,000 and fresh frozen plasma given for a prothrombin time >5 s above normal given the potential risk of intra-abdominal hemorrhage [11]. Paracentesis should be performed under sterile conditions with the patient in the supine position, typically under general anesthesia in young children. With ultrasound guidance, a site is chosen between the umbilicus and urinary bladder, commonly two fingerbreadths medial and cephalad to the anterior superior iliac spine or in the left iliac fossa below the spleen [7]. After local disinfection and local lidocaine injection, the skin is compressed down 2 cm over the abdominal muscles and a 22-gauge needle is inserted advancing slowly until ascitic fluid is visualized in the needle hub. Ascitic fluid is then collected for analysis and the needle is withdrawn.

Ascitic fluid cultures should be inoculated at the bedside, with reported increase in sensitivity from 43% to 93% when compared to agar plating [7]. It should be sent for white blood cell count and differential, fluid culture and Gram stain and albumin concentration (listed from highest to lowest priority). A coincident serum albumin should be sent for accurate calculation of the serum ascites albumin gradient (ascites albumin subtracted from serum albumin), which is >1.1 g/dL when ascites is secondary to portal hypertension.

Gram stains may be negative in SBP as demonstrated in a prospective pediatric study, in which all eight patients with SBP had negative Gram stains [10]. Gram stains that show a polymicrobial infection should prompt investigation for intestinal perforation [7]

The diagnosis of SBP is made when ascitic polymorphonuclear (PMN) cell count is >250 cells/mm^3 alone; a positive ascitic fluid culture is not necessary for the diagnosis, but is supportive and helpful in narrowing antibiotic coverage (Table 21.1). Bacterial ascites is diagnosed when an ascitic fluid culture is positive, but PMN count is <250 cells/mm^3 and may also represent culture contamination. Ascitic fluid is considered non-infected when PMN count is <250 cells/mm^3 and ascitic fluid culture was also negative.

Management

Patients with SBP should be treated with a non-nephrotoxic broad-spectrum third-generation cephalosporin, to complete a 5–7-day course [10]. In patients with SBP with positive ascitic fluid cultures, antibiotic choice is based on the sensitivities. In the adult literature, repeat paracentesis is recommended 48 h after the initial peritoneal tap with

Table 21.1 Ascitic fluid analysis and recommendations for treatment.

	Ascitic fluid PMN count	Ascitic fluid culture	Treatment
SBP	>250 cells/mm^3	Positive	Third-generation cephalosporin for 5–7-day course Consider piperacillin-tazobactam if high prevalence of multidrug-resistant organisms Consider repeat paracentesis and broaden to piperacillin-tazobactam for treatment failure
Bacterial ascites	<250 cells/mm^3	Positive	Repeat paracentesis in 48 h: if consistent with SBP, treat accordingly; if again consistent with bacterial ascites, antibiotic treatment is indicated; if consistent with non-infected ascitic fluid, no treatment necessary
Non-infected ascitic fluid	<250 cells/mm^3	Negative	None

PMN, polymorphonuclear; SBP, spontaneous bacterial peritonitis.

the recommendation to broaden antibiotic coverage typically to piperacillin-tazobactam if PMN count has not decreased by 25% indicating treatment failure. Piperacillin-tazobactam should also be considered as an initial therapy in regions with known prevalence of multidrug-resistant organisms.

Renal failure may occur in 30-40% of adult patients with SBP which may be prevented by albumin infusion (0.5–1.5 g/kg for at least 3 days). There are no existing recommendations in children on the use of albumin infusions in SBP and no evidence that it is beneficial [10].

Complications

In adults, SBP is a poor prognostic indicator with renal failure and a mortality of 10–30% [7] but similar pediatric studies are not recorded.

Prevention

Historically, Gram-positive organisms of respiratory origin, specifically *Streptococcus pneumoniae* were the most common cause of SBP. However more recent studies have found Gram-negative enteric bacilli to be the most common cause of SBP and may reflect current vaccine protocols [7]. All eligible children with chronic liver disease should receive antipneumococcal vaccination for the prevention of SBP [8].

Given the risk of recurrence of SBP, all patients should have prophylaxis. Prolonged use of fluoroquinolones is generally avoided in the pediatric population and trimethoprim-sulfamethoxazole is recommended instead for prophylaxis with demonstrated efficacy in the adult literature [7, 8].

A rise in bacterial resistance has led to further investigation of alternative candidates for prophylaxis, most recently with rifaximin, a non-absorbable antibiotic, though current pediatric recommendations are not available.

Hepatorenal syndrome

Pathophysiology

In patients with portal hypertension, vasodilation of the splanchnic vessels are mediated by nitric oxide, which is sensed by renal baroreceptors as a decreased effective circulating volume leading to activation of the renin–angiotensin–aldosterone system and renal vasoconstriction that leads to renal hypoperfusion and impairment [12]. Elevated plasma renin levels are independent predictors of HRS. Low cardiac output has also been found to be an independent predictor of HRS, with the hypothesis that a hyperdynamic circulatory state is needed to maintain renal perfusion in patients with portal hypertension. The mechanisms of decreased cardiac output in these patients have not been well described, though cirrhotic cardiomyopathy may play a role. Conditions that further decrease the effective circulating volume such as gastrointestinal bleeding and infection, specifically SBP, can also trigger HRS [12, 13].

Presentation

In adults with cirrhosis, 20% will develop acute renal failure due to HRS [12, 14]. The incidence of HRS in children is lower, around 5%, and mortality rates in the pediatric population are not known.

Diagnosis

The differential diagnosis for renal failure in patients with cirrhosis includes:

- Pre-renal etiologies of acute renal failure: 45% of patients will have pre-renal etiologies, which responds to volume expansion.
- Intra-renal etiologies (acute tubular necrosis and glomerulonephritis) present with proteinuria, microhematuria, and/or an abnormal renal ultrasound.
- Post-renal etiologies.
- HRS: adult criteria for HRS include cirrhosis with ascites; evidence of acute kidney injury with a serum creatinine >1.5 mg/dL (133 µmol/L) with no sustained improvement of serum creatinine (defined as a decrease below 1.5 mg/dL) after at least 48 h of diuretic withdrawal and volume expansion with albumin (dosed at 1 g/kg/day to a maximum of 100 g/day); absence of shock; no current or recent treatment with nephrotoxic medications and no evidence of parenchymal disease (defined as proteinuria >500 mg/day, microhematuria >50 red blood cells per higher power field, and/or an abnormal renal ultrasound) [14].

Children have lower baseline creatinine levels than adults and in a small pediatric case series, only two out of four children with HRS met the classic adult diagnostic criteria with a serum creatinine >1.5 mg/dL. However all patients had doubling of their baseline creatinine levels, suggesting that a two-fold increase in baseline creatinine is a specific diagnostic criterion for HRS [13]. There are currently no formal consensus guidelines for the diagnosis of HRS in children.

HRS is further divided into two subtypes: type 1 is characterized by acute renal impairment with a serum creatinine that doubles to >2.5 mg/dL (222 µmol/L) within 2 weeks that is often triggered by an acute process such as sepsis, gastrointestinal bleeding, and SBP [12].

Type 2 HRS is more chronic, defined as a slower rise in serum creatinine that is >1.5 mg/dL (133 µmol/L) and is considered the sequelae of end-stage liver disease and refractory ascites.

Management

All patients with HRS should have diuretics discontinued. Given the high mortality for untreated HRS, treatment should be instituted as soon as the diagnosis is suspected.

Type 1

Adults with type 1 HRS are treated with terlipressin (1 mg i.v. 4–6 times/day titrated to a maximum of 2 mg 6 times/day) with albumin (1 g/kg on the first day of treatment followed

by 20–40 g/day) [12] (Figure 21.8A). Terlipressin is a vasopressin analogue that causes vasoconstriction of the splanchnic vascular bed leading to increased renal perfusion and improved mean arterial pressure [12]. Albumin and terlipressin infusions should be continued until serum creatinine is <1.5 mg/dL or for a maximum of 14 days. Treatment is considered effective if there is a decrease in serum creatinine of 25% by day 3 of treatment.

Side effects of terlipressin are seen in 30% of patients and range from abdominal pain and diarrhea to mild vasoconstrictor effects with peripheral ischemia of the fingers and toes that can be managed with dose reduction. More serious side effects requiring discontinuation of therapy occur in 5–7% of patients and include persistent arrhythmias, myocardial infarction, and one rare report of intestinal ischemic necrosis all of which are an absolute contraindication to continued therapy.

There are no current recommendations on the appropriate dosage of terlipressin in children, although dosing extrapolated from adult studies has been suggested at 5–20 µg/kg/dose i.v. every 4 h. A recent pediatric case report used terlipressin at 30 µg/kg/day as a continuous infusion without side effects [15].

If terlipressin is not available, there are two alternatives: norepinephrine or octreotide in combination with midodrine. Both norepinephrine and octreotide plus midodrine should be given in combination with albumin (Figure 21.8B) [16].

Norepinephrine is a catecholamine with primarily α-adrenergic activity and is less expensive and more widely available than terlipressin. Side effects are less common with norepinephrine than with terlipressin and include chest pain with no electrocardiogram changes, ventricular extrasystole, and ST segment depression that improves with dose titration.

Octreotide in combination with midodrine is an alternative for the treatment of type 1 HRS. Octreotide is a vasoactive

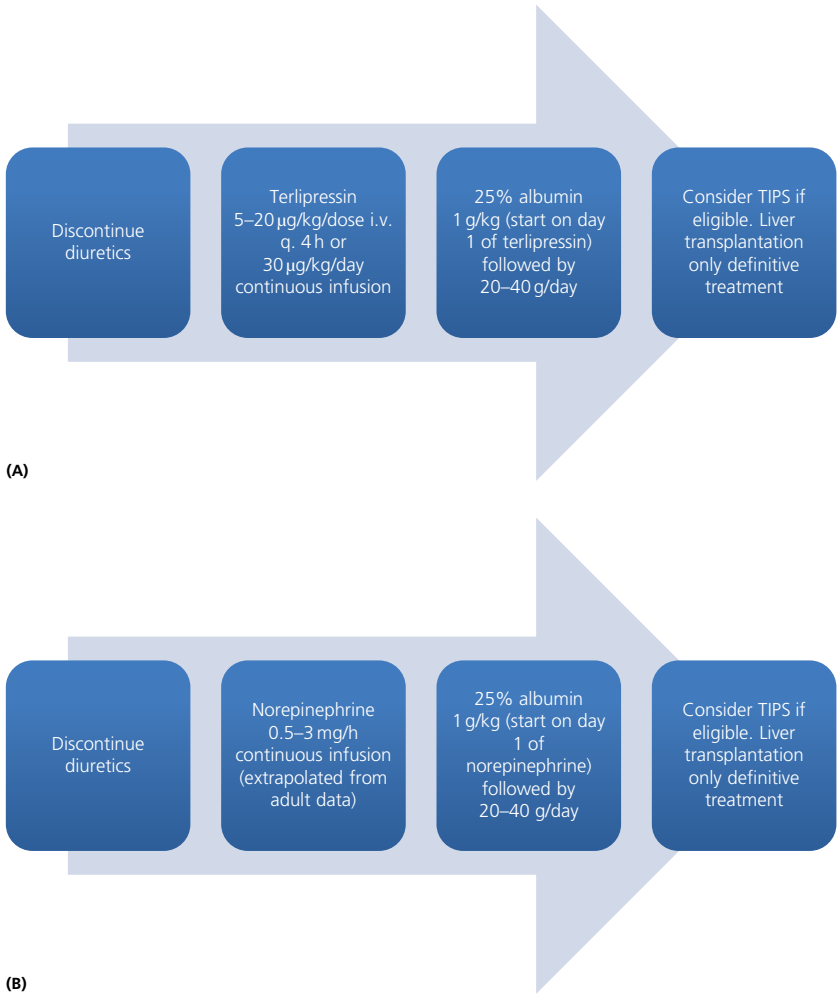


Figure 21.8 (A) Treatment of type 1 hepatorenal syndrome with terlipressin. TIPS, transjugular intra-hepatic portosystemic shunt. (B) Treatment of type 1 hepatorenal syndrome with norepinephrine. TIPS, transjugular intra-hepatic portosystemic shunt.

medication that decreases splanchnic blood flow while midodrine is an α1-adrenergic agonist that causes systemic vasoconstriction, increasing the effective circulating volume and renal perfusion [17].

There are no studies evaluating the effect of norepinephrine or octreotide plus midodrine for the treatment of HRS in children.

Type 2

There is no evidence to recommend terlipressin and albumin or other pharmacological management as a treatment for type 2 HRS [12].

Both type 1 and type 2

Patients should be considered for TIPS if eligible. However, liver transplantation is the only definitive treatment for long-term survival and should be considered for patients with both type 1 and type 2 HRS who are non-responsive to medical management.

Outcomes

In adults, average survival without treatment is a few weeks for type 1 HRS and 6 months for type 2 HRS, equivalent data are not available for children [12].

In adult patients with type 1 HRS, terlipressin plus albumin improved renal function in up to 50% of patients with an associated reduction in mortality particularly if started early. Large studies evaluating the efficacy of terlipressin in children have not been completed.

About 6% of children with HRS will require renal replacement therapy (RRT) as a bridge to liver transplantation. A retrospective case–control study of infants and children with HRS requiring RRT as a bridge to liver transplantation showed good recovery of renal function with no significant need for diuretics or anti-hypertensive medication 1 month following liver transplantation [13]. Comparatively, 90% of adults with type 2 HRS have reversal of their renal impairment, but type 2 HRS is also an independent predictor of post-transplant chronic kidney disease stage 3. Similar long-term outcome studies in children have not been completed.

Patient survival after liver transplantation for children with HRS requiring RRT is 63% compared to 100% at 1 year for controls (liver transplantation without HRS) [13].

Hepatic encephalopathy

Pathophysiology

The complete pathophysiology of hepatic encephalopathy has not been fully elucidated, though hyperammonemia is felt to play a significant role. When serum ammonia levels are elevated, they cross the blood–brain barrier activating the glutamate/glutamine pathways in astrocytes leading to increased glutamine production. Glutamine is osmotically active causing astrocyte swelling and brain edema. The extent of brain edema on MRI findings correlates with clinical findings of minimal, covert, and overt hepatic encephalopathy.

Clinical presentation

Hepatic encephalopathy can be classified as being overt or covert and episodic; recurrent if there are repeat episodes within 6 months; or persistent with constant behavioral abnormalities and only occasional relapses of overt hepatic encephalopathy.

Hepatic encephalopathy is a diagnosis of exclusion and therefore it is prudent to consider other etiologies of encephalopathy including the effects of medications (benzodiazepines and opioids), presence of intracranial bleeding, uremia, metabolic encephalopathy, or other comorbid psychiatric disorders.

Hepatic encephalopathy can be precipitated by infection, gastrointestinal bleeding, diuretic overdose, electrolyte disorders, and constipation all of which require active evaluation and treatment.

In cirrhosis, chronic encephalopathy presents as a spectrum of neurological and psychiatric disturbances. Initially, minimal hepatic encephalopathy may present only as abnormalities on neuropsychiatric testing including deficits in working memory, attention, and visuospatial relationships. Minimal hepatic encephalopathy, occurs in 20–80% of adult patients with cirrhosis though the incidence has not been well defined in children [18].

Covert hepatic encephalopathy includes minimal hepatic encephalopathy and grade 1 hepatic encephalopathy under the West Haven criteria and may manifest in children as deteriorating school performance.

Overt hepatic encephalopathy, classified as grade 2–4 is defined by the onset of asterixis and/or disorientation, both of which have good inter-rater reliability. Asterixis is best elicited in a patient whose arms are extended with hyperextension at the wrists and fingers and are unable to maintain this postural tone leading to a "flapping tremor."

Other neurological findings in patients with hepatic encephalopathy may include hypertonia and hyporeflexia (in grade 2 hepatic encephalopathy) and hyperreflexia and a positive Babinski sign (in grade 3 hepatic encephalopathy). Seizures are rarely seen. Progression of encephalopathy includes personality changes, irritability, and sleep–wake disturbances including excessive daytime sleepiness. Finally, a confusional state can develop with irritability, somnolence, and stupor progressing to coma.

Diagnosis

In adults, the diagnosis of hepatic encephalopathy is defined by the American Association for the Study of Liver Disease, which incorporates the West Haven criteria as the gold standard and is useful in older children (Box 21.3) [18]. Additional coma scales have been adapted for use in children 0–3 years old for further evaluation of hepatic encephalopathy [19].

Box 21.3 Progression of hepatic encephalopathy. (Adapted from Vilstrup *et al*. 2014 [18]. Reproduced with permission of John Wiley & Sons.)

- **Grade 0**: unimpaired: minimal hepatic encephalopathy (covert): neuropsychiatric testing abnormalities in executive and psychomotor functions, no clinical abnormalities
- **Grade 1 (covert)**: euphoria or anxiety, shortened attention span, impairment of addition or subtraction, altered sleep patterns
- **Grade 2 (overt)**: lethargy or apathy, disorientation for time, obvious personality change, inappropriate behavior, dyspraxia, may become combative, asterixis, hypotonia, hyporeflexia
- **Grade 3 (overt)**: somnolence to semi stupor, confusion, gross disorientation, bizarre behavior, combative, responsive to stimuli, hyperreflexia, positive Babinski sign
- **Grade 4 (overt)**: coma (non-responsive to verbal and painful stimuli)

Neuropsychological tests in children include the Revisie Amsterdamse Kinder Intelligentie test in children less than 12 years old and figure connection tests A and B and the performance subset of the modified Wechsler Adult Intelligence Scale for children greater than or equal to 12 years old. In adults, neuropsychiatric testing is recommended to detect minimal hepatic encephalopathy in patients with poor cognitive function or quality of life every 6 months [18]. No current recommendations exist for the pediatric population who may be at high risk for cognitive sequelae given the importance of brain maturation in this age group.

Elevated serum ammonia levels alone are not diagnostic for hepatic encephalopathy in chronic liver disease and may be falsely elevated secondary to hemolysis, temperature of the blood specimen, and time before the specimen is analyzed. Therefore, blood samples for ammonia should be sent in a pre-chilled, ammonia-free tube and delivered immediately on ice to the laboratory as samples are stable for <15 min at 4 °C. Normal ammonia levels should prompt an investigation for other etiologies of encephalopathy

An electroencephalogram (EEG) may demonstrate slow waves in grade II or III hepatic encephalopathy but may be non-specific.

MRI may demonstrate alterations in protein concentrations, but is currently a research investigation.

In children with a severely depressed level of consciousness, the Pediatric Glasgow Coma Scale may be more useful than the West Haven criteria in classifying the severity of hepatic encephalopathy [18].

Treatment

It is essential to determine if there is a precipitating event such as infection, gastrointestinal bleeding, diuretic overdose, electrolyte disorders, and constipation and treat accordingly [20]. In up to 90% of adults, hepatic encephalopathy can be treated with correction of precipitating factors alone; though similar data are not available for children [18].

Primary prophylaxis prior to the first episode of hepatic encephalopathy or for the treatment of minimal hepatic encephalopathy is generally not required, however it may be of benefit in a select patient population to help improve quality of life, particularly in those with cognitive complaints, or in patients with cirrhosis where future risk of overt hepatic encephalopathy is high.

Episodes of overt hepatic encephalopathy (grade 2–4) should be treated with lactulose, a non-digestable non-absorbable disaccharide that creates an acidic environment in the colon, decreasing ammonia production by reducing urease-producing bacteria and transforming ammonia to a less absorbable form (ammonium) that can be excreted in stool. Lactulose (0.3–0.4 mL/kg 2–3 times/day, titrated to a goal of two to three semi-formed acidic stools (pH <6.0) per day) [20] may be effective. Doses should be titrated up or down accordingly as patients having a higher frequency of watery stools are at risk for dehydration and electrolyte imbalance that may worsen encephalopathy. In patients who are hospitalized and unable to protect their airway, lactulose should be delivered by nasogastric tube. In a retrospective pediatric study, 73% of patients had complete resolution of hepatic encephalopathy with lactulose monotherapy.

After the first episode of hepatic encephalopathy, lactulose should be continued as secondary prophylaxis at the same dosing though efficacy studies are currently only available in adults [18]. In patients with recurrent episodes of hepatic encephalopathy despite lactulose prophylaxis, the addition of rifaximin is recommended, replacing older recommendations for oral aminoglycosides such as neomycin. Rifaximin is an antibiotic with minimal systemic absorption that is used to reduce ammonia-producing enteric bacteria and was initially approved in the pediatric population for traveler's diarrhea. The recommended starting dosage for rifaximin is 10–30 mg/kg/day [20].

Malnutrition is a significant problem in patients with cirrhosis and along with decreasing muscle mass increases the risk for hepatic encephalopathy. Generally, 1.5 g/kg/day of protein are recommended to be given as small meals throughout the day. More severe protein restriction may occur in the first few days of overt hepatic encephalopathy, but should not be sustained as it leads to catabolism and protein breakdown, which may further exacerbate hepatic encephalopathy.

Intubation is necessary for airway protection in patients with grade 3 hepatic encephalopathy.

In eligible patients with recurrent hepatic encephalopathy and liver failure, liver transplantation is recommended for definitive therapy.

Hepatopulmonary syndrome

Epidemiology and pathophysiology

Patients with hepatopulmonary syndrome have intrapulmonary vascular dilatation driven by increased pulmonary nitric oxide production by a mechanism that has not yet

been fully delineated [21]. In animal models, endothelin 1 (ET-1) released following liver injury increases expression of endothelin receptor type B (ET-B) which leads to the upregulation of nitric oxide synthase by pulmonary endothelial cells increasing production of nitric oxide. Intrapulmonary vascular dilatation mediated by nitric oxide leads to arteriovenous shunting, ventilation perfusion mismatch and diffusion perfusion defects all of which lead to arterial hypoxemia. Angiogenesis may also play a role in the pathogenesis of hepatopulmonary syndrome.

The pathogenesis of hepatopulmonary syndrome in patients without liver dysfunction, such as in EHPVO has been less well studied. In animal models, shear stress secondary to hyperdynamic circulation in portal hypertension is thought to increase the expression of ET-B in the pulmonary vasculature leading to increased production of nitric oxide.

Hepatopulmonary syndrome is more severe and frequent in children with cirrhosis with a prevalence of 40% compared to 13% of children with EHPVO.

Clinical presentation

Patients may be asymptomatic or present with increasing breathlessness.

Physical examination demonstrates chronic hypoxemia with spider nevi, clubbing, and cyanosis. Children with cirrhosis and hepatopulmonary syndrome are more likely to present with findings of clubbing (84%), cyanosis (21%), and dyspnea (21%) while patients with EHPVO and hepatopulmonary syndrome are more likely to present with clubbing alone (38%) (see Figure 21.10) [21].

Diagnosis

Hepatopulmonary syndrome is diagnosed in patients with portal hypertension with or without liver dysfunction, who have evidence of intrapulmonary vascular dilation (IPVD) and arterial hypoxemia in the absence of restrictive lung disease.

- IPVD is diagnosed most often by transthoracic contrast-enhanced echocardiography though transesophageal echocardiography is the gold standard. In a healthy patient, injected hand-agitated saline typically appears in the right atrium and ventricle then is absorbed by the pulmonary capillary vasculature. However in patients with no known intracardiac communications who have IPVD, intrapulmonary shunting leads to air bubbles noted in the left atrium and ventricle between the 3rd and 8th cardiac cycles after they first appear in the right atrium (Figure 21.9) [21].
- Arterial hypoxemia is most accurately diagnosed on an arterial blood gas when PaO_2 (arterial partial pressure of oxygen) is <80 mmHg and/or alveolar–arterial oxygen gradient ($PAaO_2$) is >15 mmHg. Notably, the $PAaO_2$ may become abnormally increased before PaO_2 declines below 80 mmHg. The severity of hepatopulmonary syndrome is further categorized as:
  - mild for $PaO_2 \geq 80$ mmHg and $PAaO_2 \geq 15$ mmHg
  - moderate for PaO_2 between 60 and 80 mmHg and $PAaO_2 \geq 15$ mmHg
  - severe for $PaO_2 < 60$ mmHg and $PAaO_2 \geq 15$ mmHg (Figure 21.9).
- Hyperemic arterialized capillary blood gas analyses have been investigated as an alternative to arterial blood gas for screening in children. However, falsely elevated $PAaO_2$ occurs in very young infants and children making this a useful tool only in children older than 2 years old. Abnormal findings should be confirmed with an arterial blood gas.
- Pulse oximetry is not an accurate diagnostic tool for early screening for hepatopulmonary syndrome as most children with mild to moderate hepatopulmonary syndrome will have normal pulse oximetry values (>98%).

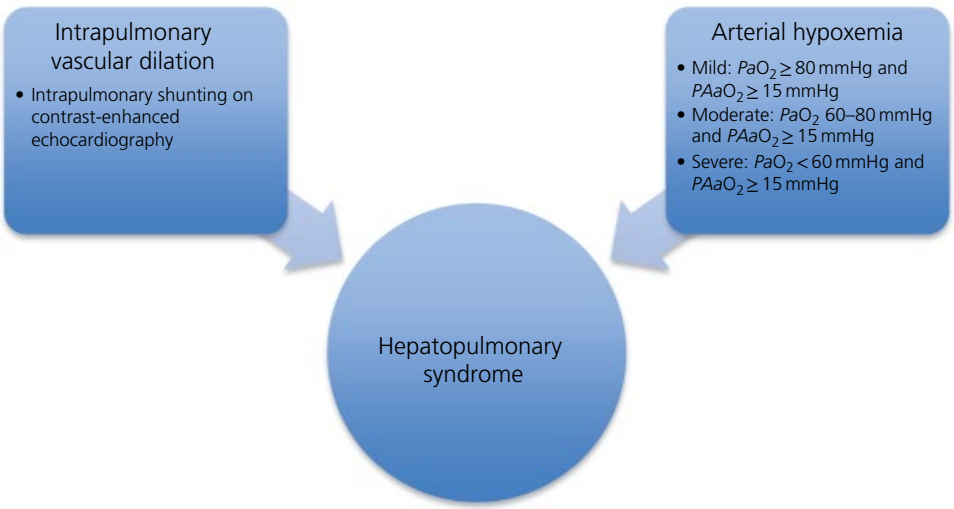


Figure 21.9 Diagnostic criteria for hepatopulmonary syndrome.

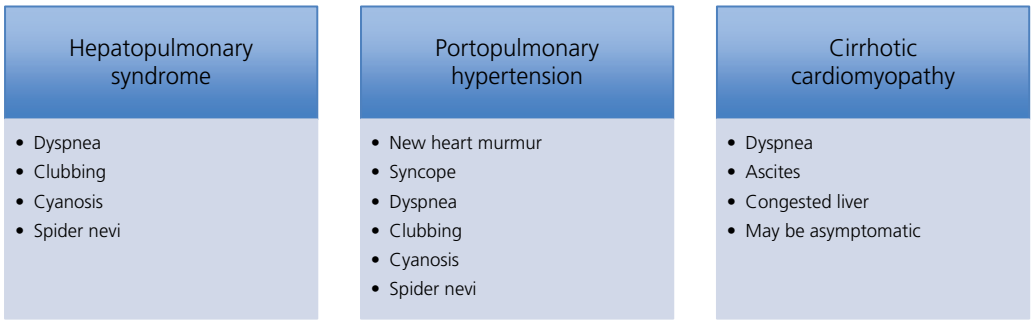


Figure 21.10 Clinical findings in hepatopulmonary syndrome, portopulmonary hypertension, and cirrhotic cardiomyopathy.

Hypoxemia alone is not enough to diagnose hepatopulmonary syndrome as patients with cirrhosis may have hypoxemia secondary to ascites, pleural effusions, and pulmonary edema.

Patients may have IPVD without hypoxemia, which is termed subclinical hepatopulmonary syndrome. The prevalence of subclinical hepatopulmonary syndrome is 6–10% in adults with cirrhosis and 2–20% in EHPVO, but has not been well studied in the pediatric population [21].

Management and outcomes

In patients with EHPVO, hepatopulmonary syndrome is an absolute indication for the consideration of a meso-Rex bypass or an alternative shunt (see Chapter 27) [3].

In patients with cirrhosis, the early diagnosis of hepatopulmonary syndrome is critical as definitive treatment is with liver transplantation and pre-transplant mortality in children ranges from 25 to 46% [22]. Supplemental oxygen is often required while awaiting liver transplantation.

Resolution of hepatopulmonary syndrome occurs within 3 months post-transplant but may persist up to 14 months. One-year survival after liver transplantation in children with hepatopulmonary syndrome is 93% which is comparable to other patients. However, retrospective studies have suggested that patients with hepatopulmonary syndrome may have higher rates of post-liver transplant complications including portal vein and hepatic artery thrombosis secondary to polycythemia so venesection may be required. A higher rate of biliary complications including anastomotic strictures has also been reported.

Portopulmonary hypertension

Pathophysiology and epidemiology

Portopulmonary hypertension is a rare but severe complication in children with hepatic and extra-hepatic causes of portal hypertension with an estimated prevalence of 0.9% [23]. Increased cardiac output is hypothesized to increase sheer stress in the pulmonary vasculature leading to vasoconstriction, increased pulmonary vascular resistance, and remodeling of endothelial and smooth muscle cells. Histopathologic findings in portopulmonary hypertension include plexogenic arteriopathy.

Clinical presentation and diagnosis

Clinical findings that suggest portopulmonary hypertension include a new heart murmur, syncope, dyspnea, and hypoxemia in a patient with portal hypertension (see Figure 21.10).

Investigations include the following:

- Chest radiograph may show a prominent pulmonary artery or cardiomegaly, but this is not a sensitive screening tool for portopulmonary hypertension and a normal chest radiograph does not reduce the need for further investigation.
- Doppler echocardiography is highly suggestive when pulmonary artery systolic pressure (PASP) is ≥40 mmHg with either right ventricular hypertrophy or interventricular wall flattening.
- Right-heart catheterization is indicated if an abnormally elevated PASP is noted on echocardiography, and is diagnostic when the mean pulmonary artery pressure is >25 mmHg, pulmonary vascular resistance index is >3 Wood units • m^2 and pulmonary capillary wedge pressure is <15 mmHg.

Management

In patients with cirrhosis, the definitive treatment for portopulmonary hypertension is liver transplantation. Patients with higher mean pulmonary artery pressures prior to liver transplantation have an increased risk of right-sided heart failure leading to increased morbidity and mortality in the postoperative period. In adults, a mean pulmonary artery pressure >45 mmHg may preclude liver transplantation due to the high rate of intra- and postoperative mortality; in children, a similar cut-off has not yet been defined. Combined heart and liver transplantation may be indicated.

In children, mean pulmonary artery pressures ≤35 mmHg prior to liver transplantation correlated with an improved postoperative survival, therefore preoperative vasodilator therapy is recommended.

In a small case series prostacyclin ± sildenafil decreased the mean pulmonary arterial pressure by 13 mmHg [23]. Vasodilator therapy has only been studied in children with primary pulmonary hypertension with normal liver function making it difficult to extrapolate appropriate

dosing for treatment in children with portopulmonary hypertension and liver dysfunction. Epoprostenol is a prostacyclin that causes pulmonary and systemic vasodilation. Pediatric doses for epoprostenol are a starting dose of 1–3 ng/kg/min by continuous intravenous infusion, increasing by 1 ng/kg every 30 min to a maximum of 50–80 ng/kg/min [24]. Side effects of epoprostenol include flushing, nausea, jaw pain, hypotension, headache, and thrombocytopenia.

Bosentan (2 mg/kg b.i.d.) is an endothelin receptor antagonist that decreases pulmonary vascular resistance and blocks receptor-mediated systemic vasodilation. More specifically, dosages recommended by weight are: 31.25 mg b.i.d. for patients 10–20 kg, 62.5 mg b.i.d. for patients 20–40 kg, and 125 mg b.i.d. for patients >40 kg [24]. Side effects include a dose-dependent transaminitis from inhibition of canalicular bile salt export that requires monthly monitoring. Elevations in aminotransaminase levels greater than three times the upper limit of normal occur in 3–16% of children. Other side effects include anemia, seminiferous tubular atrophy, abdominal pain, flushing, nausea, and headache. Bosentan is a teratogen and two forms of contraception should be provided, when appropriate, as the effectiveness of oral contraception is also decreased with endothelin receptor antagonist therapy.

Sildenafil is a phosphodiesterase type 5 inhibitor and increases vasodilation and antiproliferation of the pulmonary vasculature by increasing cyclic guanosine monophosphate (cGMP). It is metabolized primarily by cytochrome P450 enzymes. Sildenafil should be used with caution as recent Food and Drug Administration and European Medicines Agency guidelines differ. Consensus guidelines from pediatric pulmonary hypertensive experts currently recommend the cautious use of oral sildenafil in pediatric patients and avoidance of high-dose sildenafil. Recommended doses are: 0.5–1.0 mg/kg/dose t.i.d. in children <8 kg (with a maximum dose of 10 mg t.i.d.), 10 mg t.i.d. for patients 8–20 kg, and 20 mg t.i.d. for children >20 kg [24]. Side effects include flushing, hypotension, headache, and diarrhea. In a small case series prostacyclin ± sildenafil decreased the mean pulmonary arterial pressure by 13 mmHg [23].

Treatment in patients with portopulmonary hypertension without cirrhosis has not been well defined and may include congenital portosystemic shunt closure, Rex shunt, or pulmonary vasodilators.

Outcomes

If untreated, portopulmonary hypertension leads to right-sided heart failure and death with a median survival time of 3 months in children [23]. In patients treated with liver transplantation, 5-year survival was approximately 80% and did not differ between those treated with closure of a congenital portosystemic shunt and/or with pulmonary vasodilators.

Portopulmonary hypertension in patients with EHPVO is an indication for consideration of meso-Rex bypass or an alternative shunt.

Cirrhotic cardiomyopathy

Pathophysiology and epidemiology

Cirrhotic cardiomyopathy is a broad term for the specific cardiac complications in patients with cirrhosis, which include conduction abnormalities, diastolic dysfunction, and/or systolic dysfunction in the absence of known cardiac disease [25]. In portal hypertension, peripheral arterial vasodilation is mediated by nitric oxide and lowers systemic vascular resistance, decreasing cardiac afterload, which triggers an increase in cardiac output. The pathophysiology of cirrhotic cardiomyopathy has not been fully defined, but decreased β-adrenergic receptor density and function as well as increased serum nitric oxide levels may both play a role in decreasing cardiac contractility.

The prevalence of latent cirrhotic cardiomyopathy is about 18% in children with portal hypertension while 2% had manifest cirrhotic cardiomyopathy [25].

Diagnosis

Diagnostic criteria are extrapolated from the World Congress of Gastroenterology adult guidelines, though this modified criteria has not been validated in children. Major diagnostic criterion for cirrhotic cardiomyopathy include: prolongation of a corrected QT interval (≥0.45) in the absence of drug effect or electrolyte imbalance; resting left ventricular ejection fraction of <55% (systolic dysfunction); or signs of diastolic dysfunction which include E/A (E = mitral valve velocity, A = mitral valve A velocity) ratio <1.0 or >2.0 (in a restrictive pattern), prolonged deceleration time >200 ms or prolonged isovolumetric relaxation time >80 ms [25]. Minor diagnostic criterion for cirrhotic cardiomyopathy included: tachycardia as defined by age group, an enlarged left atrium, increased myocardial mass, electromechanical uncoupling, and abnormal chronotropic response.

In children, latent cirrhotic cardiomyopathy is diagnosed when there is prolongation of a corrected QT interval plus at least one minor criterion. Manifest cirrhotic cardiomyopathy is diagnosed when two major criteria are fulfilled. In patients with cirrhosis, diastolic dysfunction typically precedes systolic dysfunction.

Physical findings may be subtle and in a small retrospective pediatric study, one child with cirrhotic cardiomyopathy presented with exercise dyspnea while the remainder of patients were asymptomatic (see Figure 21.10) [25]. No child presented with respiratory distress, cyanosis, edema, or angina. Given the subtlety of presentation, periodic cardiac examinations are warranted in all children with cirrhosis. Ascites and high Child-Pugh scores may also be associated with an increased risk of cirrhotic cardiomyopathy.

Management and outcome

Medical management of cirrhotic cardiomyopathy has not been well studied in children. Medications such as inhibitors of angiotensin-converting enzyme, diuretics, and β-blockers may have deleterious effects in patients with cirrhosis.

The definitive treatment for cirrhotic cardiomyopathy is liver transplantation. In adults with cirrhotic cardiomyopathy, blunted hemodynamic response intraoperatively has a risk of poor graft function and death, but improvement in systemic vascular resistant has been reported by 2 weeks post-transplantation, improvement of diastolic dysfunction by 6 months, and normalization of QTc by 3 months post-transplant.

Cutaneous manifestations

Pruritus

Pruritus is frequently seen in chronic liver disease, most commonly in intra-hepatic cholestastic liver disease such as Alagille syndrome and familial intra-hepatic cholestasis. Pruritus can be generalized or localized most commonly to the palms and soles. Pruritus significantly affects the quality of life of children leading to poor school performance and automutilation, requiring treatment for severe manifestations.

The pathophysiology of pruritus is not clearly defined, however suggested mechanisms include bile salt accumulation and deposition in the skin and increased histamine levels acting as pruritogens; however patients with cholestatic pruritus may also have normal levels of bile salts and histamine therefore other mechanisms must play a role [26].

Combination therapies with different mechanisms of action are usually necessary (Figure 21.11) and approximately 80% of patients require the addition of a second agent or a switch to an alternative medication. There are currently no guidelines on the selection of initial agents for the treatment of pruritus.

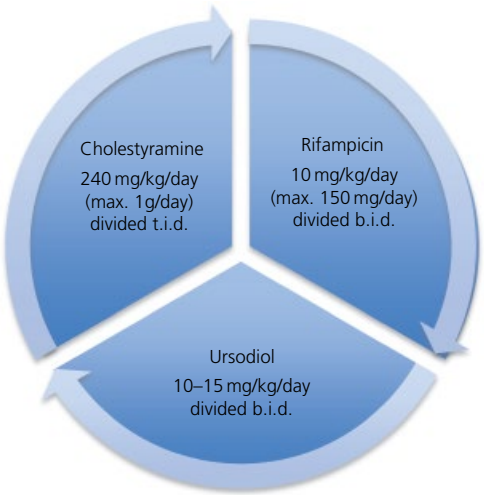


Figure 21.11 Pharmacological treatment for pruritus and xanthoma in chronic liver disease.

Ursodiol (ursodeoxycholic acid) is a hydrophilic (non-toxic) bile acid that works as a choloretic by competitively inhibiting the absorption of endogenous bile acids from the intestines. Ursodiol (10–15 mg/kg daily or divided b.i.d.) may be effective in patients with intra-hepatic cholestasis. The most common side effect is diarrhea.

Cholestyramine is a resin that binds bile acids and is typically started at 240 mg/kg/day (to a maximum daily dose of 1 g/day) divided t.i.d. This dose can then be increased to effect with a maximum dosage of 4 g/day for children ≤10 years old and maximum of 8 g/day in children >10 years old. Relief of pruritus typically occurs between 7 and 30 days after initiation of therapy [26]. Side effects are mild and include fat malabsorption and constipation.

Rifampicin enhances the degradation of toxic bile acids, which may release pruritogenic substances from damaged hepatocytes, by inducing the cytochrome P450 system. Pediatric doses start at 10 mg/kg/day divided b.i.d. (to a maximum of 150 mg/day) increased to effect to a maximum of 600 mg/day. Rifampicin may be more effective in children with extrahepatic cholestasis. Side effects include orange-red bodily secretions, hemolytic anemia, renal failure, and thrombocytopenic purpura as well as an increased risk of drug-resistant organisms with long-term therapy; though this has not yet been demonstrated in pediatric studies.

In cases of refractory pruritus, most commonly described in patients with progressive familial intra-hepatic cholestasis (PFIC) and Alagille syndrome, biliary diversion, and ileal exclusion are alternatives, but liver transplantation should be considered in these patients.

Xanthoma

Hypercholesterolemia (>200 mg/dL) occurs in 81% of patients with Alagille syndrome, and severe hypercholesterolemia (>800 mg/dL) in 24% of patients due to cholestasis and impaired bile flow [27]. When serum cholesterol levels are >500 mg/dL, xanthomas develop in the skin in 27–29% of patients with Alagille syndrome. Xanthomas typically occur over the extensor surfaces of the fingers, palmar creases, popliteal fossae, neck, inguinal and gluteal regions with areas of confluence occurring over the elbows and knees; the face is also occasionally involved. Xanthomas can be further classified as being minimal with less than 20 scattered individual lesions; moderate with more than 20 lesions that do not interfere with activities; disfiguring with either large number of lesions or large size of lesions that cause distortion of the extremities or face; or disabling with xanthomas that interfere with function such as extremity usage due to large size or number.

Medical management for the treatment of xanthomas is challenging, but ursodiol is the most effective in decreasing

serum cholesterol levels with subsequent resolution of xanthomas. Medication dosage is the same as for pruritus (see Figure 21.11). Cholestyramine is useful in the treatment of familial hypercholesterolemia, but is less useful in patients with Alagille syndrome who have bile duct paucity, cholestasis and regurgitation of biliary phospholipids into plasma. Medication dosage is also the same as for pruritus. 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) carry a risk of hepatotoxicity and do not have a role in the treatment of pediatric hypercholesterolemia in Alagille syndrome.

In patients with Alagille syndrome who undergo liver transplantation, hypercholesterolemia resolves between 3 and 14 days after transplantation with subsequent resolution of xanthomas [27]. The risk of early atherosclerotic vascular disease secondary to hypercholesterolemia has not yet been fully evaluated in children and is not an indication for earlier liver transplantation.

Oncological considerations

Hepatocellular carcinoma (HCC) has an overall incidence of 0.05 cases per 100,000, with 75% of these cases being in children more than 10 years old and a higher incidence in adolescents of 0.08 cases per 100,000. In contrast to adults, 60–70% of children with HCC do not have liver cirrhosis [28]. HCC is associated with chronic hepatitis B and C infection, Alagille syndrome, α1-antitrypsin deficiency, PFIC2, Wilson disease, tyrosinemia, and glycogen storage disease. HCC is less often described in patients with biliary atresia after hepatoportoenterostomy.

Clinical symptoms of HCC can be subtle and include abdominal distension and discomfort. Screening recommendations are not uniform for patients with chronic liver disease, though screening AFP and liver ultrasound are suggested every 6–12 months, especially in patients with PFIC2 and hereditary tyrosinemia type 1 who are at higher risk for HCC [28] (Figure 21.12).

α-Fetoprotein (AFP) may not be raised, but is typically >400 ng/dL in patients with HCC. In children with an abnormal or rising AFP level, cross-sectional imaging with CT or MRI is indicated for further evaluation (see Chapter 28).

In contrast to hepatoblastoma, HCC is less responsive to chemotherapy with a 50% response rate and an overall 5-year survival of 24%. Complete tumor resection is required for cure and tumors that are not amenable to surgical resection should prompt evaluation for liver transplantation. Contraindications to liver transplantation for HCC may include radiologic evidence of extra-hepatic disease, gross vascular invasion, or rapid disease progression despite chemotherapy. Overall 5-year survival for children with HCC undergoing surgical resection or liver transplantation is 59% compared to 7% for children without operative intervention (see Chapter 28).

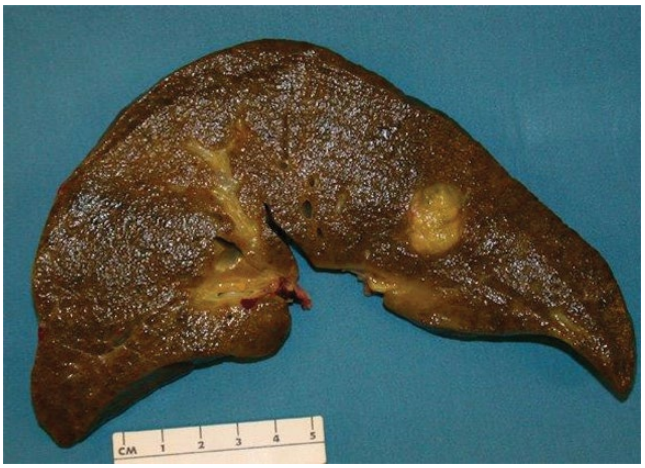


Figure 21.12 Hepatocellular carcinoma in the native liver of a patient with progressive familial intra-hepatic cholestasis type 2.

Coagulopathy

The liver plays an important role in the maintenance of hemostasis by a complex balance between the production of coagulation proteins, inhibitors of coagulation, and removal of fibrin degradation products and coagulation factors. Thus, coagulation disorders are common in chronic liver disease due to a combination of vitamin K malabsorption and deficiency, reduced synthesis of coagulation factors and inhibitors of coagulation, thrombocytopenia secondary to hypersplenism, or from intravascular coagulopathy. These disturbances are particularly important in prognostic assessment, and in the genesis and management of gastrointestinal bleeding, and may lead to serious complications such as intracerebral bleeding and intravascular coagulopathy.

Pathophysiology

There are abnormalities of primary hemostasis (interaction between platelets and vessel wall), coagulation (thrombin generation), and fibrinolysis in patients with chronic liver disease, but there is poor correlation between the risk of bleeding and the peripheral indices of hemostasis. Normal hemostasis is affected by reduced numbers of circulating platelets and platelet function but, platelet counts >20,000 rarely cause problems.

Management

This is directed at prevention or correction of vitamin K deficiency by regular administration of vitamin K. All patients should have oral vitamin K supplements (parenteral vitamin K; 2–10 mg i.v. daily for 3 days or 5–10 mg/week i.m.) should be given to cholestatic patients with a prolonged prothrombin time. Infusions of fresh frozen plasma (5–10 mL/kg), cryoglobulin, and/or platelet transfusions are effective for transient correction, and should be reserved for invasive procedures such as liver biopsy and for bleeding episodes.

Malnutrition

Methods of assessment

The cause of malnutrition in children with chronic liver disease is multifactorial and includes decreased oral intake, malabsorption, and increased caloric requirements. Decreased oral intake may be secondary to early satiety from ascites or organomegaly. Malabsorption of fats occurs in the cholestatic infant or child with decreased delivery of bile to the small intestine. Vascular congestion in the setting of portal hypertension may further contribute to enteropathy in these patients exacerbating a malabsorptive state [8]. Finally, children with end-stage liver disease have a resting energy expenditure that is 27–29% higher than healthy children [8, 28]. Combined, these factors place infants and children with chronic liver disease at high risk for malnutrition.

Assessment

- Clinical history should include the frequency, volume, and content of feeds. Food diaries may be helpful in the older child.
- Height, weight-for-age, and head circumference in children younger than 3 years old should be plotted on age and gender-appropriate percentile charts and standardized by conversion to a z-score (the number of standard deviations above or below the 50th percentile). Weight may be a less accurate measurement due to the presence of ascites, edema, and organomegaly as suggested by the finding that children with chronic liver disease are noted to have more depressed height z-scores than weight z-scores [8].
- Arm anthropometrics are a more sensitive measure of the nutritional status of children with chronic liver disease. Triceps skinfold thickness, which estimates adipose tissue (energy) reserve, is measured most often by an experienced pediatric dietitian using calipers over the back of the arm, mid-way between the acromion and olecranon processes with the arm held in a relaxed state; which is then converted to a z-score. Mid-upper arm circumference is also measured at the same location and converted to a z-score to estimate muscle bulk [29]. In a study of children with chronic liver disease without overt findings of ascites or edema, triceps skinfold thickness z-scores was significantly more depressed than weight/height z-scores suggesting that weight and height measurements may overestimate the nutritional status in these patients. Serial arm anthropometrics therefore are necessary in the nutritional evaluation of children with chronic liver disease.

Requirements: caloric, carbohydrate, protein, fats

To compensate for increased resting energy expenditure and fat malabsorption, caloric goals are approximately 130–180% of the recommended daily allowance in children with chronic liver disease (see Chapter 6) [29]. Nasogastric tube placement is recommended when patients are unable to meet these additional caloric requirements orally. Nasogastric tube feeding initiation may specifically be considered when triceps skinfold thickness and mid-upper arm circumference z-scores are less than –2 standard deviations below normal, or continue to fall despite increase in caloric density and volume of oral feedings [28]. Parenteral nutrition may be required in patients awaiting liver transplantation and should be considered in patients who have recurrent variceal bleeds or do not have normalization of triceps skinfold thickness and mid-upper arm circumference z-scores despite maximal nasogastric tube feeding.

Patients with chronic liver disease also have a decreased capacity for gluconeogenesis and glycogen storage with a risk of fasting hypoglycemia particularly in infants and small children. Inadequate gluconeogenesis and glycogen storage leads to an increased reliance on amino acids for fuel with a resulting negative nitrogen balance. Protein intake is therefore recommended at 2–4 g/kg/day. In patients with overt hepatic encephalopathy, severe protein restriction may occur in the acute setting though this should not be sustained and generally 1.5 g/kg/day of protein are recommended to be given as small meals throughout the day.

In infants and children with cholestasis, there is decreased bile flow into the intestine. Bile acids are needed for the emulsification and absorption of long-chain fatty acids. As an alternative, medium-chain triglycerides (MCT) are more water soluble and do not require micellar emulsification, they therefore can be absorbed in the absence of bile flow. About 30–60% of daily fat should be provided as MCT oil. Due to fat malabsorption, children with chronic liver disease are also at risk for essential fatty acid deficiency (linoleic and linolenic acid), which can be detected on routine testing and avoided by the provision of at least 40% of total daily fat from long-chain fatty acids.

Vitamin deficiencies and supplementation

Fat malabsorption in patients with chronic liver disease also leads to fat-soluble vitamin (A, D, E, and K) deficiency. Vitamin A deficiency occurs in 43–69% of children with cholestatic liver disease. Abnormal serum retinol levels are defined as <20 μg/dL (<0.7 μmol/L) with a 90% sensitivity and 78% specificity for the detection of vitamin A deficiency and is an appropriate first screening study for vitamin A deficiency in children with cholestatic liver disease. Clinically, vitamin A deficiency in children with liver disease is asymptomatic and night blindness is rare, but supplementation is recommended at 5000–25,000 IU/day with continued monitoring of vitamin A levels given risk of hepatotoxicity and teratogenicity in hypervitaminosis A.

Vitamin D deficiency, defined as a serum 25-OH vitamin D level of <20 ng/mL, occurs in 25% of children with chronic liver disease, of whom 11% will have fractures. In vitamin D deficiency, supplementation with

cholecalciferol at 1200–8000 IU/day is recommended [30]. Supplementation is essential in any patient with low vitamin D levels, osteopenia, or pathologic fractures. Calcitriol is the active form of vitamin D and is needed for the intestinal absorption of calcium and phosphorous, therefore patients with vitamin D deficiency also have an increased risk of osteopenia and rickets. Co-supplementation with D-α-tocopherol polyethylene glycol 1000 succinate forms micelles in the absence of bile salts and enhances the absorption of vitamin D.

Using the ratio of serum vitamin E concentration to total serum lipid concentration, vitamin E deficiency is defined as <0.6 mg/g for children <1 year old and <0.8 mg/g in older children. Clinically, vitamin E deficiency is present in about 62% of children with end-stage liver disease and may present as peripheral neuropathy, ataxia, or proximal muscle weakness. Dosing of D-α-tocopherol polyethylene glycol 1000 succinate is recommended in increments of 25 IU/kg/day (to a maximum of 100 IU/kg/day); this water-soluble micellar preparation can be costly.

Vitamin K (see earlier) is required for the carboxylation of vitamin K dependent coagulation factors (factors II, VII, IX, X, and protein C and S). Using INR as a surrogate marker, vitamin K deficiency is diagnosed when INR is ≥1.3 (prothrombin time >3 s over control). Supplementation strategies include the following: if 1.2 < INR ≤1.5 give 2.5 mg oral vitamin K daily; if 1.5 < INR ≤1.8 give 2–5 mg vitamin K parenterally then 2.5 mg oral vitamin K daily thereafter; if INR >1.8 give 2–5 mg vitamin K parenterally and then 5 mg oral vitamin K daily thereafter.

Complications of malnutrition

Nutritional status is an important and potentially modifiable risk factor prior to liver transplantation. Children with chronic malnutrition have a higher incidence of infection, surgical complications, poorer neurodevelopmental outcomes, and mortality following liver transplantation.

Prior to liver transplantation, children have an increased risk for osteopenia, rickets, and pathologic fractures. This risk may continue post-liver transplantation as bone mass continues to decrease 3–6 months following transplantation. Bone mineral density normalizes 6–19 months following liver transplantation in children less than 2 years old, however similar data is not available in older children.

Poor growth in children with chronic liver disease and malnutrition is further exacerbated by the depressed production of insulin-like growth factor 1 (IGF-1) by the liver despite elevated endogenous growth hormone levels. In children treated with exogenous recombinant human growth hormone, IGF-1 levels remain low suggesting insensitivity to growth hormone and is therefore not a useful therapy in children with chronic liver disease and growth failure.

Children demonstrate catch-up growth for height on average 6–24 months after liver transplantation, however final adult height remains dependent on the degree of growth failure prior to liver transplantation highlighting the critical importance of nutrition in patients with chronic liver disease.

Conclusion

Chronic liver disease is multifactorial and will progress to cirrhosis and portal hypertension in the majority of children. Prevention of complications, attention to nutrition, and early consideration for transplantation are key to the optimal management of these children.

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SECTION 10

The Liver and Other Organs

CHAPTER 22

The Liver in Systemic Illness

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Key points

- The liver is exposed to a wide range of agents liable to disturb the integrity of: firstly, the portal areas signified by rises in γ-glutamyl transpeptidase and alkaline phosphatase; and secondly, hepatocytes signified by rises in aspartate amino transferase and alanine aminotransferase.
- Hypoxia is relatively well tolerated in the short term because the double vascular inlet (hepatic artery and portal vein) is protective; but an ischemic insult lasting for more than an hour or two risks inducing a steep rise in transaminases followed by cholestasis after a few days.
- Infective agents have a particular impact on the cholangiocytes affecting bile acid transporters (especially the bile salt export pump) which are inhibited by cytokines and lipopolysaccharide causing jaundice (conjugated hyperbilirubinemia).
- Abnormal liver function is common in the course of inflammatory bowel disease and may relate to drug treatment; virus activation; disease of the terminal ileum disrupting the enterohepatic re-circulation of bile salts; and to associated autoimmune disorders such as celiac disease, autoimmune hepatitis, and sclerosing cholangitis.
- Hepatomegaly may be a result of infiltrative diseases (e.g., histiocytosis, hematological malignancy), vascular congestion (especially right-sided heart lesions), endothelial damage from chemotherapy, fatty liver, or excessive glycogen storage in poorly controlled type I diabetes mellitus.
- Liver biopsy carries the risk of major bleeding (estimated at 1 in 250 to 1 in 2500), but where diagnostic uncertainty continues it can provide invaluable diagnostic information relating to the location of liver injury, disease severity, and pathognomonic features.
- The finding of granulomas should prompt an evaluation of immunodeficiency, sarcoidosis, mycobacterial disease, and adverse drug reaction.
- Abnormal liver function in children with chronic respiratory infections, or cryptosporidial infections, recurrent otitis media, and/or failure to thrive should prompt an evaluation of the immune system.

Interpretation of abnormal liver function tests in systemic illness

The volume of blood flowing through the liver and the multitude of functions performed by hepatocytes and cholangiocytes, mean that systemic events and disease states in other parts of the body frequently affect the function of the liver [1]. The liver is the largest solid organ in the body weighing about 300 g in a healthy 10-kg child and each gram contains around a 100 million hepatocytes arranged in subunits called acini [2]. The acinus is composed of three main tissue types: endothelium (blood vessels including sinusoids); two types of epithelium (cholangiocytes found in bile ducts and ductules; cuboidal hepatocytes arranged in plates) and mesenchymal cells such as immune cells; and stellate cells which secrete collagen when activated. The acinus has a central vein which collects blood flowing in to the periphery of the acinus via two blood supplies: the portal vein and the hepatic artery which feed into the acinus at the boundary plate visible on low-power microscopy, and known as portal tracts [3]. The double vascular input from the celiac axis and portal vein inlet means that the liver takes a large percentage of the cardiac output (~25%) which allows it to perform the many metabolic functions (protein synthesis, drug and hormone detoxification, glucose homeostasis, regulation of sodium balance, maintenance of cellular respiration) which are essential to health.

In addition to these vascular relationships the liver has its own excretory pathway – the biliary tree which is a unique system allowing for the re-circulation of bile salts and excretion

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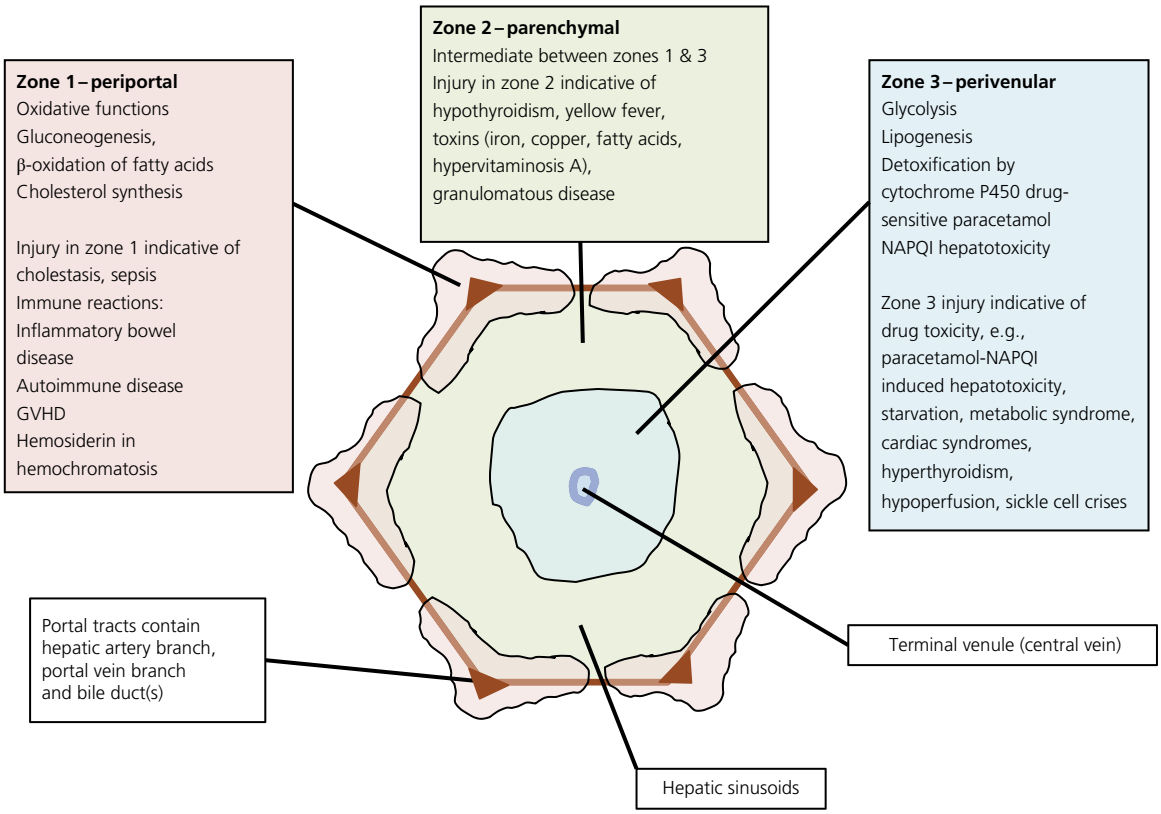


Figure 22.1 Differentiation of hepatocyte function. GVHD, graft-versus-host disease; NAPQI, *N*-acetyl-*p*-benzoquinone imine.

of a wide range of drugs especially those which are lipophilic and not so easily eliminated via the water-based excretion system in the renal tract.

Although the hepatic cell mass is often thought of as a single entity, differentiation of hepatocyte function occurs along an axis between the entry of blood into the liver in the portal tracts, and the exit of blood on the far side of the sinusoid via the hepatic veins. Zone 1 hepatocytes near the portal tracts are closely involved with biliary structures, hepatocyte regeneration and also immune phenomena, whereas zone 3 hepatocytes near the hepatic vein have a greater role in detoxification and are rich in glutathione (Figure 22.1).

This explains the different patterns of biochemical disturbance according to etiology: those diseases which have origins outside the liver often affect the cells in the portal areas first and are associated with elevations in bilirubin and the alkaline phosphatase, γ-glutamyl transferase enzymes; whilst disease affecting the hepatocytes directly such as viruses and some drugs cause the hepatic transaminases to be notably elevated: sometimes this may be gross, i.e., 10–50 times the normal range [4] (Table 22.1) (see Chapter 1).

Because changes in liver function tests are not diagnostic in themselves where there is diagnostic uncertainty, the assistance which liver biopsy can provide in terms of disease severity, correlation between zonal damage and etiology, and guidance to further treatment, can be invaluable. For example paracetamol (acetaminophen) poisoning results in perivenular necrosis in zone 3 whereas autoimmune liver disease is concentrated around the portal tracts and zone 1 (see Chapter 2). Liver biopsy has the potential to cause fatal bleeding and should always be undertaken in specialist centers with appropriate protocols [5].

Liver dysfunction according to prevailing pathophysiology

Aberrations in vascular flow include: (1) cardiac disorders; (2) perinatal circulatory disorders; and (3) arteriovenous shunting, pulmonary hypertension, and hepatorenal syndrome.

Cardiac disease and disorders of circulation

(Table 22.2)

Congestion

The hepatic veins drain into the inferior vena cava or directly into the right atrium, which means that cardiac dysfunction rapidly leads to hepatic congestion as in: pulmonary atresia, constrictive pericarditis, tetralogy of Fallot, and after the Fontan procedure carried out on univentricular hearts [1]. Sinusoidal engorgement usually causes modest elevations of transaminases, while alkaline phosphatase and bilirubin are

Table 22.1 Pathophysiological correlates of liver enzyme elevations. From Giannini *et al.* 2005 [4].

	Function	Tissue location	Implications of abnormal serum concentrations
AST	Catabolizes amino acids, permitting them to enter the citric acid cycle Has a half-life of 17 h	Widely distributed in heart, skeletal muscle, kidney, brain, red blood cell as well as liver; 20% found in the cytosol and 80% in mitochondria	During loss of hepatocyte integrity especially those cells in zone 3 where AST is concentrated, e.g., during ischemia and drug toxicity AST : ALT ratio may be 10 : 1 in Wilson disease, and other intoxications, e.g., alcohol
ALT	Catabolizes amino acids, permitting them to enter the citric acid cycle Has a half-life of 47 h	Mainly found in liver tissue and almost all in the cytosolic compartment of the cell	Longer half-life than AST and will peak later but is more specific indicator of hepatocyte necrosis, e.g., by cytopathic effect of viruses, autoimmune process, allograft rejection
ALP	Transports metabolites across cell membranes Half-life in the circulation is about 7 days Requires zinc	Found on the surface of bile duct epithelia Also bone, placenta, kidneys, small bowel and white blood cells	Cholestasis enhances synthesis and release of ALP, and accumulating bile salts increase its release from the cell surface ALP increases occur late in bile duct obstruction ALP may be reduced in zinc deficiency states and disorders of copper metabolism
GGT	Catalyzes the transfer of GGT residues to amino acids or small peptides Has a half-life of 14–26 days	Concentrated in endoplasmic reticulum, hepatocytes, biliary epithelial cells, renal tubules, pancreas and small bowel. Not very specific but highly sensitive marker of liver disease Activity induced by several drugs, e.g., anticonvulsants and oral contraceptives	Elevated GGT may occur in chronic obstructive airways disease after acute myocardial infarction and renal disease In the setting of chronic liver disease and after liver transplantation, GGT is associated with bile duct damage and fibrosis. GGT often goes up in immune reactions such as rejection where biliary epithelium is targeted by the immune system
Bilirubin unconjugated	Not applicable	Normally an intermediate metabolite of heme breakdown	Increases occur when: a) liver failure results in impaired hepatic uptake b) hemolysis results in excess production (sickle cell disease; rhesus incompatibility; Coombs positive autoimmune hepatitis; hereditary spherocytosis) c) physiological immaturity of UDP-glucuronyltransferase, or genetically determined decrease of UDP-glucuronyltransferase (Gilbert syndrome) or absence (Crigler–Najjar syndrome) d) reduced activity of UDP-glucuronyltransferase caused by hypothyroidism
Bilirubin conjugated	Disposal pathway for heme and key component of bile which is essential for digestion and absorption of dietary lipids	Synthesized in hepatocytes and excreted via active transporters into the biliary canaliculi	Increases occur when: a) the biliary tree is compromised by obstruction b) during sepsis when the activity of bile acid transporters is inhibited by lipopolysaccharide c) when fibrosis/cirrhosis have reduced the functional capacity of the liver to well below 50% d) drug reactions

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; UDP, uridine-5′-diphosphate.

Table 22.2 Features of cardiac–hepatic syndromes.

Cardiac features	Hepatic features	Hepatic disorder
Dextrocardia, ASD, VSD	Cholestasis and cirrhosis	Biliary atresia
Peripheral pulmonary stenosis; right ventricular overload	Cholestasis, pruritus	Alagille syndrome
Volume-overloaded ventricles	Edema	Hyperaldosteronism
Bounding pulses	Encephalopathy, coagulopathy, jaundice	Fulminant or subacute liver failure
Right ventricular heart failure and/or cor pulmonale	Extrahepatic portal hypertension, dyspnea, hypoxemia; liver function tests may be normal, cirrhosis not always present	Portopulmonary hypertension
Hypertrophic cardiomyopathy	High lactate, coagulopathy	Mitochondrial cytopathy, tyrosinemia type I
Cyanosis; shunting within the pulmonary capillary beds.	Cirrhosis and hypoxemia	Hepatopulmonary syndrome

ASD, atrial septal defect; VSD, ventricular septal defect.

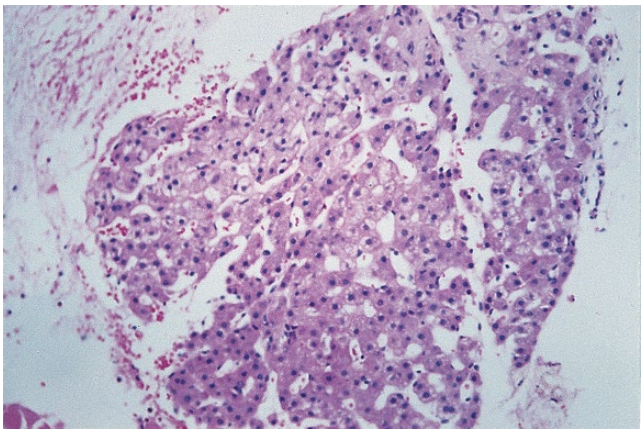


Figure 22.2 Sinusoidal dilation secondary to raised hepatic pressure from constrictive pericarditis due to tuberculosis. The patient improved following pericardectomy and isoniazid treatment.

typically normal, although there may be a rise in unconjugated hyperbilirubinemia.

The clinical features are those of hepatic vein outflow obstruction and include: hepatomegaly, which may be tender; unconjugated hyperbilirubinemia; transaminases 80–200 IU/L; and sinusoidal dilation around the central vein on histology (Figure 22.2). Long-term survivors of the Fontan operation have a relatively high prevalence of cirrhosis (25.9% after a mean of 11.5 years postoperatively) and the clinical picture also includes hypoalbuminemia exacerbated by protein-losing enteropathy, ascites and portal hypertension [1].

If constrictive pericarditis is the underlying cause of hepatic congestion, the diagnosis may be missed, as cardiac signs and symptoms are minimal. Cardiac catheterization may be required to confirm the diagnosis. In a study of 83 patients with a variety of cardiac disorders, there was a correlation between raised aminotransferases and raised hepatic venous pressures (mean wedge pressure 18 mmHg and free 15 mmHg), which was also related to the presence of centrilobular necrosis and inflammation [6]. The differential diagnosis includes Budd–Chiari syndrome, sinusoidal obstruction syndrome (see later), and tuberculous pericarditis.

Hypoxia and low cardiac output states

Hypoxia associated with neonatal asphyxiation is well recognized to cause damage to multiple organs. A retrospective study of 130 infants with neonatal asphyxia found that cardiovascular, pulmonary, renal, and hepatic dysfunction was present in >80% of infants but there was no association with severity of hypoxia as quantified by the number of organs involved in dysfunction and subsequent developmental delay [7]. Hepatic dysfunction was defined by aspartate aminotransferase or alanine aminotransferase elevated (100 IU/L) at any time during the first week after birth which is an insensitive marker of severe organ damage and may explain the lack of correlation with neurological outcome in this study – but clearly liver co-morbidity is common in neonatal hypoxia states. The prognosis of liver dysfunction is generally good provided normal circulation and gas exchange is established and biochemical changes return to normal within 1–2 weeks.

Hypoxia secondary to epileptic seizures may also cause elevated hepatic transaminases, which resolve spontaneously. The prognosis of liver dysfunction after prolonged grand mal seizures may be more serious because the mechanisms are multifactorial and include pre-existing drug-induced liver stress, steatosis, and hypoxic components.

Hypoplastic left heart and cardiomyopathy result in reduced blood flow within the liver parenchyma. This particularly affects zone 3 hepatocytes around the central vein. There may be a compensatory increase in portal vein blood flow to balance the reduction in hepatic arterial flow, so hepatic dysfunction can be minimal unless multiorgan failure has developed due to the low output state. Jaundice and considerably raised transaminases (100–10,000 IU/L) may develop and are more severe with prolonged low output states [1]. The biliary tree is particularly sensitive to acute and chronic hypoxia associated with reduced blood flow into

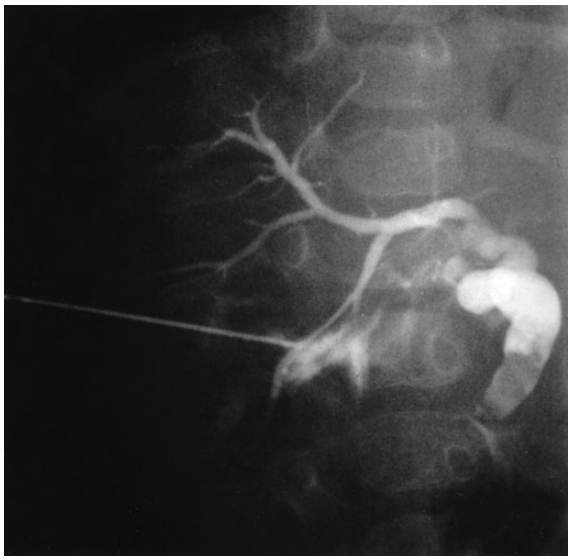


Figure 22.3 Percutaneous transhepatic cholangiogram, demonstrating obstructed biliary tree due to biliary sludge following cardiac surgery.

the liver (e.g., hepatic artery thrombosis is associated with major bile duct damage because the cystic artery supplies the major bile ducts). Cardiac surgery may lead to the formation of biliary sludge because of the acute hypoxia during cardiac bypass (if >2 h) and low output state while the heart recovers. Biliary sludge is a cause of biliary obstruction and cholestasis (Figure 22.3) which may require intervention with interventional radiology or surgery. A slow return to enteral feeding also increases the risk of biliary obstruction and accounts for the high incidence of gallstones seen in intensive care patients.

Perinatal cardiac and vascular syndromes

Arrhythmias

Fetal arrhythmias such as supraventricular tachycardia and atrial flutter lasting more than 2 weeks may cause neonatal cholestasis, but this is reversible upon resolution of the arrhythmia. Babies with arteriovenous block, however, can develop severe cholestasis associated with liver failure and death [8]. The differential diagnosis includes drugs such as amiodarone, viral hepatitis, and congenital liver disease (e.g., biliary atresia, α_1-antitrypsin deficiency; see Chapters 8, 12, 13, and 25).

Patent ductus venosus

A persistent patent ductus venosus causes a variable percentage of the cardiac output to bypass the liver and there is a correlation between the severity of the shunt (i.e., the flow volume through the patent ductus) and elevated plasma ammonia, serum bilirubin concentrations, and hepaplastin percentage [9]. In the majority of neonates, the ductus venosus closes spontaneously within weeks of birth, but important liver functions such as detoxification and the regulation of coagulation factors and bilirubin metabolism are impaired by a patent ductus and premature neonates whose physiology has more catching up to do, frequently become cholestatic and are more likely to develop long-term hepatic injury especially if they depend on intravenous nutrition in the first few weeks of life.

Box 22.1 Nutritional support for infants and children with liver disease and fat malabsorption [37].

- Fat-soluble vitamins
  - vitamin A: 5000–25,000 IU/day; Dalivit® (LPC) multivitamin drops provide 5000 IU vitamin A per 0.6 mL dose and a small dose of vitamin D (400 IU per 0.6 mL dose) is a useful starting supplement. Alternatively where vitamin A levels remain low Aquasol-A® 50,000 IU/mL is an intramuscular preparation which can also be prescribed for oral use
  - vitamin D: 100–1000 IU/kg/day or 20 ng/kg/day (alfacalcidol oral drops provide 100 ng/drop; ergocalciferol solution provides 3000 IU/mL)
  - vitamin E: 100 mg daily (α-tocopherol in vitamin E suspension provides 100 mg/mL)
  - vitamin K: 1–10 mg/day (phytomenadione in Konakion® MM Pediatric, Roche, 10 mg/mL).
- Ursodeoxycholic acid (20–50 mg/kg/day)
- Calorie supplements
  - medium-chain triglyceride: Liquigen® (Scientific Hospital Supplies)
- Specialized feed containing medium-chain triglyceride (MCT)
  - Pregestimil® (Mead Johnson) suitable for infants from birth provides 54% of fat as MCT
  - Peptisorb® (Nutricia Clinical) for children 1–10 years provides 47% of fat as MCT
  - Peptamen Junior® (Nestlé) for children 1–10 years provides 60% of fat as MCT.

Portal vein cavernoma

Portal blood flow can become compromised by sepsis, and the combination of necrotizing enterocolitis, low cardiac output state, hyperviscosity of systemic inflammatory response syndrome, and the presence of a foreign body such an umbilical vein catheter provides the ideal scenario for portal vein thrombosis. This induces a portal vein cavernoma: liver function is usually normal, but in long-term follow-up the liver may become atrophic with peri-sinusoidal fibrosis nodular regenerative hyperplasia and many patients present with gastrointestinal bleeding secondary to esophageal varices [10].

Management of cardiac disorders resulting in liver disease, including neonates

Hepatic dysfunction secondary to heart disease requires an accurate diagnosis, supportive management (see Box 22.1) and treatment of the underlying cardiac lesion which will usually lead to improvement of liver function. The management algorithm is outlined in Figure 22.4.

After complex cardiac surgery and major blood transfusion, there is an increased risk of the formation of biliary sludge and parenteral nutrition is best avoided or combined with enteral feeding to stimulate bile flow. Ursodeoxycholic

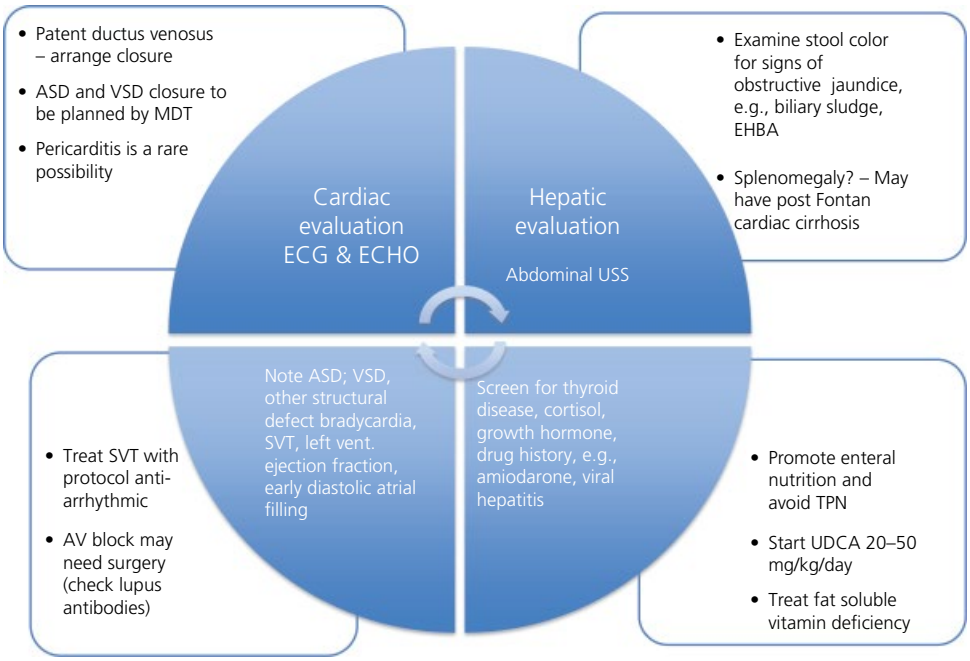


Figure 22.4 Are abnormal liver function tests present? ASD, atrial septal defect; EHBA, extrahepatic biliary atresia; VSD, ventricular septal defect; MDT, multidisciplinary team; SVT, supraventricular tachycardia; AV, atrioventricular; TPN, total parenteral nutrition; UDCA, ursodeoxycholic acid; USS ultrasound scan.

acid (20– 50 mg/kg/day) may be a useful choleretic in children with impaired biliary drainage, as it stimulates bile flow and reduces the formation of insoluble cholesterol and phospholipid aggregates. The development of the inspissated bile syndrome may be resistant to ursodeoxycholic acid, and surgical or radiological intervention with biliary lavage may be necessary. If cholestasis is prolonged, fat-soluble vitamins, calorie supplements, and medium-chain triglycerides should be prescribed (see Box 22.1). The prognosis depends on the underlying cardiac condition and is good unless a low cardiac output state persists and multiorgan failure develops.

Arteriovenous shunting in hepatopulmonary syndrome

Patients with stable chronic liver disease may present with cyanosis with saturations below 95% as a result of arteriovenous shunting in the basal segments of the lung. This intrapulmonary shunting known as hepatopulmonary syndrome (HPS) may be severe enough to warrant liver transplantation even in individuals with compensated cirrhosis [11] (see Chapters 21 and 31). The chronic hypoxia leads to increased erythropoietin production and an increased red cell mass, which persists for many months after liver transplantation. The pathophysiology of the arteriovenous shunting is considered to be a consequence of a failure of the diseased liver to clear circulating vasodilatory molecules such as tumor necrosis factor (TNF)-α and pulmonary endothelial nitric oxide production. The diagnosis is most easily made by pulse oximetry, performed in the supine position and repeated in the upright position; a supine oximetry measurement of less than 92% that decreases by more than 4% when the patient is sitting upright is indicative of HPS [12]. A perfusion scan of pulmonary and systemic circulations will also demonstrate shunting and is a useful confirmatory test. Bubble echocardiography in which blood from the patient is collected in a syringe, shaken, and immediately returned to the patient's peripheral circulation, is also a confirmatory test of A-V shunting, since blood which has not passed through the pulmonary capillary bed will retain the bubbles of CO_2 and O_2 which are detected by cardiac echocardiography. HPS is reversible after liver transplantation which means it is important to differentiate arteriovenous shunting secondary to liver disease from cardiac disease and poor respiratory function due to other causes (e.g., cystic fibrosis or pulmonary hypertension; see later). Another reversible cause of HPS is obstructed hepatic vein outflow, whether caused by stricture or by a coagulation defect, as in Budd–Chiari syndrome. Early diagnosis of Budd–Chiari syndrome – that is, before the development of ascites – is advantageous because anticoagulation and stenting the obstruction have good outcomes.

Portopulmonary hypertension

Portopulmonary hypertension (PPH) is rare and may occur in apparently well children with extrahepatic portal hypertension and normal liver function, but also occurs in cirrhosis and may be detected during evaluation for liver transplantation; dyspnea, hypoxemia, and right ventricular hypertrophy are key signs (in contrast to bilateral ventricular hypertrophy, often seen in children with fluid retention

secondary to chronic liver failure). The pathophysiology is related to failure of the liver to remove vasoactive substances, but in this syndrome the pathology is caused by vasoconstrictors, which drive up the pressure in the pulmonary arterioles and lead to cor pulmonale (see Table 22.2). Unfortunately, liver transplantation has not been successful in PPH especially when the mean pulmonary artery pressure is above 35 mmHg [11], and such patients may need to be considered for combined lung, liver, and heart transplantation.

Hepatorenal syndrome (see also Chapter 21)
Hepatorenal syndrome is an inappropriate activation of the renin–angiotensin–aldosterone system, the sympathetic nervous system, and arginine vasopressin, all contribute to sodium and water retention and the development of ascites, and occurs in fulminant and chronic liver disease [13]. The redistribution of fluid between compartments reduces renal blood flow, setting in motion several intrarenal events:

- Increased renal sympathetic activity and vasoconstricting prostaglandins.
- Acute tubular necrosis.
- Severe oliguria and inappropriately low sodium excretion.
- Functional renal failure.

Management of both types includes careful fluid balance, including the use of splanchnic vasoconstrictors and colloid volume expansion, renal dialysis, or hemofiltration in order to maintain perfusion of vital organs.

Gastrointestinal disease and liver involvement

All forms of inflammatory bowel disease (Crohn disease, ulcerative colitis, and indeterminate colitis) are associated with chronic hepatitis and/or autoimmune sclerosing cholangitis (ASC) [14, 15]. The prognosis is variable, but children who present before the onset of liver failure have a 5-year survival of over 90%. This pattern of parallel inflammatory events in the liver and bowel is also seen with celiac disease [15] in which intermittent elevations of liver amino transferases is common, although fibrosis and sclerosing cholangitis are rare (see Table 22.6). The pathogenesis of hepatic involvement is related to the close anatomic connection via the portal vein and the abnormal microbiome present in inflammatory bowel disease which triggers an intestinal immune response [16]; combined with abnormal permeability in the intestinal mucosa which allows ingress of antigens (so-called PAMPS – pathogen-associated molecular pattern) which provoke the production of proinflammatory cytokines such as TNF-α and interleukin (IL)-6.

Liver function tests especially ALT are transiently raised in up to 35% of children with inflammatory bowel disease [4] although only 10% or less had chronically raised liver enzymes [17]. In children with chronically raised liver enzymes further investigation of the liver is warranted as other important diagnoses have been reported in this group, e.g., primary sclerasing cholangitis/autoimmune hepatitis overlap (7.4%); histoplasmosis (3.7%); non-alcoholic fatty liver disease (NAFLD; 11.1%); and cholelithiasis 3.7% [17]. Table 22.3 outlines the recommended hepatic investigations and differential diagnosis for inflammatory bowel disease. With the increasing use of anti-TNF therapy to treat fistulas, it is important to be aware that acute liver failure has been described in association with anti-TNF-α antibody – infliximab appeared to trigger subfulminant hepatitis B in a patient carrying HB_s antigen. Drug-induced liver disease is an important differential diagnosis to consider in patients with inflammatory bowel disease who develop abnormal liver function tests (see Table 22.3 and Chapter 12) since many of the drugs used cause elevated transaminase and less frequently cholestasis, fibrosis, and occasionally granulomatous hepatitis [15]. Azathioprine is metabolized by the enzyme thiopurine methyltransferase (TPMT) to 6-mercaptopurine which is a dose-dependent hepatotoxin. TPMT testing can predict life-threatening myelotoxicity the 1 in 300 who are deficient and permits individualization of dosing, management of non-compliance, and thiopurine-resistant/thiopurine-refractory disease.

Management
Inflammatory bowel disease is a chronic condition with considerable morbidity, which is potentially fatal and should be supervised in a regional center by a multidisciplinary team. Remission of both bowel and liver disease can be induced with prednisolone and maintained with azathioprine. Immunosuppression is reduced slowly over months and years while liver function tests and the full blood count are monitored carefully. The course of the liver disease is variable, as the disease may have many relapses and remissions, and it is not necessarily associated with the severity of the bowel disease. Second-line drugs such as calcineurin inhibiors are reserved for use in protracted cases resistant to steroids and may constitute an indication for liver transplantation (see Chapters 11 and 31). Liver transplantation may be necessary in a small number of children with aggressive disease or who present late with subacute liver failure. Inflammatory bowel disease may deteriorate or present for the first time after liver transplantation [15, 17].

Shwachman–Diamond syndrome
Hepatomegaly and moderately elevated hepatic transaminases (80–300 IU/L) are present in over 50% of patients at the time of presentation, although there was some resolution with time. Histology is non-specific, with macrovesicular fatty change. The diagnosis is suspected when a cyclical neuropenia and pancreatic insufficiency occur and can be confirmed in 90% of patients who carry bi-allelic mutations in the *SBDS* gene. Shwachman–Diamond syndrome is now considered to be one of a heterogeneous class of diseases known as ribosomopathies which also include: Diamond–Blackfan anemia; 5q syndrome; dyskeratosis congenita;

Table 22.3 Differential diagnosis and key management points of liver disease and inflammatory bowel disease.

Symptoms/investigations	Diagnosis	Management
Raised amino transferases Cholestasis (raised bilirubin, ALP, GGT, jaundice) Endothelial cell injury (peliosis, peri-sinusoidal fibrosis, ascites in advanced cases) – full work-up including endoscopy Liver biopsy may be needed	Drug-induced liver disease See Chapter 12 e.g.: • infliximab • 5-aminosalicylates • thiopurines • methotrexate • azathioprine	Transaminase usually settles with dose reduction or withdrawal of drug (NB: routine screening for HBsAg before starting infliximab). TPMT testing is useful when starting azathioprine Cholestasis can progress rapidly to liver failure – prompt drug withdrawal required Withdraw drug promptly in endothelial injury (thiopurines) Post-chemotherapy fibrosis may require management of ensuing portal hypertension
Serology (hepatitis A, B, C, and E, EBV, CMV, adenovirus, human herpes virus 6, parvovirus, coxsackievirus)	Viral hepatitis See Chapter 13	Consider reduction in steroids and other immune suppressants and refer to regional center. Hepatitis B virus is treated with pegylated interferon but nucleoside/tide analogues (e.g., entecavir, telbivudine, tenofiovir) are being evaluated in children. Current standard treatment for hepatitis C virus is dual therapy pegylated interferon and ribavirin. Newer direct-acting antiviral therapies are being evaluated in trials
Stool color, hepatic ultrasound; small bowel contrast study or MRE to assess terminal ileum, SeHCHAT study	Abnormal enteropathic circulation of bile salts	Trial of ursodeoxycholic acid 10–20 mg/kg/dose Bile salt binding agents (if bile salt colitis present)
Autoantibodies (SMA, ANA, ANCA) IgA-tTG (celiac disease) Immunoglobulins Complement levels Fasting blood glucose Endoscopic retrograde cholangiogram MRI cholangiogram Liver biopsy	Autoimmune disease, e.g., autoimmune hepatitis Celiac disease Hypergammaglobulinemia IDDM (see also Table 22.6) See Chapter 11	Should be managed in a regional center; first-line treatment is with prednisolone and azathioprine For celiac disease - recheck liver enzymes 6–12 months after a strict gluten-free diet, if enzymes remain abnormal further investigations for hepatic fibrosis primary sclerosing cholangitis are warranted

ANA, antinuclear autoantibodies; ANCA, antinuclear cytoplasmic antibodies; IgA-tTG, anti-tissue transglutaminase; CMV, cytomegalovirus; EBV, Epstein–Barr virus; GGT, γ-glutamyl transpeptidase; IDDM, insulin-dependent diabetes mellitus; MRE, magnetic resonance enterography; MRI, magnetic resonance imaging; SMA, smooth muscle autoantibodies; TPMT, thiopurine methyltransferase.

cartilage–hair hypoplasia; and Treacher Collins syndrome [18]. The SBDS protein is involved in several cellular pathways including: mitotic spindle stabilization, DNA metabolism and reactive oxygen species regulation, and chemotaxis, in addition to ribosomal biogenesis. Treatment is symptomatic, with hematopoietic stem cell transplantation in cases of progression to cancer or severe bone marrow failure.

Disorders of the immune system and hematopoiesis: primary immunodeficiency, bone marrow transplant, acquired immunodeficiency (including human immunodeficiency virus)

Primary immunodeficiency

Inherited immunodeficiency syndromes associated with liver disease include severe combined immune deficiency (SCID), common variable immunodeficiency (CVID), hyper-IgM syndromes, CD40 ligand deficiency, and chronic granulomatous disease. New genetic technologies, such as whole-genome and whole-exome sequencing, have uncovered an ever-increasing and diverse range of immunodeficiencies (Table 22.4). In 2012, 19 new genetic abnormalities were identified as responsible for causing a variety of disorders: defects in innate immunity; antibody defects; combined immunodeficiencies; abnormalities of immune regulation; and autoinflammatory disorders [19]. For example defects in the pro-stimulatory cytokine IL-12 and interferon (IFN)-γ results in a clinical phenotype resembling autoimmune hepatitis sufficiently closely that the patient received immunosuppression for over 10 years and then was found to have intra-abdominal mycobacterial granulomas. Gene sequencing studies confirmed IL-12RB1 deficiency in this patient [19]. IL-21 receptor-α (IL-21R) deficiency is a congenital immunodeficiency state which mimics primary biliary cirrhosis – this and CD40 ligand deficiency can be successfully treated with allogenic stem cell transplantation or bone marrow transplant, respectively. The gene mutations underlying IL-21R deficiency and CD40 ligand deficiency and Bruton's hypogammaglobulinemia have now been identified (see Table 22.4).

In addition to presentations with interstitial lung disease, chronic diarrhea, and failure to thrive (Figure 22.5), abnormal liver functions tests are common in immunodeficiency because recurrent bacterial, viral, or opportunistic infections which ascend the biliary tree from the intestinal tract cause sclerosing cholangitis. The differential diagnosis of sclerosing cholangitis includes primary immunodeficiency as well as human immunodeficiency virus (HIV), autoimmune pancreatitis, portal biliopathy, eosinophilic cholangitis, intra-arterial chemotherapy, and intraductal stone disease. CD40 ligand deficiency is an X-linked inherited immunodeficiency in which atypical organisms such as cryptosporidia, eventually

Table 22.4 Examples of immunodeficiencies, immune defects, and clinical associations. (From Chinen *et al*. 2014 [19]. Reproduced with permission of Elsevier.)

Immunodeficiency	Immune defects	Clinical associations
Severe combined immunodeficiency (SCID)	Around 20 gene mutations identified so far – some X linked and some autosomal recessive, all affect proliferation and maturation of B and T cells	Enteropathy; hemolytic anemia; glomerulonephritis; cytomegalovirus and adenovirus hepatitis
Common variable immunodeficiency (CVID)	Hypogammaglobulinemia; deficits in cell-mediated immunity	Immune thrombocytopenic purpura; hemolytic anemia; rheumatoid arthritis; lupus; primary biliary cirrhosis; autoimmune hepatitis in rare cases leading to cirrhosis, ascites, and rarely hepatocellular carcinoma
Bruton hypogammaglobulinemia	Hypogammaglobulinemia, X-linked mutations in XIAP (G466X) cosegregates polymorphism in CD40LG (CD40 ligand (G219R).	Epstein–Barr virus-driven lymphoproliferation, splenomegaly, colitis, and liver disease
CD40 ligand deficiency	X linked (G219R); hyper-IgM syndrome; also mutations in *NFκ* gene	Chronic hepatitis, cirrhosis, and opportunistic infections of the biliary tree
Interleukin (IL)-12	IL-12RB1 deficiency	Autoimmune hepatitis; mycobacterial granulomas
IL-21	Mutation in *IL21* gene	Very early onset inflammatory bowel disease and recurrent respiratory tract infections
IL-21 receptor (IL-21R) deficiencies	Reduced numbers of B cells impaired B-cell proliferation and immunoglobulin class switch; reduced T-cell effector functions; reduced natural killer cell functions	IL-21R deficiency associated with cryptosporidial infections associated with chronic cholangitis and liver disease
Abnormal signaling through IL-21R/γc/JAK3/STAT3	Failure to respond to vaccination	Susceptible to encapsulated bacterial infections
Veno-occlusive disease with immunodeficiency (VODI)	Combined B- and T-cell dysfunction Autosomal recessive mutations in *SP110*, a gene encoding a PML nuclear body-associated protein Can be corrected by bone marrow transplantation	Fever, hepatomegaly, and pancytopenia Hepatic injury may progress to liver failure
Chronic granulomatous disease	Neutrophil dysfunction X-lnked and autosomal recessive forms – mutations in the gene coding for the NADPH oxidase 2 complex	Hepatic abscesses with fungal and/or encapsulated organisms especially staphylococci Abscesses and fistulae of the gastrointestinal tract which mimic Crohn disease

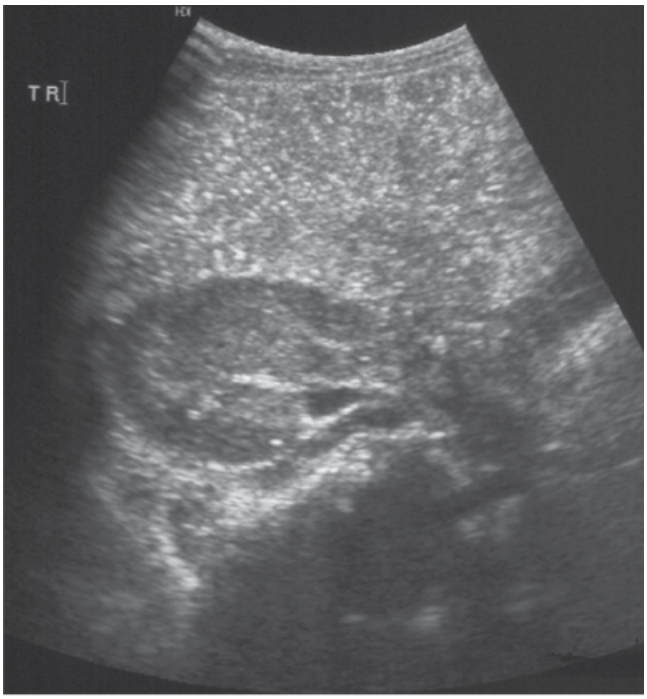
causes a sclerosing cholangiopathy which leads to liver failure (Figure 22.6). The incidence of liver disease increases with age: by the age of 20 years, 75% of survivors with CD40 ligand deficiency have liver disease, which recurs following liver transplantation. A better outcome for bone marrow transplant was associated with younger age at transplant, normal liver histology, and absence of lung damage.

Immunodeficiency is a risk factor for sinusoidal obstruction syndrome (SOS; see later) in children after hematopoietic stem cell transplantation [20].

Hemophagocytic lymphohistiocytosis (HLH), is primarily a hematological disease but it has a profound effect on liver function and is may present as an immunodeficiency phenotype. In fact a loss of control of macrophage function, including hepatic Kupffer cells, results in the liver becoming infiltrated with excessively activated macrophages which consume erythrocytes, and secrete large quantities of inflammatory cytokines. The disorder may be sporadic, associated with viruses, for example, parvovirus 19, echovirus, or Epstein–Barr virus (EBV). There is a familial form (FLH) with specific genetic mutation defects, which are usually inherited in an autosomal recessive fashion, although some show X-linked inheritance. Patients develop hepatosplenomegaly and jaundice, and often follow a rapidly progressive course to fulminant liver failure which can be difficult to distinguish from septicemia. The diagnosis is made by demonstrating erythrophagocytosis in the liver or bone marrow. Management consists of support for acute liver failure (see Chapter 18) and cytotoxic therapy, but liver transplantation is not indicated because of the risk of recurrence.

Management primary immunodeficiency and liver complications

SCID is most commonly treated with bone marrow transplant in infancy if possible, or gene therapy or enzyme replacement therapy in suitable cases, e.g., adenosine deaminase deficiency, enzyme replacement, although the latter are still being trialled [19]. Treatment of liver disease is focussed on preventing and treating infections such from *Cryptosporidium* and cytomegalovirus (CMV) using anti-infective agents and screening blood products. Patients who develop colitis as a result of chronic granulomatous disease may benefit from TNF-α inhibitors (e.g., infliximab) but this approach needs caution as there is a risk of virus reactivation. Patients who develop hepatic abscesses may require surgical drainage and prolonged intravenous antibiotics.



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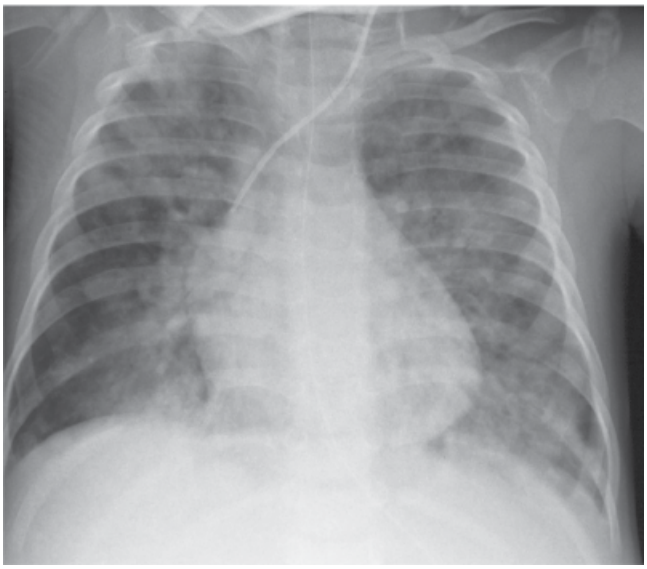
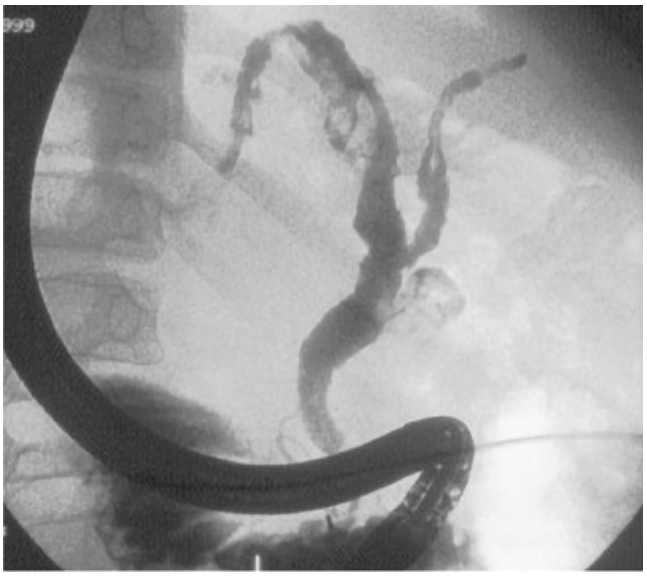


(B)

Figure 22.5 This infant with severe combined immunodeficiency (SCID) presented with acute liver failure due to fatal *Pneumocystis carinii* infection of the liver with numerous abscesses (A) and interstitial lung disease (B).



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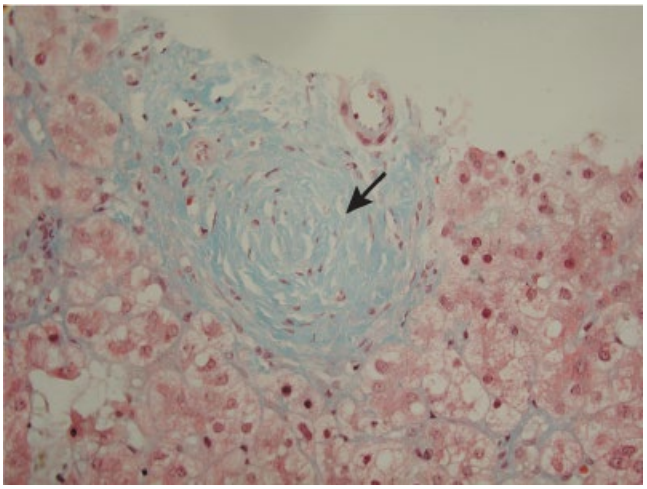


(B)

Figure 22.6 CD40 ligand deficiency is a rare X-linked immunodeficiency syndrome that is treatable by bone marrow transplantation. (A) Sclerosing cholangitis secondary to *Cryptosporidium* infection in the liver may develop and is shown in this endoscopic retrograde cholangiopancreatogram, which demonstrates the characteristic dilation and beading of bile ducts. (B) This boy developed liver failure after bone marrow transplantation, due to graft-versus-host disease and cryptosporidial cholangitis with loss of bile ducts. (Masson trichrome, original magnification × 400.)

Bone marrow transplantation

There are two major syndromes which affect the liver after bone marrow transplantation: graft-versus-host disease (GVHD) and sinusoidal obstruction syndrome (previously known as veno-occlusive disease). GVHD represents a failure to establish tolerance between the recipient tissues and the newly engrafted marrow although GVHD may also be seen in pediatric patients after small bowel and liver transplantation where the pathophysiology is somewhat different in that there appears to be an excessive suppression of the recipient's own lymphocytes, while at the same time the lymphocytes which are transplanted with the bowel retain their cytotoxicity despite systemic immunosuppression and attack host tissue especially skin and lungs [21] (and liver, if bowel only is transplanted) (see Chapter 32).

Graft-versus-host disease

GVHD after bone marrow transplantation is a complex systemic disorder involving the skin, gut, lung, eye, pancreas, and liver, typically occurring 7–50 days after bone marrow

transplantation (and in up to 10% of cases of liver or small bowel transplantation). The acute form presents with a desquamating skin rash and diarrhea, and the liver is involved in 40% of cases, manifested by mild jaundice and hepatomegaly [22]. The immune damage in the liver is directed towards the small bile ducts. The biliary epithelium becomes irregular, with nuclear pleomorphism and vacuolated cytoplasm. There may also be endothelitis and mild portal tract inflammation, with bile duct loss and cholestasis; the parenchyma is relatively spared (Figure 22.7). Chronic GVHD is defined as continuing poorly controlled acute GVHD after 100 days. Eighty percent of patients are cholestatic and have complete loss of all bile ducts. Histology of the liver shows bile duct loss, bridging fibrosis, and occasionally cirrhosis (Table 22.5).

The differential diagnosis of both acute and chronic GVHD includes:

- Viral hepatitis – CMV, EBV, hepatitis A, B, C, and E, other.
- Drug toxicity (including parenteral nutrition).
- Biliary obstruction from biliary sludge (occurs in 20% of bone marrow transplantation recipients).
- Hepatobiliary infection.
- SOS also known as veno-occlusive disease.

The diagnosis of GVHD can be made by biopsy of symptomatic tissue (e.g., intestinal mucosa, skin if rash present) and noting a high percentage of donor T-cell chimerism in peripheral blood, although the latter may be absent. Serum complement, autoantibodies forming immune complexes, have also been detected in GVHD, and the success of the B-cell inhibitor rituximab in some patients with chronic GVHD suggests an important role of B cells in the disease [23].

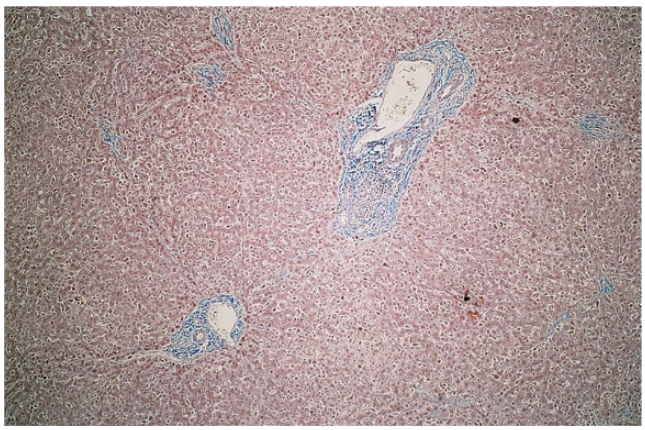


Figure 22.7 Graft-versus-host disease is most common after bone marrow transplantation. It may affect the skin, intestine, and liver. Liver histology demonstrates loss of bile ducts, which may be irreversible.

Systemic treatment of GVHD is usually indicated if ≥3 organs are affected or if involvement in any single organ is severe and debilitating. Systemic treatment is also considered for patients with mild overall GVHD severity if they present characteristics associated with poor prognosis (i.e., platelets below 100,000/mm^3, progressive onset). Steroids and/or tacrolimus are commonly used in bone marrow transplantation GVHD, but in GVHD after small bowel transplantation reduction of immune suppression in mild cases of GVHD is recommended, with early recourse to extracorporeal plasma photophoresis if there is no improvement [21].

Sinusoidal obstruction syndrome

SOS is a serious complication of bone marrow transplantation that occurs in 20–30% of patients and presents within 30 days of bone marrow transplantation. The clinical features mimic the Budd–Chiari syndrome and are shown in Table 22.5. Patients with a history of pre-transplant viral hepatitis, radiotherapy, or busulfan conditioning are more likely to develop SOS. The use of ciprofloxacin and vancomycin prophylaxis, 6-thioguanine intensification regimens, methotrexate treatment for GVHD, are associated with injury to the hepatic endothelial which increases the risk of SOS [15, 20]. In patients considered to be at high risk of SOS, prophylactic ursodeoxycholic acid and vitamin E have been reported to be helpful.

The diagnosis is based on clinical criteria:

- Exclusion of other causes of hepatic dysfunction post-bone marrow transplantation (opportunistic infection, drug toxicity).
- Abdominal ultrasound of the portal vein flow to identify retrograde flow and or an increase in hepatic resistance.
- Liver biopsy, if coagulation permits. Liver histology demonstrates narrowing or occlusion of the terminal hepatic venules, sinusoidal congestion, and necrosis of hepatocytes, with a mild inflammatory infiltrate in the centrilobular zone.
- Fibrosis and cirrhosis may develop.

The management is supportive: diuretics for ascites, ursodeoxycholic acid, fat-soluble vitamins and calorie supplements if jaundice is prolonged, and thrombolysis. Historically,

Table 22.5 Clinical features of graft-versus-host disease (GVHD) and sinusoidal obstruction syndrome (SOS).

	Acute GVHD	Chronic GVHD	SOS
Onset	7–50 days	>100 days	1–30 days
Clinical	Skin rash	Skin rash	Tender hepatomegaly
	Diarrhea	Pale stools	Ascites, elevated jugular venous pressure
Biochemistry	Bilirubin >50 µmol/L	Bilirubin >200 µmol/L	Bilirubin >35 µmol/L
		Raised alkaline phosphatase	Raised alkaline phosphatase
Histology	Vanishing bile ducts	Bile ducts absent	Narrow or occluded vessels

SOS-induced liver failure had a mortality of 50%, but this is improved with the use of thrombolytic defibrotide, combined with antithrombin III. However, SOS remains a difficult problem: the Cochrane database review in 2015 concluded that further high-quality randomized controlled trials are needed before this becomes standard treatment as the evidence for benefit was poor and the optimal regimen not established. Defibrotide has also been used to treat SOS after liver transplantation. Of the other treatments evaluated in the Cochrane review (heparin, glutamine, antithrombin III, PGE1), only ursodeoxycholic acid 12 mg/kg/day showed an all-cause reduction in mortality and mortality caused by SOS.

Budd–Chiari syndrome

Budd–Chiari syndrome is a rare disorder caused by hepatic venous outflow obstruction or obstruction in the hepatic portion of the inferior vena cava and hepatic vein which lead to hepatic dysfunction. It may be due to a congenital web or gradual obstruction due to an underlying coagulation disorder or myeloproliferative disorder.

Clinical features include the development of hepatomegaly, ascites, and acute liver failure. The diagnosis is suggested by detecting the blocked venous outflow on ultrasound, angiography, or venography.

It is important to perform a thrombotic screen including measuring levels of protein C or S, factor V Leiden, factor II mutation, antiphospholipid syndrome, and excluding myeloproliferative disorder.

Treatment includes anticoagulation, vascular stents, transjugular intra-hepatic portosystemic stent–shunt, and liver transplantation if interventional radiology is unsuccessful.

Acquired immunodeficiency/human immunodeficiency virus

HIV-induced liver disease is characterized by a wide range of pathologies including hepatitis, steatohepatitis, endothelialitis, necrosis and granulomatosis, and occasionally non-cirrhotic portal hypertension as a result of portal obliteration and nodular hyperplasia [24]. Aplastic anemia may develop weeks or months after an episode of hepatitis caused by HIV and other hepatotrophic viruses (hepatitis A–E and G, EBV, CMV, parvovirus B19, and echovirus have all been implicated).

In addition, HIV disables T-helper cells and makes patients susceptible to infections which would normally be controlled by this clone of immune cells. Mothers who are HIV positive have a 1 in 4 risk of infecting their babies unless they are given antiretroviral therapy during pregnancy and avoid breastfeeding which reduces vertical transmission to <5%. Of the reported 26% of babies who acquire HIV from their mothers, approximately 90% of the infants have hepatomegaly and abnormal liver function tests, usually as a consequence of opportunistic infection [25]. These babies are also at risk of hepatitis B and C. Other potential causes of hepatic disease include *Mycobacterium avium-intracellulare*, CMV, lymphomas, and Kaposi sarcoma, all of which may develop in children with HIV.

As with primary immunodeficiency, recurrent or chronic infection of the biliary tree with *Cryptosporidium* or other organisms may produce a clinical picture resembling sclerosing cholangitis. Myocarditis and congestive cardiac failure can occur in HIV-infected patients and produce secondary changes in the liver (abnormal liver function tests and fibrosis of the central vein). Clinically fungal infections are important in HIV and can present insidiously evolving over many days and weeks, often in the context of malnutrition, broad-spectrum antibiotics, and a high swinging fever (temperature typically 39–40 °C). Visceral candidiasis has been reported in which *Candida* species invaded the liver, biliary tract, and portal vein, producing cholestasis and gross hepatosplenomegaly. The positive identification of fungi is very difficult, except in overwhelming infections, and treatment with amphotericin (1–3 mg/kg/day) and/or flucytosine (100–200 mg/kg/day) is therefore often empirical, based on risk factors and clinical suspicion. In systemic candidiasis, prolonged treatment may be required.

Management

The long-term medical outlook for children with HIV has improved considerably with triple treatment with nucleoside analogues, non-nucleoside analogues, and protease inhibitors [25]. However, coinfection with hepatitis C or B is still a key issue and there are several studies evaluating combination therapies [24] with agents such as the nucleoside reverse transcriptase inhibitor tenofovir, which is converted to the pharmacologically active metabolite after only two phosphorylation steps and is capable of producing synergistic effects against HIV. Tenofovir is less hepatotoxic than non-nucleoside analogue reverse transcriptase inhibitors and is reported to suppress hepatitis B viral replication in patients coinfected with HIV and also to improve synthetic parameters of liver function such as prothrombin time and albumin. Vaccination against hepatitis A and B is recommended although a satisfactory immune response may be difficult to achieve.

Multisystem disorders including granulomatous disease, hepatotoxic disorders including obesity, malnutrition, and sepsis

Multisystem disorders

There are a number of multisystem disorders shown in Table 22.6, which affect the liver.

The pattern of abnormalities in liver function tests can provide useful pointers to etiology in clinical scenarios where multiple co-morbidities may be present for example:

- A septic patient with sickle cell disease may have biliary obstruction and cholestatic liver functions tests secondary to gallstones, or abnormal parameters of synthetic function caused by necrotic liver as a result of repeated sickling and infarction of parts of the liver.

Table 22.6 Multisystem disorders which may present with neonatal hepatitis and/or late-onset liver disease.

Condition	Clinical features	Hepatic features
Celiac disease	Gluten intolerance Enteropathy associated with weight loss, poor appetite, diarrhea, and/or constipation	Moderately elevated amino transferases (80–300 IU/L) Acute hepatitis, fatty liver, rarely liver fibrosis, cirrhosis and case reports of primary sclerosing cholangitis in adults [15]
Shwachman–Diamond syndrome	Exocrine pancreatic dysfunction Growth failure (malabsorption) cyclical neutropenia; metaphyseal dyschondroplasia of the femur, humerus Autosomal recessive mutations in the *SBDS* gene which affects ribosomal biogenesis [18]	May present as "neonatal hepatitis" Moderately elevated amino transferases (80–300 IU/L) Hepatomegaly
Systemic juvenile idipathic arthritis	Fever, arthritis, skin rash Fibrosing alveolitis; pericarditis; autoimmune gut disease Mutations in the cytolytic pathway genes are association with macrophage activation [26]	Jaundice; pruritus Acute liver failure (rare) – may be part of macrophage activation syndrome with pancytopenia and coagulopathy Moderately elevated amino transferases (50–500 IU/L) Raised immunoglobulin levels Raised complement levels
Turner syndrome	XO genotype; short stature	Most patients (90%) develop transiently abnormal amino transferases during follow-up; some obese patients develop non-alcoholic fatty liver disease [27]
Alström syndrome	Congenital retinal dystrophy; blindness; hearing impairment; hypertriglyceridemia; truncal obesity; insulin resistance and type II diabetes mellitus; dilated cardiomyopathy Autosomal recessive: 70% of cases have mutations in the *ALMS1* gene which is involved in intracellular trafficking, regulation of cilia signaling pathways, and cellular differentiation [28]	Amino transferases may be normal or moderately increased (ALT: normal to 150 IU/L) Hepatosplenomegaly and steatohepatitis may occur. Occasionally cirrhosis and portal hypertension can lead to hepatic encephalopathy and life-threatening esophageal varices
Type I insulin-dependent diabetes mellitus	Poorly controlled type I diabetes Adolescents with type I insulin-dependent diabetes mellitus more likely to experience ketoacidosis and high exogenous insulin administration making glycogen deposition more likely [29]	Moderately elevated amino transferases (80–150 IU/L) Hepatomegaly (excess glycogen and fat stores) Abdominal pain secondary to: • gallstones, or • stretching of the liver capsule caused by swings in insulin and glucose levels and increased glycogen and fat stores Steatosis may lead on to cirrhosis
Lipodystrophy	Congenital or acquired autoimmune disease associated with destruction and loss of fat cells, severe insulin resistance and hypertriglyceridemia, low high-density lipoprotein cholesterol, low leptin, adiponectin, ectopic fat accumulation	Non-alcoholic fatty liver disease/steatohepatitis Portal fibrosis and zone 1 steatosis Autoimmune hepatitis is reported with acquired lipodystrophy Steatosis may lead on to cirrhosis [30]
Hypopituitarism	Partial/total failure to produce adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone, growth hormone, and gonadotropins Micropenis; septo-optic dysplasia Mutations have been found in *HESX1* and *FGF8* which encode a transcription factors for the development of the pituitary and hypothalamus [31]	Moderately elevated amino transferases (80–300 IU/L) Conjugated hyperbilirubinemia (30–150 µmol/L) Hypoglycemia Neonatal hepatitis and failure to thrive followed by hyperphagia in childhood

- A teenager with type I diabetes mellitus with elevated aminotransferases with abdominal pain who may have developed huge glycogen deposits as a result of recurrent ketoacidosis and high insulin requirements.
- A patient with celiac disease may develop an autoimmune hepatitis with modestly elevated amino transferase enzymes (AST and ALT).

All these disorders start with minor changes in liver function tests often associated with some degree of growth failure, but all have the capacity to progress to advanced liver disease with fibrosis and, in systemic juvenile idiopathic arthritis a macrophage activation syndrome (MAS) resembling acute liver failure has been reported.

In addition to the general supportive management of these multisystemic conditions detailed later, there are some specific measures as follows:

- Celiac disease hepatitis usually resolves on a gluten-free diet and the rare patient who develops a sclerosing cholangitis may benefit from ursodeoxycholic acid [15].
- Shwachman–Diamond syndrome may be treated with pancreatic enzyme replacement and nutritional supplements

including fat-soluble vitamins, and hematopoietic stem cell transplantation is indicated if bone marrow failure or malignancy develop [18].

- Systemic juvenile idiopathic arthritis is managed with a range of immune modifying agents: corticosteroids, ciclosporin, anticytokine agents, etoposide, and admission to the intensive care unit may be required for established cases of MAS where the mortality rate is around 8% [26]. Acute liver failure in the absence of MAS has also been described and treated with plasmaphoresis, pulsed methyl prednisolone, and ciclosporin.
- Turner syndrome is associated with high risk of short stature, cardiovascular diseases, ovarian failure, hearing loss, and hypothyroidism. Liver dysfunction is mild and usually a result of growth hormone and estrogen; no specific treatment for the raised amino transferases is needed unless it is related to obesity and fatty liver [27], in which case lifestyle changes to diet and activity levels are recommended.
- Alström syndrome has a wide clinical variability. Treatment is based on: (1) assessing the range and severity of organ/tissue involvement; (2) prevention of complications, e.g., fatty liver and progression to type II diabetes mellitus can be modified by avoidance of severe obesity with healthy diet and exercise; and (3) surveillance and treatment of portal hypertension by regular testing of liver function and abdominal ultrasound [28].
- When abnormal liver function occurs in type I insulin-dependent diabetes mellitus it is usually a result of excess glycogen synthesis and reflects poor diabetic control – the drivers for glycogen synthesis are excess insulin and ketoacidosis [29]. This complication can be treated with continuous subcutaneous insulin often resulting in complete remission, but if episodes of ketoacidosis continue then pancreatic transplantation is an option.
- Lipodystrophy is characterized by loss of adipose tissue, low leptin levels and markers of metabolic syndrome such as insulin resistance, and hypertriglyceridemia. Fatty liver is inevitable and may progress to cirrhosis and portal hypertension; autoimmune liver disease has been reported as well (in which steroid responsive hepatitis and steatosis coexist). Metreleptin therapy has been used successfully in adults and children with liver disease and fibrosis and leptin replacement therapy should also be considered in cases of worsening liver disease [30].
- Hypopituitarism (septo-optic dysplasia) is managed by ensuring a regular supply of calories in infancy via nasogastric tube if necessary. After the age of 2 years, linear growth is more dependent on growth hormone, which is therefore given by subcutaneous injection. Treatment with hydrocortisone and thyroxine is associated with improvement in liver function and growth. Mutations in several genes involved in the development of the pituitary have been identified [31]. The prognosis for septo-optic dysplasia depends on the severity of the initial lesion and is not related to hormone replacement.

Chronic granulomatous disorders

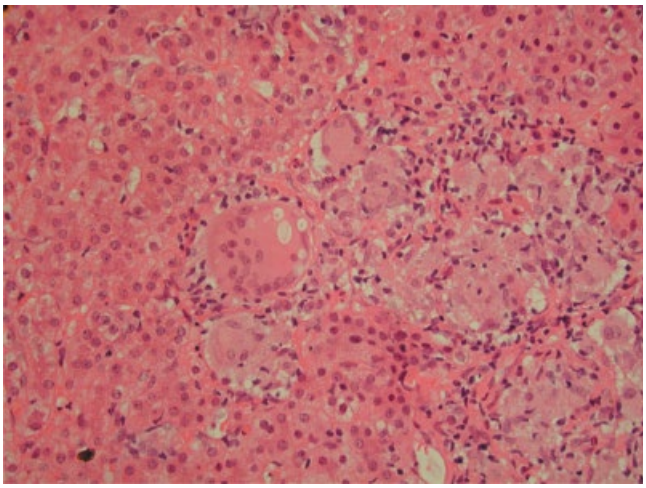
Granulomas are collections of specialized immune cells such as Kupffer cells, macrophages, or neutrophils, which – in response to infection, toxic injury (drugs and alcohol), or abnormal regulation of the immune system – fuse to form large epithelioid cells with multiple nuclei. Granulomas may appear anywhere in the body, although particular diseases have characteristic patterns (Table 22.7).

Numerically the most important causes of granuloma in the liver are tuberculosis, sarcoid, schistosomiasis, drug-induced liver disease, and intrinsic liver disease (Figure 22.8). Hepatic granulomas are often clinically silent, but established disease may present as a chronic intra-hepatic cholestatic syndrome, portal hypertension, and Budd–Chiari syndrome. Even in patients with no respiratory symptoms, exclusion of tuberculosis is extremely important.

Sarcoidosis is a systemic disease characterized by non-caseating granulomas and is the most frequent etiology of

Table 22.7 Granulomatous conditions affecting the liver: common sites and characteristics.

	Primary site	Associated sites	Special features
Tuberculosis	Lung, lymph node	Intestine, liver	Caseating granulomas secondary to infection with *Mycobacterium tuberculosis*
Sarcoidosis	Lung, ocular, joints	Liver, spleen, bone, heart	Extrapulmonary disease more common in pediatric age; 40% have elevated angiotensin-converting enzyme, often long delay between onset of malaise, weight loss, stiffness and muscle pain, and the diagnosis; etiology unknown
Schistosomiasis	Veins of the colon	Portal veins infested by *Schistosoma mansoni*	Ova from adult worms evoke delayed-type hypersensitivity portal hypertension, so-called "pipe stem" fibrosis
Crohn disease	Intestinal tract	Liver, skin, oral mucosa	May be part of overlap syndrome including chronic active hepatitis, juvenile chronic arthritis
Wegener granulomatosis	Naso-oral cavity	Liver, skin, intestine, heart, kidney	Fibrinoid necrosis of medium-sized arteries, especially of midline structures, resulting in ulcerating granulomas
Chronic granulomatosis disease	Lung, neutrophil dysfunction	Intestine (diarrhea), skin, nodes, liver	Rare X-linked disorder of neutrophil hydrogen peroxide dismutase Foamy macrophages Liver abscesses *Aspergillus* pneumonia



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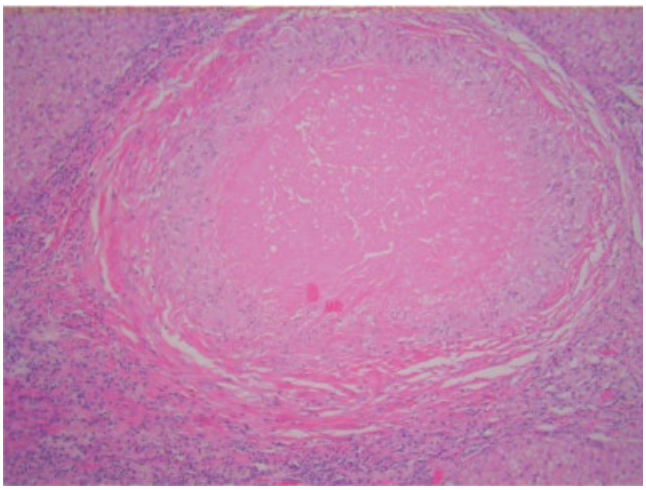


(B)

Figure 22.8 Granulomas in the liver may be due to many different causes. The lesions range from small aggregates of macrophage-like cells, as in this child with sarcoid (A), to caseating granulomas as in this case of tuberculosis (B).

hepatic granuloma. The liver is the third most affected organ in sarcoidosis. From a clinical standpoint, hepatic sarcoidosis may lead to cholestasis and portal hypertension and its associated complications.

Langerhans histiocytosis (LCH) is a rare disorder of histiocytes: it may occur as a primary problem or secondary to acute leukemia rhabdomyosarcoma, neuroblastoma, Hodgkin disease, and non-Hodgkin lymphoma (NHL). In LCH, the Langerhans cells are abnormal and may be found in different parts of the body, including the bone marrow, skin, lungs, liver, lymph glands, spleen, and pituitary gland.

Presenting symptoms depend on the organ affected and include skin rash, fever, anemia, diabetes insipidus, and lymphadenopathy. Hepatic symptoms include jaundice, hepatomegaly, and hypertriglyceridemia. Biliary obstruction and biliary cirrhosis are also described. Liver biopsy may show large bile duct obstruction, the presence of eosinophilic granulomas, but multiple nodules in several organ systems containing histiocytes distinguishes it from the granulomatous conditions listed in Table 22.7. The prognosis is highly variable and is worse with multisystem disease including liver involvement – patients with single system or skeletal involvement tend to have the best outcomes [32]. Multisystem disease is treated with vinblastine, prednisolone, and 6-mercaptopurine for 6–12 months depending on the extent of the disease. Liver transplantation is indicated for end-stage liver failure without active multisystem disease.

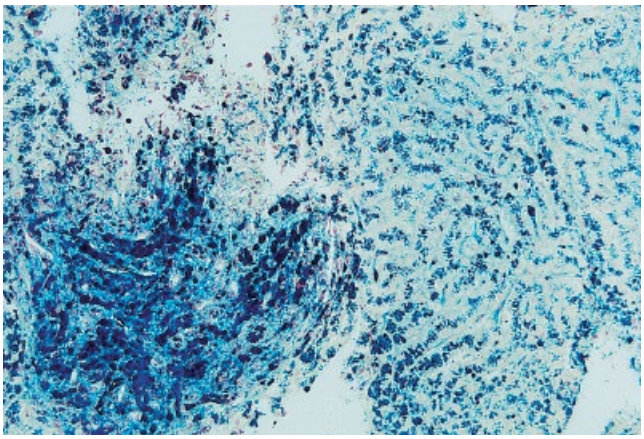
Toxic accumulation disorders including iron overload (e.g., thalassemia), copper overload (Wilson disease), manganese overload, vitamin A toxicity, and obesity

(see also Chapter 15)

Iron overload (hemosiderosis) occurs where excess iron enters the body usually as a result of blood transfusions, sometimes compounded by oral intake of iron. Mild iron overload can be seen after as little as two transfusions (iron-laden macrophages), and is increasingly recognized in survivors of childhood cancer [33], but does not usually become clinically important until more than 10–20 transfusions have occurred. MRI is now preferred to liver biopsy as a screening test for iron overload as it is not prone to sampling error and modern techniques (Ferriscan®) also quantify the amount of iron. The impact of excess iron can be greatly reduced by regular venesection, or treatment with an iron-chelating agent, such as desferrioxamine or deferasirox, are used in children with thalassemia before they undergo bone marrow transplantation. Despite iron chelation treatment, hemosiderosis may progress to fibrosis and cirrhosis. Provided bone marrow transplantation is carried out before fibrotic liver disease is established, the iron overload can be reversed after bone marrow transplantation as shown in Figure 22.9.

Copper overload is a recognized cause of liver disease which occurs when there is a genetic susceptibility caused by abnormalities in the gene coding for the natural copper carrying protein ceruloplasmin (very low levels are found in Wilson disease; see Chapter 20). The liver may take some years to become damaged – the histological changes are initially mild with steatosis progressing to micronodular cirrhosis and a sudden descent into acute liver failure typically after the age of 8 years characterized by low alkaline phosphatase levels and relatively high AST : ALT ratio. The liver disease and associated neurological disease (copper accumulates in the basal ganglia) can be prevented by oral zinc which prevents the absorption of copper from the diet by competitive inhibition of intestinal transporters.

Manganese is excreted almost exclusively by the biliary tree which is why it may accumulate in cholestatic liver disease especially in the setting of intestinal failure associated liver disease. It does not lead to liver disease per se but high



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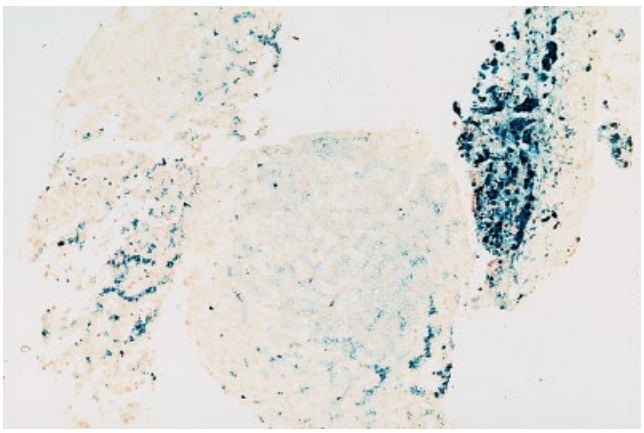

(B)

Figure 22.9 Thalassemia. There is marked hemosiderosis involving both hepatocytes and Kupffer cells (A), which has almost disappeared 3 years after bone marrow transplantation (B).

levels of plasma manganese are deposited in the basal ganglia and can cause an ataxic syndrome similar to that of copper accumulation.

Hypervitaminosis A is rare but a recognized cause of elevated aminotransferases and in time can lead to parenchymal liver disease: it has been reported even when oral intakes are only just above the daily recommended intakes. Chronic renal failure and artificial nutritional support may be a risk factors for accumulation of vitamin A in the liver [34]. Vitamin A is stored in hepatic stellate cells, activation of which is key to the development of hepatic fibrosis and cirrhosis. The abnormalities can be reversed by stopping vitamin A supplements.

Although obesity is a result of environment, genetic susceptibility, and behaviour, in the context of liver disease it is another example of an toxic overload disorder – in this instance of adipocytes. Obesity rates have increased dramatically and led directly to the sharp rise in NAFLD which in the developed nations is estimated to affect around 9% of children [27]; and up to 25% of cases progress to fibrosis and cirrhosis (see Chapter 15). The presenting features are central obesity, hyperlipidemia, insulin resistance (metabolic syndrome) and an elevated ALT level at least 30 IU/L above the upper limit of normal, and an abnormal abdominal ultrasound suggesting fatty infiltration. The pathophysiology is a complex interplay of excess refined sugars and fats in the Westernized diet, a proinflammatory microbiome and dysregulation of the gut–liver axis [16, 35]. A range of treatments have been evaluated including lifestyle changes; bariatric surgery; and obeticholic acid (a farnesoid X agonist which appears to limit the accumulation of fat by reducing hepatic lipogenesis and improves insulin sensitivity) all of which have shown some promise in mainly adult studies [27].

Malnutrition

Malnutrition has profound effects on the functioning of all organs, including the liver. Drug and toxicant clearance is reduced and myocardial and respiratory function are significantly impaired in malnourished intensive care patients. Hepatic and plasma concentrations of glutathione and associated antioxidant systems are reduced in protein energy malnutrition [36]. The liver has a large population of Kupffer cells and therefore has a major role in the immune system, which is depressed in malnutrition. In particular, cell-mediated responsiveness and peripheral T-cell counts are reduced, rendering malnourished patients with acute or chronic liver disease especially susceptible to fungal and enteric infections. Malnutrition also appears to be a risk factor in major surgical procedures, with an increased operative morbidity and mortality after liver transplantation. The consequences of malnutrition are profound, so it is important to institute appropriate nutritional support as soon as possible (see also Table 22.9).

Sepsis

Sepsis is probably the most common cause for abnormal liver function. Clinically, a non-specific elevation in transaminases occurs early in sepsis with jaundice and hepatomegaly developing after a few days in cases of chronic sepsis. At a cellular level, lipopolysaccharide and proinflammatory cytokines (TNF-α, IL-6, IL-1b) cause direct impairment of bile flow through alterations of both transcriptional and post-transcriptional gene expression of bile acid transporters, resulting in the downregulation of transporter proteins including the energy-dependent transporter BSEP which lies on the canalicular membrane. Recurrent sepsis leads to activation of stellate cells via stimulation of the Toll-like receptors by lipopolysaccharide causing hepatic fibrosis [33].

Septic shock may lead to a hypoxic hepatitis (aminotransferases >500 IU/L) associated with a coagulopathy and a loss of control of glucose metabolism, whilst a more chronic infection especially with Gram-negative bacteria which have abundant lipopolysaccharide may cause cholestasis [37]. A direct myotoxic effect of infections such as hepatitis C,

Table 22.8 Relationships between presenting symptoms, liver function tests, affected cell types within the liver, and disease. See text for abbreviations.

Typical symptoms and changes in liver function tests	Indicative tissue	Pathophysiology	Examples of diseases
Jaundice, pruritus Raised ALP, GGT Bilirubin	Bile ducts	*Inflammatory response* Cytokines, TNF-α, IL-6, IL-1β Lymphocytes VAP-1	Gram-negative sepsis; autoimmune activation as in inflammatory bowel disease; GVHD; liver allograft rejection; necrotizing enterocolitis
Jaundice and abdominal pain Raised AST/ALT, GGT and ALP followed by raised bilirubin	Bile ducts	*Biliary obstruction* Impaired enterohepatic recirculation of bile Impingement by tumor	Gallstone disease; thalassemia; hereditary spherocytosis; sickle cell disease; inspissated bile pancreatitis; inflammatory bowel disease especially ulcerative colitis in association with sclerosing cholangitis; cystic fibrosis; fat malabsorption – fat-soluble vitamin deficiency
Hepatomegaly Raised AST and ALT – other liver function usually well preserved	Hepatocytes	*Infiltrative* Histiocytosis and other immune system cells Adipocytes (excess calorie intake)	Acute lymphoblastic leukemia; granulomatous disorders; hemophilia A coinfected with bloodborne viruses; NAFLD; Shwachman–Diamond syndrome; IFALD early stages; insulin-dependent diabetes mellitus
Liver may be atrophic, spleen enlarged if significant fibrosis Raised AST, ALT; PT prolonged Albumin reduced Glucose levels may be raised (metabolic syndrome) or low if massive hepatocyte loss has taken place	Hepatocytes	*Ischemia/fibrosis/necrosis* Heptotrophic viruses Proinflammatory lipids (linoleic fatty acids found in soya oil) Drugs Hormones Sequestration of iron, copper Activated immunocytes	Hepatitis A, B, C, EBV, CMV, HIV; IFALD advanced stage; NASH; methotrexate, doxorubicin, actinomycin; sodium valproate (esp. if less than 6 years) Fructose; hypothyroidism; hypervitaminosis A, thalassemia; hereditary spherocytosis; sickle cell disease; Wilson disease Hemophagocytic lymphohistiocytosis Aplastic anemia
Hepatomegaly ± splenomegaly if portal hypertension Ascites, raised AST and ALT	Endothelium	*Vascular disorders* Congestion; blood flow impaired (in or out of the liver) occlusive disease (conditioning regimens for bone marrow transplantation esp. 6-thioguanine) Impingement by tumor	Right ventricular disorders including pulmonary atresia, post-Fontan; tuberculous pericarditis; HIV-induced myocarditis; thalassemia and transfusion-related iron overload; sickle cell crisis; Budd–Chiari syndrome

dengue and varicella-zoster which can cause a myocarditis, the HIV virus known to cause cardiomyopathy – may lead to cardiac failure and worsening of multiorgan failure.

Management

There should be a low index for suspecting sepsis in vulnerable patients (pre-term infants, babies, and children stressed by complicated births or surgery, children on immunosuppression treatment, individuals with indwelling catheters especially feeding catheters and central venous line) and blood cultures, viral studies (serology, polymerase chain reaction) obtained. The search for the source of infecting agents should include ultrasound scanning of the abdomen (abscesses, fluid collections, obstructed biliary tree) and contrast CT where there is a possibility of retroperitoneal fluid collection/abscess.

The commonest hepatic ultrasound finding in septicemia is an "echo-bright" liver due to hyperplasia of the reticuloendothelial system or fatty change. Acute hydrops of the gallbladder (massive distension of the gallbladder in the absence of stones, or congenital malformations) is rarely seen but is particularly associated with Kawasaki disease. Aspiration of the gallbladder may be required in order to prevent perforation.

Patients who develop hepatic decompensation as a result of sepsis should receive full supportive care which includes broad-spectrum antibiotics, aciclovir, and intravenous fluid to maintain plasma glucose and support circulating volume as required. Patients should be closely monitored for signs of coagulopathy, encephalopathy, and cardiorespiratory compromise and transferred to pediatric intensive care if necessary.

The major pathologies underpinning liver disease in systemic illness – inflammation, biliary obstruction, infiltrations, ischemia/fibrosis and vascular disorders – are summarized in Table 22.8.

Principles of management of liver disease in systemic illness

The management of liver disease which is a consequence of disease in other organs clearly depends on treating the disease in that organ, e.g., the effects of tuberculous pericarditis on the

liver are best managed by administering antitubercular drugs and pericardectomy. Diseases with multisystemic manifestations such as sarcoidosis, GVHD, and NAFLD will require input from other specialists such as a rheumatologists, ophthalmologists, immunologists, endocrinologists, and gastroenterologists. It is beyond the scope of this chapter to detail all the treatments in the organ or tissue which is affecting the liver, but the general principles of management are:

- Diagnostic precision.
- Exclusion of exacerbating factors such as drug intoxication and infections.
- Supportive treatment especially nutritional support and blood products as required.
- Evaluate the enterohepatic circulation and biliary tree and treat cholelithiasis if present.
- Support for acute liver failure as required including liver transplantation.

Diagnostic precision

Diagnostic precision involves carrying out the full range of liver function tests and an abdominal ultrasound. A liver biopsy is especially helpful where there are autoimmune conditions and infiltrative lesions and as well as confirming the diagnosis allows the severity of the hepatic co-morbidity to be graded (see Chapter 2). Other tests which are available include new modalities of imaging such as the FibroScan® and MRS and SeHCAT (selenium-labelled taurocholate) which provides information on the stiffness of the liver; the liver substance and blood vessels; and the enterohepatic circulation, respectively (see Chapter 5).

Exclusion of exacerbating factors

A detailed history of drug consumption and screening tests for drug reactions which may include eosinophilia; hemolysis, fatty change, or fibrosis on liver biopsy should be performed and the drug excluded. A septic screen and serology for hepatrophic viruses (hepatitis A, B, C, and E, HIV) and polymerase chain reaction for other viruses such as EBV, CMV, and adenoviruses should be performed where the clinical picture is of a hepatitis (i.e., markedly elevated amino transaminases accompanied by only modest increase in bilirubin). Patients who are receiving chemotherapy or long-term intravenous nutrition are at increased risk of bacterial septicemia and there should be a low threshold for consideration of sepsis as a complicating factor when liver function deteriorates.

Supportive treatments

Patients who are undernourished will need additional calories delivered via nasogastric tube if necessary and, if the patient is cholestatic, will benefit from medium-chain tryglyceride supplements as an energy source which is more easily absorbed even when the amount of intraluminal bile salts is low. Bigger doses of fat-soluble vitamins should also be provided to compensate for the fat malabsorption of 50–90% commonly seen in jaundiced patients (Box 22.1). Calories intake may need to be increased to 150–200% of the recommended intake.

Ascites is treated with diuretics and according to cause, i.e., stents are used in Budd–Chiari syndrome, or thrombolysis with defibrotide, combined with antithrombin III in veno-occlusive disease. Some patients will require drainage of ascites and infusion of salt-poor albumin.

Immunotherapy may be needed to treat the liver and the initiating condition, e.g., sarcoidosis, is treated with oral prednisolone (1–2 mg/kg/day). Patients who are septic may require treatment with antifungals and antibiotics from the carbapenem class which have a wide spectrum of effect including Gram-negative and anaerobic organisms which are especially associated with hepatobiliary pathology.

Evaluation and management of enterohepatic circulation

The formation of biliary sludge is a risk with any patient requiring complex surgery, blood transfusion, and intensive care, especially after cardiac surgery. Nutrition support via the enteral route is preferred to parenteral nutrition because enteral feeding stimulates bile flow. Ursodeoxycholic acid (20–50 mg/kg/day) also stimulates bile flow and reduces the formation of insoluble cholesterol and phospholipid aggregates, and is used in children with impaired biliary drainage. Ursodeoxycholic acid appears to protect against further deterioration in liver function in cystic fibrosis (see Chapter 16). The development of the inspissated bile syndrome may be resistant to ursodeoxycholic acid, and surgical or radiological intervention with biliary lavage may be necessary (see Chapter 25). If cholestasis is prolonged, fat-soluble vitamins, calorie supplements, and medium-chain triglycerides should be prescribed (see Chapter 8 and Box 22.1). In children with terminal ileal disease (short bowel syndrome or Crohn disease), metronidazole (20 mg/kg/day), which selectively decontaminates the intestinal tract and reduces deconjugation of bile salts by bacteria, may be useful in reducing abnormalities in liver function tests.

Acute liver failure (see Chapter 18)

The impact of diseases elsewhere in the body is occasionally catastrophic and lead to liver failure. For example inflammatory bowel disease and autoimmune hepatitis may necessitate liver transplantation, but children who present before the onset of liver failure have a 5-year survival of over 90% when managed with immunosuppression (oral prednisolone and azathioprine) [14]. The abnormal accumulation of copper in the brain and liver in Wilson disease can present with either a neurological or hepatic presentation and the use of copper chelation therapies may prevent further deterioration even in acute liver failure – thus diagnostic precision

alongside supportive treatment is essential in cases of acute liver failure (see Chapter 20). Recovery without liver transplantation can be achieved with supportive care which includes monitoring and management for encephalopathy, hypoglycemia, and coagulopathy – fluid and sodium restriction, 10% dextrose, and blood products as required and withdrawal of hepatotoxic drugs (see Table 22.3 and Chapter 12). Specific treatments for encephalopathy such as lactulose which acidifies gut contents and reduces ammonia production by intestinal bacteria as well as hastening evacuation of high protein dietary loads are useful, also mannitol which can temporarily reduce intracranial pressure by reducing circulating blood volume through an osmotic diuresis. Children with acute liver failure should be referred to a specialist unit where further support including ventilation can be provided and, if indicated, liver transplantation.

Conclusion

The recognition of new classes of disease, e.g., ribosomopathies [18] and ciliopathies [28], and the developments in genetic diagnoses especially in the field of immunology [19], has enabled phenotypically similar disorders to be distinguished and has led to targeted treatments of the primary disorder and associated hepatic morbidity. Advances in the understanding of the microanatomy of the liver and the pathophysiological correlations associated with elevations in liver enzymes and symptoms of liver disease (jaundice, pruritus, hepatomegaly, ascites, encephalopathy) have facilitated earlier and more precise diagnoses of cardiac/hepatic syndromes, overlap immune disorders, and multisystemic disorders [1, 3, 4, 14, 15, 17].

Although the treatment of hepatic dysfunction caused by disease elsewhere is mostly supportive and the outcome largely dependent on treating the primary problem, there are circumstances when interventions such as endoscopic ligation of varices or liver transplantation are required [10, 15, 20]. An appreciation of distinct cellular pathways which induce steatohepatitis, stellate cell activation, and fibrosis (e.g., cytokines, Toll-like receptors which mediate the inflammatory response) and the concept of fibrogenic microbiotome has generated new therapeutic models and novel therapeutic agents which will improve the management and outcome of multisystem disorders in the future [16, 27, 35, 38].

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CHAPTER 23

Skin Disorders in Liver Disease

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Key points

- Skin disorders pose a significant diagnostic and therapeutic dilemma for the non-dermatologist.
- The majority of skin disorders in children with liver disease are common conditions such as acne, warts, and eczema.
- Certain skin manifestations form important diagnostic clues and may signal underlying liver disease.
- Other skin conditions may occur following liver transplantation either as a direct side effect of medication or as a consequence of an immunosuppressed state.

Pediatric skin disorders account for 10–15% of family practitioner consultations. Their prevalence in children with liver disease is unknown, but may be even higher. The enormous variety of skin diseases poses a considerable diagnostic challenge to the non-dermatologist, and appropriate treatment may be delayed.

In the context of pediatric hepatology, skin disorders can be separated into three groups:

- Common incidental skin conditions such as acne, warts, eczema, drug rashes, and infections due to common bacteria, viruses, or fungi.
- Dermatological manifestations of an underlying systemic disorder such as xanthelasma, associated with cholestasis or spider nevi in chronic liver disease.
- Skin disorders following liver transplantation, including:
  - side effects of immunosuppressant drugs
  - direct results of immunosuppression, including bacterial, viral, fungal, and protozoal infections
  - graft-versus-host disease
  - cutaneous malignancies.

This chapter first deals with the structure and function of normal skin as a basis for understanding skin pathophysiology, dermatological manifestations of liver disease in general, and skin conditions in the immunocompromised host.

Structure and function of normal skin

Skin structure

The skin is divided into three layers: the epidermis, dermis, and subcutaneous layer.

Epidermis

The epidermis is a multilayered structure that renews itself continuously by cell division in the basal layer. It consists of:

- Keratinocytes, which move peripherally from the basal layer, giving rise to successive layers of cells that lose their nuclei and eventually die as they reach the surface.
- Melanocytes, melanin-producing dendritic cells.
- Langerhans cells, important antigen-presenting cells bearing major histocompatibility (MHC) class II antigens.
- Merkel cells, involved in sensation and mainly seen on the digital pads, lips, and in the oral cavity.

The epidermal appendages are apocrine sweat glands, hair, sebaceous glands, nails, and teeth.

Dermis

The dermis forms the bulk of the skin and contains connective tissue fibers (mainly collagen, with some elastin and reticulin) lying beneath the epidermis, giving the skin its

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ability to stretch and mold. Blood vessels, nerves, lymphatics, and muscles, as well as cells such as leukocytes, histiocytes, fibroblasts, and mast cells, are found in this layer.

Subcutaneous layer

The subcutaneous layer mainly contains fat, sweat glands, and blood vessels.

Skin function

The skin is a vital part of the body with numerous functions, including:

- Physical barrier to antigens or bacteria (intact stratum corneum, lipids in sebaceous glands, suppressant effect of normal skin flora on pathogens, presence of granulocytes, macrophages, and killer T cells).
- Prevention of excessive absorption or loss of water and regulation of internal temperature (sweat glands).
- Pigmentation, preventing injury from ultraviolet light.
- Vitamin D synthesis by sunlight in the epidermis.
- Sensation of pain, touch, and temperature.
- Involvement in immunological reactions (Langerhans cells, secretory immunoglobulin A (IgA), T cells, and B cells).

Dermatological manifestations of liver disease

Jaundice

Jaundice (icterus), a yellow discoloration of the skin and mucous membranes, is the most obvious sign of liver disease and is best seen in the conjunctivae. It is detectable when the serum level of bilirubin exceeds 2 mg/dL (34 μmol/L), although in neonates it may not be detected unless the level exceeds 5 mg/dL (85 μmol/L). The differential diagnosis and management are discussed elsewhere (see Chapter 4).

Palmar erythema

Palmar erythema, or "liver palms," is a non-specific red discoloration of the palms and fingertips indicative of a hyperdynamic circulation, which is associated with chronic liver disease and cirrhosis. The erythema blanches with pressure. Patients may complain of throbbing and tingling. The exact pathogenesis is not known.

Spider nevi

Spider nevi, or spider angiomas, are telangiectases consisting of a central arteriole with superficially radiating small vessels, resembling spiders' legs, and are mainly observed in the superior vena cava distribution area (i.e., above the nipple line) (Figure 23.1). Characteristically, the whole lesion will blanch when pressure is applied to the center of the spider, and during episodes of severe or prolonged hypotension they may disappear. They can be successfully treated with laser therapy.

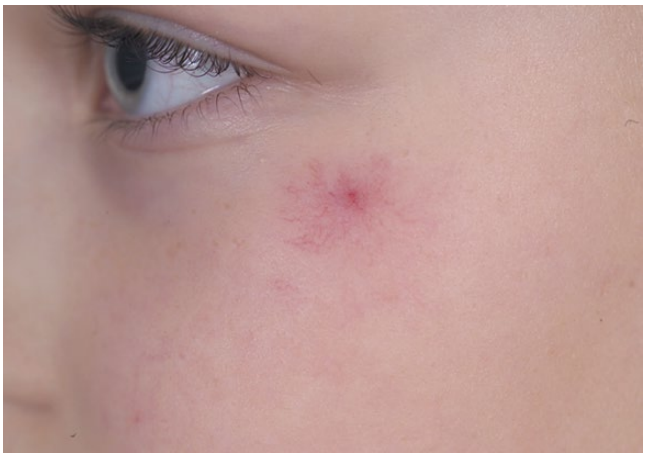


Figure 23.1 Spider nevus on a young girl's cheek suggestive of chronic liver disease. Spider nevi are also found in pregnancy, at puberty, and in girls taking the contraceptive pill, although usually in fewer numbers.

Although spider nevi do occur in healthy people, especially at puberty, their presence may be suggestive of chronic liver disease. An increase in the number or size of spider nevi may suggest progressive liver damage (cirrhosis), but also occurs in pregnancy or in girls taking the contraceptive pill.

Nail changes

Nail changes are a common feature of chronic liver disease (cirrhosis) in adults, but are less frequently seen in children. Nail changes include clubbing, thickening, longitudinal ridging, white bands, and brittle nails.

Pruritus

Pruritus is a distressing symptom of cholestasis or inborn errors of bile acid metabolism. Symptoms range from mild to severe intractable pruritus, which interferes with the patient's daily activity and sleep. Intractable pruritus may be an indication for liver transplantation.

The exact mechanism of pruritus is uncertain. Cholestasis leads to an accumulation in plasma of substances which are normally excreted into bile (e.g., bilirubin, cholesterol, and bile acids). High levels of bile acids may damage hepatocyte membranes, triggering the release of pruritogenic substances, which interact with nerve endings in the skin [1].

The skin in patients with generalized pruritus is often very dry and varies from a normal appearance, through mild flakiness with a few scratch marks, to severe excoriation with scars and nodules.

Treatment of pruritus is empirical and unsatisfactory. Good skin care is essential. As dryness of the skin is a prominent feature, avoidance of soap and liberal use of emollients are strongly recommended. Alternative treatments such as evening primrose oil and aromatherapy, and a wide range of drugs and therapies including ursodeoxycholic acid, phenobarbitone, rifampicin, cholestyramine, histamine

antagonists, ondansetron, plasmapheresis, and phototherapy (see Chapter 8) are used with variable success. Partial external diversion of bile has been attempted in patients with pruritus unresponsive to medical treatment, again with highly variable success rates [2].

More recent studies have implicated increased opioid neurotransmission/neuromodulation in the central nervous system as a contributor to pruritus in cholestasis, suggesting that oral opiate antagonists – for example, naloxone – may provide long-term relief from pruritus [3]. Although the exact mechanism of opiate involvement is still unknown, the beneficial effects of opiate antagonists have been shown in different randomized, blinded, placebo-controlled trials [4]. Finally, subjective amelioration of pruritus following intravenous administration of ondansetron to cholestatic patients may suggest that altered serotonergic neurotransmission may also contribute to this form of pruritus [3].

Xanthelasma

Orange-yellow lipid deposits of cholesterol in the skin are known as xanthelasmas. They are seen in children with elevated plasma cholesterol due to chronic cholestasis secondary to intrahepatic biliary hypoplasia such as Alagille syndrome. They rarely present before the age of 16 months. The commonest sites are areas of mild trauma, the elbows, knees, and at flexures. Xanthelasmas regress with management of hypercholesterolemia and following liver transplantation.

Purpura

Purpura is extravasation of red cells in the skin, which presents with red patches that do not blanch on pressure. Purpura in liver disease usually reflects thrombocytopenia secondary to hypersplenism and portal hypertension, or skin fragility due to steroids.

Photosensitivity

Photosensitivity with abnormal liver function may be due to porphyria cutanea tarda. Early cutaneous features are blister formation and erosions on the backs of the hands, the forearms, and the face following exposure to sunlight. The areas heal with scarring and milia formation. Later, there may be hyperpigmentation, hypertrichosis, and pseudoscleroderma-tous thickening of the skin.

Similar photosensitive blistering eruptions, associated with elevated plasma and urinary porphyrins (raised coproporphyrin isomer I and III fraction), have been described in children with Alagille syndrome (Figure 23.2). It is unclear whether the elevated porphyrins are secondary to liver dysfunction or associated with the deletion of chromosome 20 in this condition. The lack of similar lesions in other hepatic disorders associated with comparable porphyrin abnormalities does suggest that other factors may be involved in the pathogenesis of these cutaneous lesions [5].

Carotenemia

This yellow/orange discoloration of the skin, in particular of the palms, soles, and feet, may occur in otherwise healthy children due to excess dietary β-carotene. Its importance is that it may be mistaken for jaundice. In some children, it may be associated with low serum retinol-binding protein (RBP4) levels, resulting in slow uptake and release of vitamin A by the liver and subsequent inhibition of conversion of carotene to vitamin A. The resulting hypercarotenemia and low vitamin A levels do not respond to vitamin A supplementation.

Hemangiomas

Rarely, infants are born with multiple angiomas of the skin and internal organs. The skin lesions look like small strawberry nevi, which are present at birth or arise shortly afterwards and, although they may appear anywhere on the body, have a predilection for the head, neck, and napkin area (Figure 23.3).

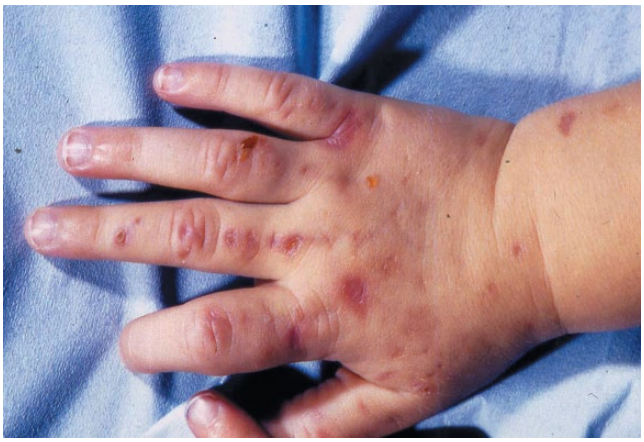


Figure 23.2 Photosensitive porphyria-like skin lesions in a child with Alagille syndrome. She had elevated urinary porphyrins, which were thought to be secondary to liver dysfunction.

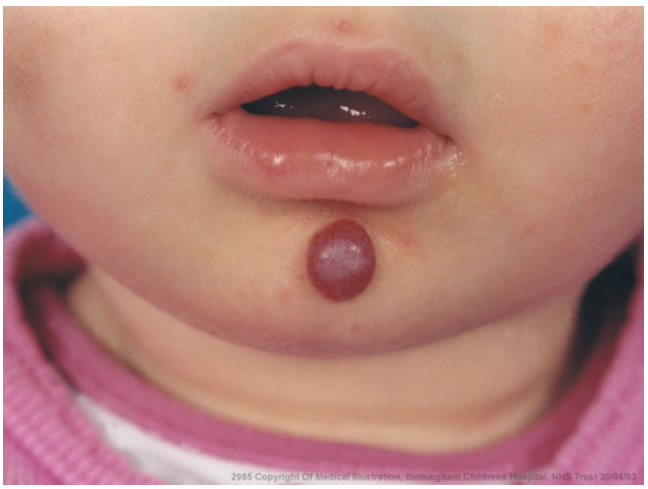


Figure 23.3 Hemangioma on a child's chin. Hemangiomas may be single or multiple. They may be a sign of other hemangiomas elsewhere, e.g., the liver.

The lesions contain both capillary and "cavernous" vascular elements, rapidly increase in size to often dome-shaped, red/purple extrusions, and may bleed if traumatized. Although the skin lesions would normally undergo spontaneous resolution with time, the associated hepatic and intestinal angiomas may lead to severe complications and, depending on the extent of disease and organs affected, often have a poor prognosis (see Chapters 14 and 27).

Gianotti–Crosti syndrome

Gianotti–Crosti syndrome, or papular acrodermatitis of childhood, is a non-specific viral exanthema that may accompany hepatitis B infection. There are erythematous, non-itchy, small, umbilicated papules on the face, buttocks, and extremities. Other clinical findings are generalized lymphadenopathy, and in children with hepatitis there is hepatomegaly and biochemical and histological evidence of acute or chronic hepatitis.

Lichen planus and hepatitis C infection

Lichen planus is an idiopathic inflammatory disease of the skin and mucous membranes. The pathogenesis is not fully understood, but several reports have suggested a relationship between oral lichen planus and chronic liver disease, especially hepatitis C virus (HCV) infection. A recent meta-analysis has reported that there is a significant increased risk of lichen planus patients being HCV positive [6]. Therefore, screening patients with oral lichen planus for antibodies to HCV is recommended.

Neonatal lupus erythematosus

Neonatal lupus erythematosus (NLE) is an autoimmune disease characterized by congenital heart block, thrombocytopenia, hepatobiliary disease, and/or transient skin lesions of subacute cutaneous lupus. In contrast to adult lupus erythematosus, the lesions have a predilection for the face, especially the periorbital region [7]. The skin lesions typically resolve without scarring, although dyspigmentation may persist for many months and some may have residual telangiectasias. Children with skin signs of neonatal lupus should be evaluated for cardiac, hepatobiliary, and hematologic manifestations.

Skin manifestations of malnutrition

Infants with chronic liver disease are particularly at risk of malnutrition (see Chapters 8 and 21). Deficiencies in calories, protein, essential fatty acids, minerals, and trace elements (particularly zinc and selenium) may eventually lead to skin abnormalities.

Dietary protein deficiency (kwashiorkor) may result in non-specific skin changes, ranging from pigmentary changes (hyperpigmentation or hypopigmentation) to flexural erosions, desquamation, and dry, depigmented, and pluckable hair. In children with reduced calorie intake (marasmus), the skin may appear dry and wrinkled. Similar changes can be found as a result of longstanding essential fatty acid deficiency, but the main feature is a dry, flaky skin (ichthyosis) with hyperpigmentation (Figure 23.4).

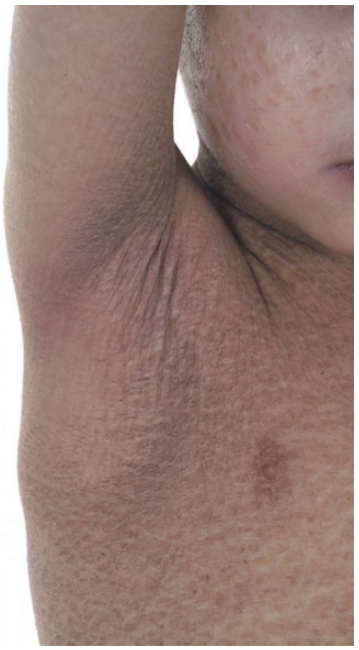


Figure 23.4 Dry, flaky skin (ichthyosis), often seen in long-standing essential fatty acid deficiency, which is secondary to fat malabsorption in liver disease.

Zinc deficiency occasionally complicates liver disease and may lead to exudative eczematous lesions around the orifices and on the hands and feet, similar to those found in acrodermatitis enteropathica, an inborn error of zinc metabolism. Selenium deficiency may lead to loss of hair pigment.

All of these conditions are reversible with appropriate dietary supplementation.

Dermatological complications of liver transplantation

Skin in the immunocompromised host

Environmental conditions and skin microorganisms, which have little significance in the normal host, may have quite devastating effects in the immunocompromised patient. Protection by an intact well-functioning skin is essential, but may be compromised by various factors uniquely associated with the immunosuppression. Firstly, the integrity of the skin may be breached by the use of long-term central lines, intravenous devices, and invasive diagnostic procedures, allowing easy invasion of pathogens. Secondly, immunosuppressant drugs such as corticosteroids cause atrophy of the skin, thus

compromising the first-line barrier, while other immunosuppressant drugs (ciclosporin, tacrolimus, etc.) lead to granulocytopenia, neutropenia, and neutrophil defects, or alteration of T-cell and B-cell function, thereby reducing the second-line defenses against pathogens. Finally, longstanding antimicrobial therapy may alter the normal skin flora (e.g., staphylococci, coryneforms, and some Gram-negative bacilli such as *Acinetobacter* spp.), allowing colonization with potential pathogens. Once colonized, the immunocompromised host is at continuous risk of acquiring infection with these organisms.

Cutaneous side effects of immunosuppressive drugs

Hypertrichosis (hirsutism)

Ciclosporin therapy causes a reversible, dose-dependent increase in the growth of body hair, more frequently seen in younger transplant patients (Figure 23.5) [8]. Vellus hair (thin, short hair without pigment, which takes over from lanugo hair in hair follicles after birth) converts to terminal hair, which is longer, coarser, and darker, and existing terminal hair becomes thicker. The mechanism of this follicular stimulation is unknown. It may be exacerbated by the use of systemic steroids [9]. Laser hair removal is effective, but repeated treatment is required in order to maintain improvement [10].

Retrospective studies in adult renal transplant recipients receiving ciclosporin found that 40–60% had hirsutism, with a slightly higher incidence in dark-skinned patients, suggesting predisposing genetic factors. The incidence of hypertrichosis in children receiving ciclosporin after liver transplantation is approximately 30%, and the impact of its cosmetic effect, particularly in teenagers, is substantial. However, with availability of newer immunosuppressant drugs ciclosporin is no longer first choice in the treatment of prevention of graft rejection, and ciclosporin-related hypertrichosis is therefore less frequently seen. Ciclosporin is rarely used now [11].

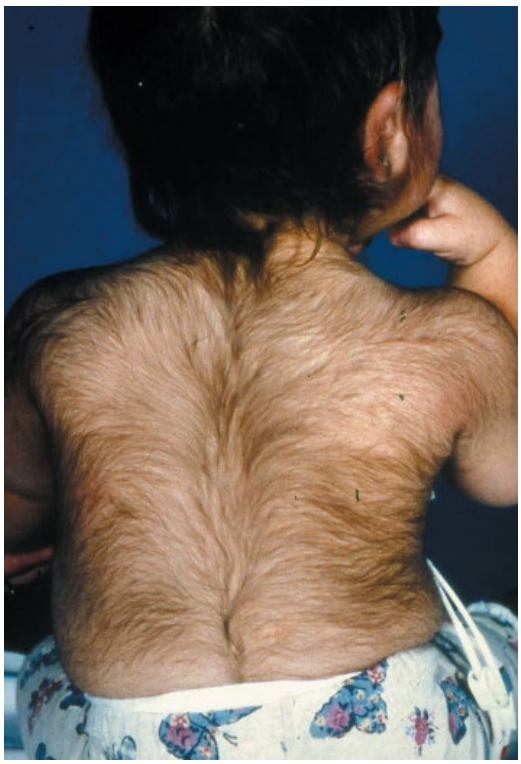


Figure 23.5 Immunosuppression with ciclosporin commonly causes excessive hirsutism, which resolves on reduction of the drug.

Gingival hyperplasia

Gingival hyperplasia is a recognized side effect of ciclosporin and of the antihypertensive drug nifedipine, which is frequently used after liver transplantation (see Chapter 31).

Eczema

Atopic dermatitis, or eczema, is a common skin disorder in infancy and childhood, affecting 5–7% of children before the age of 5 years. It may be particularly troublesome in children with liver disease before or after transplantation.

In infants and younger children, the main affected areas are the face and extensor surfaces of the extremities, while in older children and adults, the flexural areas are predominantly involved. Chronic eczema (Figure 23.6) appears red, scaly, and lichenified (thickened, with exaggerated skin markings), while acute exacerbations are characterized by edema, oozing, crusting, and excoriation. The main symptom is pruritus, which may be not only difficult to manage, but also difficult to differentiate from pruritus due to cholestasis in children with both conditions.

Immunocompromised children with atopic dermatitis have an increased risk of secondary bacterial infection of the eczematous lesions, leading to crusting and weeping.

Occasionally, eczema appears for the first time or becomes more severe in children after transplantation who are receiving ciclosporin or tacrolimus. This is paradoxical,

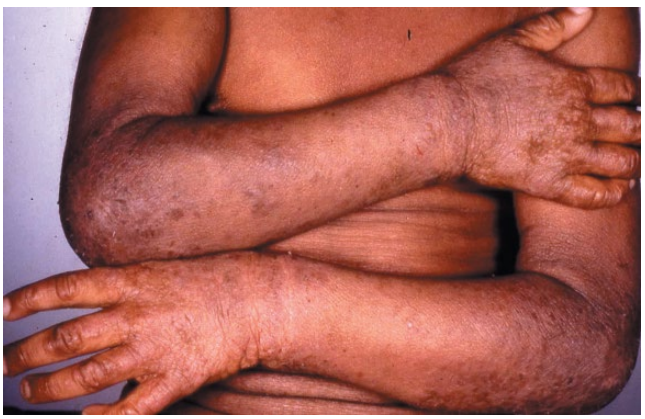


Figure 23.6 Red, scaly skin with exaggerated skin markings (lichenification) are a sign of chronic eczema. It is difficult to treat in children who also have pruritus due to cholestasis. Eczema may also become exacerbated after transplantation, particularly with ciclosporin treatment.

because both drugs are used to treat eczema, and may indicate a role for infection in the pathogenesis of eczema. There is no clear relationship with dose, but the eczema may improve when immunosuppression is reduced or changed.

First-line treatment of eczema involves soap substitutes, bath oils, and emollient creams (e.g., aqueous cream and combinations of white soft paraffin and liquid paraffin) to moisturize the skin and reduce pruritus secondary to xeroderma (dry skin). More inflamed areas should be treated with topical steroids, which are available in increasing strengths from hydrocortisone 0.5–1%, to Eumovate (clobetasone butyrate 0.05%) or Betnovate (betamethasone 0.1%). In superimposed bacterial or fungal infection, an ointment combining a steroid and antibacterial or anticandidal agent is necessary, such as Canesten HC, Terra-Cortril, or Vioform HC. A body suit made of tubular bandages is comforting at night. Sometimes a double layer of bandages, with the inner layer wet ("wet wraps"), is helpful for extreme pruritus. Ichthammol-impregnated bandages are useful for lichenified eczema on the limbs.

In more resistant cases of chronic eczema, long-term use of topical and systemic corticosteroids is limited because of numerous side effects. In recent years, other immunomodulatory forms of treatment have been explored, including topical tacrolimus ointment. Large multicenter studies have recently shown that its long-term use in both adults and children with severe atopic eczema is both efficacious and safe [12].

Acne vulgaris

Acne vulgaris affects most adolescents during puberty, when changes in the hair follicle and sebaceous glands are stimulated by hormones. Drug-induced acne rarely occurs before puberty, in the "unprimed" prepubertal follicles. Adolescents and adults treated with high-dose corticosteroids for autoimmune hepatitis or after transplantation may develop acne for the first time, or experience an exacerbation of pre-existing acne [8].

Steroid-induced acne is reversible, and generally appears 2–3 weeks after initiation of high-dose prednisolone therapy. It may be mild or severe, extending beyond the usual distribution sites to the arms, the whole central back, and down to the buttocks. Acne vulgaris is characterized by comedones, papules and pustules, nodules and cysts, and finally scar formation (Figure 23.7). In steroid-induced acne, pustules predominate, and the eruption is often strikingly monomorphic. Ciclosporin may also provoke acne as part of its effect on the hair follicle.

Mild forms of acne should be treated with topical preparations such as benzoyl peroxide or retinoic acid, alone or in combination with topical antibiotics (tetracyclines, erythromycin, or clindamycin). In moderate cases, treatment should include a combination of topical preparations with systemic oxytetracycline or erythromycin [13]. Minocycline, an oral preparation of tetracycline, should be avoided, as it can cause a lupus-like hepatitis. The more potent anti-acne drugs, cyproterone acetate and isotretinoin, are potentially hepatotoxic and therefore contraindicated.

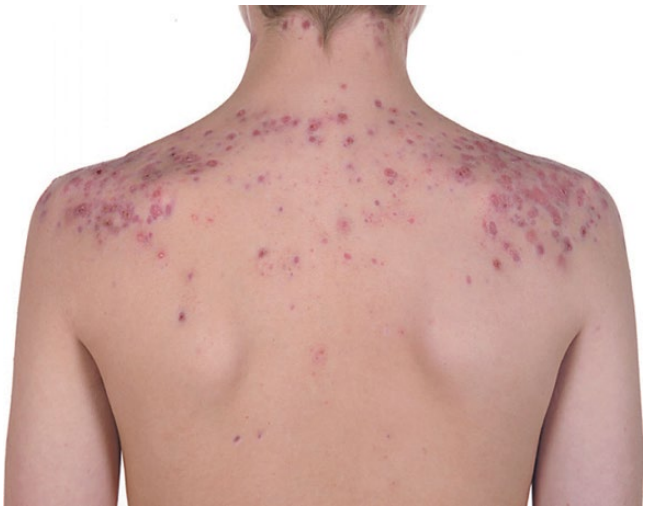


Figure 23.7 Treatment with steroids after transplantation for autoimmune hepatitis can lead to severe acne with extensive scarring.

Other steroid-induced skin manifestations

Striae, facial erythema, atrophic and friable skin, purpura, and telangiectasia are all well-recognized cutaneous side effects of prolonged high-dose steroid use. Apart from erythema, these changes are irreversible.

Cutaneous lesions in immunocompromised hosts

Viral infections

Cutaneous viral infections in healthy individuals are common, benign, and self-limiting, but they may be devastating in immunocompromised hosts, so early diagnosis and treatment are vital. The most important cutaneous virus infections in the context of liver transplantation are human papillomaviruses (HPV), molluscum contagiosum, herpes simplex virus (HSV), varicella-zoster virus (VZV), and cytomegalovirus (CMV) [14].

Human papillomavirus. Viral warts are one of the commonest cutaneous manifestations of long-term immunosuppressive therapy and may be so numerous as to be disfiguring (Figure 23.8). The warts may reflect primary infection or reactivation of previously acquired latent virus. Studies in adult renal transplant recipients have shown a link between HPV and cutaneous malignancies, although other factors such as ultraviolet exposure and the type of immunosuppressive drug also play a role.

Common warts, palmar and plantar warts (verrucae). Warts can be treated conservatively with keratolytic paints or destructively by cryotherapy. In immunosuppressed children, cryotherapy is often ineffective, as well as painful. If the child requires a general anesthetic for another reason, the warts may be curetted or frozen at the

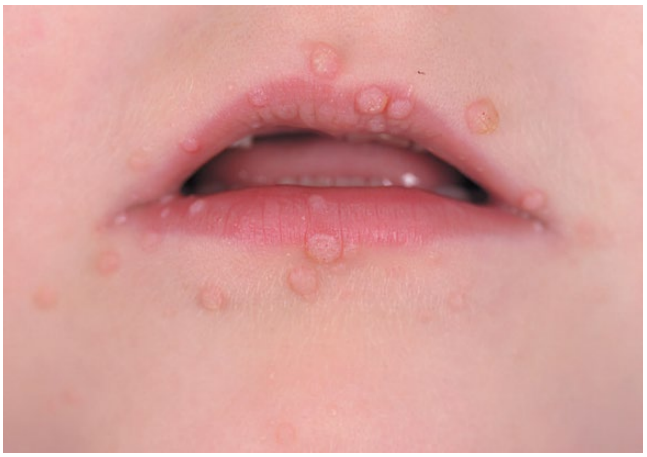


Figure 23.8 Warts are common in post-transplantation patients because of immunosuppression. This child receiving ciclosporin therapy had warts on the hands that spread to the face. Warts can be treated conservatively with keratolytic paints or destructively using cryotherapy.

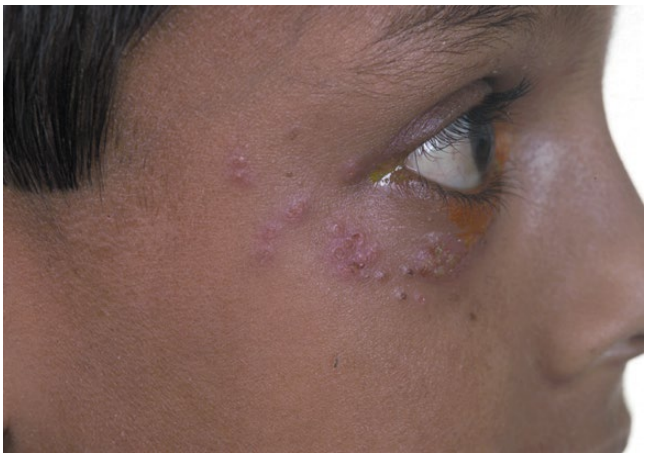


Figure 23.9 Herpes simplex infection around the eye is characterized by monomorphic vesicles and erosions. Treatment is with aciclovir and reduction in immunosuppression, if relevant.

same time, but unfortunately they may recur. Repeated treatment may be required until immunosuppression can be reduced. If the warts are asymptomatic, treatment may not be indicated. There is some evidence that other treatment such as topical imiquimod 5% cream [15], pulse dye laser [16], intralesional bleomycin, and acitretin may benefit immunosuppressed adults with recalcitrant warts, but studies in children with transplants have not been performed.

Plane warts. These are tiny, flat, flesh-colored warts, usually occurring on the back of hands and on the face. They usually resolve spontaneously, and treatment is not indicated.

Condyloma acuminatum. These are genital warts, located on the penis, vulva, or perianal area, which usually present as small, cauliflower-like lesions. They are common in childhood, particularly in children undergoing immunosuppression. They are best treated with podophyllin paint under general anesthetic if there are numerous warts.

Molluscum contagiosum. Molluscum contagiosum is caused by a poxvirus. The umbilicated, white, or whitish-yellow papules occur anywhere on the body and may be very extensive in immunosuppressed patients, responding to reduction in immunosuppressive therapy. There is no specific treatment, as they eventually resolve spontaneously. If symptomatic, cryotherapy or individual enucleation with a needle is possible but it does not shorten the time to resolution.

Herpes simplex virus. Primary herpes simplex infection due to HSV type 1 presents in immunocompetent children as a cold sore with mild to moderate stomatitis. Immunocompromised patients suffer primary HSV-1 infection as severe herpetic gingivostomatitis with extensive blisters and erosions on buccal mucosa and lips. Conjunctivitis and keratitis may also occur (Figure 23.9).

As the virus persists in sensory ganglia, the patient remains at risk of recurrent herpes infection, which usually presents as cold sores on the lips, with occasional spread to the esophagus.

The diagnosis is based on the clinical features and positive electron microscopy of cultured fluid from vesicles. Serology is helpful only in primary HSV infection.

Cutaneous HSV infection in immunosuppressed patients should always be treated systemically (aciclovir), with reduction of immunosuppression in more extensive infections.

Varicella-zoster virus. The presentation of varicella (chickenpox) in immunosuppressed patients resembles that in healthy children, with fever, malaise, and a characteristic skin rash, but the disease may be more severe and lead to serious complications such as hemorrhagic varicella, postinfectious encephalomyelitis, and pneumonia. The virus remains dormant in dorsal root ganglia and may reactivate, leading to herpes zoster (shingles). In immunosuppressed hosts, reactivation is more likely and may lead to recurrent varicella and/or disseminated disease with visceral involvement, which may be fatal. Treatment of both forms of varicella includes a reduction in immunosuppressive therapy, intravenous acyclovir, and zoster immunoglobulin (ZIg).

Cytomegalovirus. CMV infection in immunocompromised hosts rarely presents with cutaneous manifestations, but as a febrile illness with arthralgia and myalgia or disseminated disease with visceral involvement. A few patients with primary or reinfection with CMV have presented with erythema multiforme with the typical "target lesions" or blisters.

Fungal infections of the skin

Fungal infections are common in immunocompromised patients, some reviews reporting an incidence as high as 70–85%. They include: (1) infections that commonly affect normal individuals but in immunocompromised hosts present in a more severe and extensive form; and (2) "opportunistic" fungal infections with organisms unlikely to invade a normal host.

This chapter covers only the superficial mycoses involving the outermost layers of the skin, the nails, the hair, and mucous membranes. The main pathogens in this group are the dermatophytes and yeasts [17].

Dermatophytoses. Infections with dermatophytes, or ringworm fungi, are confined to the superficial stratum corneum, nails, and hair and are usually acquired from contact with keratin debris carrying fungal spores. There are three genera of dermatophytes: *Trichophyton*, *Microsporum*, and *Epidermophyton*, of which more than 40 species are recognized worldwide. Some are anthropophilic (i.e., transmitted from person to person), while others are zoophilic (i.e., passed from animals to humans).

Diagnosis is confirmed by microscopic detection of fungal hyphae in skin scrapings, nail clippings or plucked hair, or a positive culture. Treatment includes topical broad-spectrum

Table 23.1 Drug dosages in dermatological conditions.

Drug	Dosage	Frequency (route)
Antibacterial		
Co-trimoxazole	18 mg/kg/dose	12 h (i.v.)
	120 mg (up to 6 months of age)	b.d. (oral)
	240 mg (6 months – 6 years)	b.d. (oral)
	480 mg (6–12 years)	b.d. (oral)
	960 mg (12–18 years)	b.d. (oral)
Erythromycin	125 mg (1 month – 2 years)	6 h (oral)
	250 mg (2–8 years)	6 h (oral)
	250–500 mg (8–18 years)	6 h (oral)
Flucloxacillin	12.5–25 mg/kg/dose (max. 1 g/dose)	4–6 h (i.v.)
	62.5–125 mg (1 month – 2 years)	6 h (oral)
	125–250 mg (2–10 years)	6 h (oral)
	250–500 mg (10–18 years)	6 h (oral)
Gentamicin	7 mg/kg/dose	Single (i.v.)
Oxytetracycline	250–500 mg/dose (12–18 years)	6 h (oral)
Penicillin		
Benzylpenicillin (penicillin G)	25–50 mg/kg/dose (max. 2.4 g/dose)	4–6 h (i.v.)
Phenoxymethylpenicillin (penicillin V)	62.5 mg (1 month – 1 year)	6 h (oral)
	125 mg (1–6 years)	6 h (oral)
	250 mg (6–12 years)	6 h (oral)
	500 mg (12–18 years)	6 h (oral)
Ticarcillin (in combination with clavulanic acid)	90 mg/kg (1 month – 12 years)	6–8 h (i.v.)
	2.25-4.5 g/dose (12–18 years)	
Vancomycin	15 mg/kg/dose (max. daily dose 2 g)	Every 8 h (i.v. over 2 h), adjusted according to plasma concentration
Antifungal		
Amphotericin (liposomal)	250 µg/kg/dose, increased over 2–4 days to 1 mg/kg/dose	Daily (i.v. over 1 h)
Fluconazole	3 mg/kg (1 month – 12 years), max. 100 mg/dose	Daily (oral/i.v.)
	50–100 mg/dose (12–18 years)	Daily (oral/i.v.)
Flucytosine	50 mg/kg/dose (1 month – 18 years)	6 h (oral./i.v.)
Griseofulvin	10 mg/kg (1 month – 12 years), max. 500 mg	Daily (oral)
	1 g/dose (12–18 years)	Daily (oral)
Itraconazole	3–5 mg/dose (1 month – 12 years), max. 100 mg	Daily (oral)
	100 mg/dose (12–18 years), max. 200 mg	Daily (oral)
Ketoconazole	100 mg/dose (weight <30 kg)	Daily (oral)
	200 mg/dose (weight >30 kg)	Daily (oral)
Antiviral		
Aciclovir	10–20 mg/kg (1–3 months)	8 h (i.v. over 1 h)
	250 mg/m^2 (3 months – 12 years)	8 h (i.v. over 1 h)
	5 mg/kg (12–18 years)	8 h (i.v. over 1 h)
	100 mg/dose (1 month – 2 years)	5 times daily (oral)
	200 mg/dose (2–18 years)	5 times daily (oral)
Ganciclovir	5 mg/kg (1 month – 18 years)	12 h (i.v. over 1 h)

antifungal agents such as miconazole (Daktarin®) or clotrimazole (Canesten®) in cases of very limited infection, or oral griseofulvin where the fungal infection is more extensive. More potent systemic antifungals include itraconazole and terbinafine, but these are not licensed in children at present and are potentially hepatotoxic. For drug doses see Table 23.1.

Tinea pedis (athlete's foot). This presents as scaling, itchy skin between the toes, often spreading to the entire sole. A foul odor may be present. Vesicles may occur, particularly during warm weather, rupturing to leave a ring-like ragged border. It is a relatively uncommon condition in immunocompetent prepubertal children, where the diagnosis of contact dermatitis is more likely. However, the incidence is increasing in immunosuppressed children in this age group. The condition may resolve without treatment, but often recurs.

Tinea corporis. Dermatophyte infection of the trunk, legs, arms, or face produces annular scaly, itchy lesions with an inflammatory edge and central clearing (Figure 23.10). It may have spread from another site (e.g., tinea cruris) or from an external source, animal or human.

Tinea capitis. Dermatophyte infection of the scalp and hair produces varying degrees of scaling, patchy hair loss, and areas of suppuration (kerion) (Figure 23.11). The few hairs in the affected areas are usually broken just above the surface of the scalp, and in some cases the fungi can be detected by their yellow-green fluorescent appearance under Wood's light (long-wave ultraviolet light). This infection always requires systemic treatment for at least 6 weeks.

Tinea unguium. Onychomycosis due to dermatophyte infection more often affects toenails than fingernails, and is frequently associated with tinea pedis. It usually starts as yellow-white, irregular distal nail dystrophy, which spreads slowly proximally, eventually producing a thickened, friable, opaque, yellow nail (Figure 23.12). If treatment is required, it must be systemic.

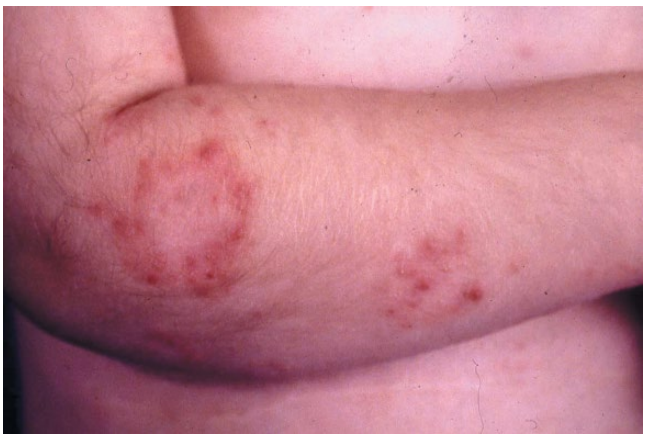


Figure 23.10 Tinea corporis (ringworm) is common in children, but may be more severe in immunosuppressed children after liver transplantation. The annular lesion with an inflammatory edge and central clearing should be noted.

Tinea cruris. Uncommon in children and usually occurring in young adult males, tinea cruris is a symmetrical, red, itchy eruption with a scaly edge and central clearing, which spreads from the groin and pubic region to the inner thighs.

Superficial yeast infections

Pityriasis (tinea) versicolor. This common condition is characterized by light-brown, oval, scaly patches on the trunk, neck, shoulders, and upper arms. In immunosuppressed patients, the rash may extend to the scalp, face, abdomen, groin, and legs. The causative organism, *Malassezia furfur*, is a normal skin commensal. The diagnosis is clinical and includes demonstration of a greenish, golden-yellow, or pink fluorescence under Wood's light. Treatment with topical agents such as selenium sulfide, miconazole, clotrimazole, or

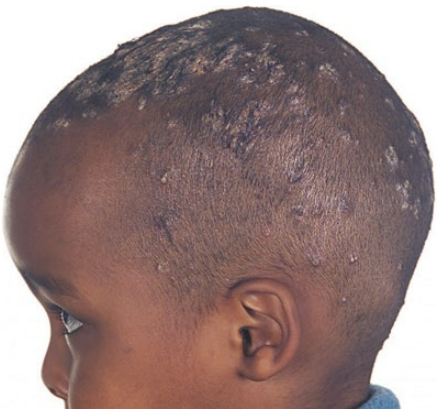


Figure 23.11 Tinea capitis is a dermatophytic infection of the head, with scaling, patchy hair loss, and areas of suppuration (kerion). It requires systemic therapy.

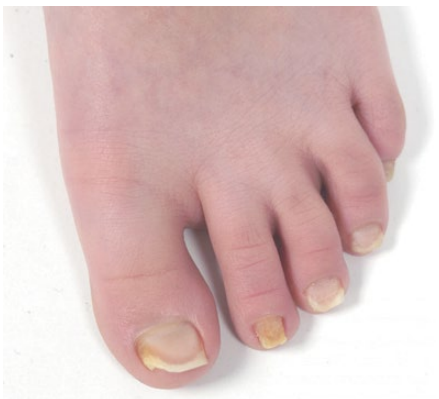


Figure 23.12 Tinea unguium is frequently associated with tinea pedis. The lateral and distal discoloration of the large toenail and a thickened, dystrophic second toenail should be noted.

econazole is usually effective, although the infection may relapse in places where the topical agents have not been applied properly. Oral itraconazole may be indicated for cases resistant to topical treatment.

Candidiasis. *Candida albicans* is a normal commensal of the human digestive tract and can be isolated from the mouth and intestinal tract in 30–50% of the normal population and from the genital tract in up to 20% of normal women. Infection of skin and mucous membranes (candidiasis) is usually derived from the patient's own reservoir. Topical and systemic steroid treatment, immunosuppression, and long-term use of broad-spectrum antibiotics predispose to candidiasis.

Adequate treatment of superficial candidiasis is usually achieved with topical antifungal preparations such as nystatin, clotrimazole, amphotericin, or miconazole. In more widespread infections, systemic antidermatophytes such as ketoconazole, miconazole and fluconazole (see Table 23.1) are indicated. Griseofulvin is ineffective against *Candida* spp. Liposomal amphotericin is the drug of choice for severe or intractable candidiasis. Flucytosine, a synthetic antifungal drug, is only active against yeasts, but has been demonstrated to have a synergistic effect with amphotericin.

Oral candidiasis. This is characterized by white, curd-like plaques inside the mouth, which can be scraped off, leaving inflamed and friable mucosa. In severe cases, the disease may spread to the oropharynx and esophagus. Localized lesions should clear within 2 weeks of the start of topical treatment with nystatin oral suspension, amphotericin, or miconazole gel (Daktarin). In extensive infections, systemic treatment is indicated.

Chronic paronychia. This is a chronic inflammatory process affecting the proximal nail fold and nail matrix, caused by bacteria or *C. albicans*. Simple measures such as keeping the fingers dry and avoiding finger-sucking and nail-biting will help. Treatment should be with topical or, if this is unsuccessful, with oral preparations.

Cutaneous candidiasis. Cutaneous candidiasis occurs in moist areas, such as body flexures (intertrigo) and skin that has been occluded with bandages or adhesive tape. Lesions start as vesicles or pustules, which may coalesce and erupt, leaving an erythematous area surrounded by an irregular, scaling margin. The clinical diagnosis of candidiasis is confirmed by microscopic demonstration of *C. albicans* in scrapings of the lesions or by culture from swabs. Topical treatment is usually adequate, but systemic therapy is indicated for widespread infection.

Pityrosporum folliculitis. In this acneiform condition, which is seen frequently in immunosuppressed children, small follicular papules and pustules are present on the trunk, in the absence of other features of acne. It responds well to treatment with antifungal agents such as miconazole.

Other opportunistic fungal infections

Cutaneous aspergillosis. Aspergillosis is a common fungal infection in immunocompromised hosts. The primary cutaneous form of aspergillosis, however, is uncommon and in most cases develops at sites of intravenous cannula insertion, venepuncture, catheters or where splints were strapped to the skin. The lesions are erythematous or violaceous, edematous, indurated plaques that evolve into necrotic ulcers covered with a black eschar. They may be painful and pruritic, in contrast to ecthyma gangrenosum, which looks identical and evolves in quite a similar way. The diagnosis is confirmed by demonstrating dermal hyphae in a skin biopsy. Cultures of biopsied lesions may yield *Aspergillus flavus*, *A. fumigatus*, *A. niger*, or, less commonly, *A. terreus*. Blood cultures are rarely positive.

Secondary cutaneous aspergillosis may follow hematogenous spread of invasive aspergillosis in immunocompromised hosts, and presents as cutaneous maculopapular lesions, which eventually become pustular and evolve into ulcers covered with a black eschar. Diagnosis is again made by microscopy and culture of a skin biopsy, and blood cultures are more likely to be positive.

Serological tests for the presence of *Aspergillus* antibodies (precipitin test to detect precipitating antibodies) or *Aspergillus* antigens (latex particle agglutination test for *Aspergillus* galactomannan, a cell wall glycoprotein) in immunocompromised hosts are not very helpful in establishing the diagnosis and are frequently false negative, due to a delayed or absent immune response.

Treatment includes removal of any foreign body such as intravenous lines and catheters, local wound care, and intravenous antifungal treatment with liposomal amphotericin B (see Table 23.1).

Cutaneous cryptococcosis. This uncommon condition in immunocompromised patients may present as primary cutaneous cryptococcosis or the more common secondary cutaneous disease, which occurs in 10–15% of patients with disseminated disease.

There may be single or multiple nodules, vesicles, ulcers or abscesses, predominantly located on the head, trunk, or limbs, or small maculopapular lesions resembling molluscum contagiosum. The diagnosis is confirmed by demonstrating the organism, *Cryptococcus neoformans*, in aspirates from blister fluid and in cultured fluid, ulcer drainage, or skin biopsy specimens. In most patients with disseminated disease, latex agglutination tests for the presence of cryptococcal antigen in body fluids will be positive, although serology is usually negative. All patients with cutaneous cryptococcosis should be investigated for disseminated infection. Treatment with systemic antifungal preparations such as amphotericin B, with or without flucytosine, should be commenced as soon as possible.

Cutaneous histoplasmosis. This very rare infection with *Histoplasma capsulatum* is usually self-limiting in the general population, but in immunocompromised hosts it frequently progresses to disseminated disease. Cutaneous involvement is rare and presents as papules, plaques, and ulcers, which may progress to purpuric lesions and abscesses. Occasionally, aggressive erysipelas or cellulitis-like eruptions develop. The diagnosis is confirmed by demonstrating the organism in skin biopsies, as culture growth is too slow to establish an early diagnosis. Serological precipitin and agglutination assays are unreliable and often negative in immunocompromised patients. Treatment should consist of amphotericin B, often given in combination with flucytosine for its additive and synergistic effect.

Unusual fungal skin infections

Cutaneous trichosporonosis. In its mild form, infection with *Trichosporon beigelii* causes a superficial hair infection, which presents as firmly attached, irregular, soft, light-brown nodules along the midshafts of the hairs (white piedra). It particularly affects young adults and is found worldwide, although it is most common in tropical and subtropical regions. The diagnosis is confirmed by microscopic detection of the hyphae on the hairs, as well as culturing of the organism. The simplest treatment option is to shave or clip the hair in the affected area, followed by use of antifungal shampoos and topical application of clotrimazole or miconazole cream.

Secondary cutaneous trichosporonosis usually presents as multiple, erythematous, maculopapular or papulovesicular lesions, which may develop into necrotic ulcers. Histopathological examination of skin biopsies reveals the organism, and the diagnosis can be confirmed by positive blood cultures. Serological tests are often false negative. Intravenous amphotericin B is an effective treatment, provided the patient is not neutropenic.

Others. Cutaneous infections in the immunocompromised host may also be caused by an array of unusual fungi which were previously often considered as contaminants and whose classification creates great confusion. Examples are certain yeasts, such as *Geotrichum candidum*, *Rhodotorula*, *Saccharomyces*, and *Torulopsis* species, dermatophytic fungi such as *Alternaria*, *Curvularia*, and *Drechslera*, and hyaline molds such as *Fusarium*, *Penicillium*, and *Paecilomyces*. Skin lesions vary enormously, but many begin as a pigmented papule or vesicle, which subsequently progresses to necrosis. A positive culture is essential for the correct diagnosis, as on histological examination of skin biopsies it is often impossible to differentiate between the various types of fungal organisms. Amphotericin B is the drug of choice, with imidazoles for amphotericin-resistant cases.

Bacterial skin lesions

Gram-negative infections. Gram-negative bacilli such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are responsible for most serious skin infections in immunocompromised patients. Skin manifestations include cellulitis, subepidermal bullae, subcutaneous abscesses, and rarely toxic epidermal necrolysis (TEN).

Ecthyma gangrenosum, usually due to *P. aeruginosa*, is commonest in severely neutropenic patients. It begins as a painless, round, erythematous macule, which may have a small vesicle on its surface. Later it becomes indurated and bullous or pustular, and subsequently the skin sloughs and forms a gangrenous ulcer with a gray-black eschar surrounded by an erythematous halo. It is commonest in intertriginous regions, but may also appear on the extremities, face, or trunk. It may be associated with a high temperature, tachycardia, tachypnea, and hypotension. The diagnosis is confirmed by positive cultures from a skin biopsy specimen or blood cultures. Treatment is aggressive with high doses of intravenous carboxypenicillins, such as carbenicillin or ticarcillin, in combination with an aminoglycoside (see Table 23.1). Persistent neutropenia during treatment and multiple skin lesions are poor prognostic features.

Gram-positive infections. Skin infections by Gram-positive bacteria do not differ in character from those in immunocompetent patients, but may be more severe and are more often associated with systemic involvement.

Streptococcal infections. Streptococcal infection of the skin and subcutaneous tissue presents as cellulitis, an erythematous, hot, swollen area, often on the leg. There may be blisters and even skin necrosis with malaise, high fever, and rigors. Treatment consists of intravenous penicillin, although in some cases surgical debridement of severely necrotic areas may be necessary.

Staphylococcal infections. These can take various forms, as outlined below.

- Folliculitis. This is an infection of the superficial part of a hair follicle with *Staphylococcus aureus*, leading to a small pustule on an erythematous base, centered on the follicle. In mild forms, treatment consists of antibacterial washes and topical preparations (e.g., benzoyl peroxide, alone or in combination with topical antibiotics similar to those used in the treatment of acne). In severe cases, systemic antibiotics such as flucloxacillin or erythromycin may be required (see Table 23.1).
- Furunculosis ("boils"). This is characterized by painful, inflammatory nodules due to deep infection of hair follicles by *S. aureus* (Figure 23.13). Over a period of time, the lesion becomes fluctuant and once the central necrotic core has been discharged, the boil resolves. In immunosuppressed patients, treatment is with intravenous flucloxacillin.

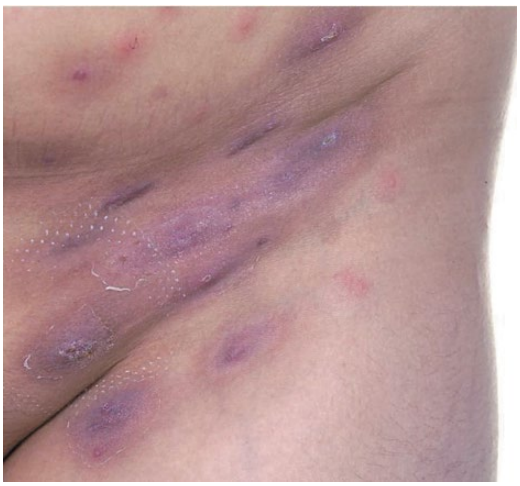


Figure 23.13 Staphylococcal infection leads to furunculosis (boils). Systemic treatment is required in immunosuppressed patients.

- Impetigo. This is a superficial skin infection caused by *S. aureus*, alone or in combination with hemolytic streptococci. There is an initial small blister or pustule, which rapidly increases in size, ruptures, and leaves a raw, exuding surface, which then dries and forms the typical golden-yellow crust. Impetigo may appear anywhere on the body, even in immunocompetent patients, and in immunosuppressed patients should be treated with systemic flucloxacillin or erythromycin.
- Staphylococcal scalded skin syndrome (SSSS). This is generally thought of as a disease of healthy infants and children, but when seen in adults it predominantly occurs in immunosuppressed individuals. It is caused by the production of a toxin by some staphylococcal phage types, which splits the dermis, causing the superficial epidermis to peel off and leaving a skin resembling severe scalding. In immunocompromised patients, it is often associated with severe sepsis, with positive blood cultures for *S. aureus*, and treatment should include a course of intravenous antibiotics (flucloxacillin).

Corynebacterial infections. Coryneforms, or diphtheroids, are normal skin commensals which cause minor problems in healthy individuals (e.g., erythrasma). Cutaneous infections in immunocompromised patients occur in severely neutropenic patients previously treated with broad-spectrum antibiotics. They mainly present as cellulitis or subcutaneous abscess at the site of a puncture wound or intravenous catheter. A few cases have been reported in which corynebacteria infection presented as an erythematous maculopapular rash, initially on the trunk, followed by diffuse spread and a more pustular appearance. Clinical diagnosis requires confirmation from blood and skin biopsy cultures. Treatment is difficult, as the organism is resistant to most antibiotics except vancomycin.

Mycobacterial infections. Both *Mycobacterium tuberculosis* and atypical mycobacteria may cause cutaneous disease. Primary skin infections, usually caused by the atypical mycobacteria *M. marinum*, *M. chelonae*, *M. kansasii*, and *M. haemophilium*, occur in normal and immunosuppressed hosts. The commonest presentation is a pigmented, granulomatous nodule with an erythematous halo on an extremity, although a wide variety of lesions, such as cellulitis or panniculitis, may occur. Systemic symptoms are not usually present, although in immunocompromised hosts the infection may disseminate to multiple cutaneous sites or ulcerate, leading to superinfection.

Skin manifestations of disseminated *M. tuberculosis* infection in immunocompromised hosts include recurrent episodes of skin and soft tissue abscesses of the extremities with symptom-free intervals in between and often minimal systemic symptoms. The diagnosis is confirmed by histological identification of acid–fast bacilli and positive cultures. Treatment should be with appropriate antituberculous chemotherapy.

Nocardial infections. *Nocardia* spp. infection, especially with *N. asteroides*, is strongly associated with immunosuppression and may be focal or disseminated. Skin manifestations include pustules, cellulitis, or ulcers, and are usually secondary to disease in the lungs. The diagnosis is often difficult, as *Nocardia* is not easily detected in cultures or histological sections. Treatment consists of adequate surgical drainage, if required, in combination with co-trimoxazole.

Cutaneous malignancies

Susceptibility to skin cancer after transplantation is multifactorial. Predisposing risk factors include older age at the time of transplant, lighter skin type, solar keratosis, greater sunlight exposure, higher rejection rate in the first year after transplant, and level of immunosuppression [18]. Research, mainly in adult renal transplant recipients, has indicated that genetic variation in the enzymes involved in free radical metabolism in the skin might also be associated with the development of skin cancer [19].

Cutaneous lymphomas occur with increased incidence in transplant recipients. They are predominantly of B-cell origin and in many cases are associated with EBV infection [20]. Cutaneous T-cell lymphomas have been rarely described in this group, and their etiology is ill understood. Reduced immune surveillance, chronic antigenic stimulation caused by transplant grafts, and the direct oncogenic effect of immunosuppressive drugs have all been suggested as mechanisms [21]. It is likely that immunosuppressive treatment (ciclosporin, tacrolimus) causes an imbalance in the T-cell regulatory systems, resulting in an expanded T-cell subpopulation. In both conditions, the early skin changes are

non-specific (Figure 23.14), but the later stages are characterized by exfoliative generalized erythroderma and lymphadenopathy. The definitive diagnosis is made by skin biopsy.

Non-melanoma skin cancer (NMSC) is increasingly recognized as a complication of long-term immunosuppression in solid organ transplant recipients [22]. Major contributing factors are high levels of sun exposure, drug-induced immunosuppression, and HPV infection. Skin cancer in transplanted children is extremely rare during childhood. In a study of pediatric transplant recipients in the UK, atypical pigmented lesions were identified, but skin cancer was not detected [23].

Dysplastic skin lesions in adult transplant recipients include actinic or solar keratosis, Bowen disease, squamous cell carcinoma, and, less commonly, basal cell carcinoma and malignant melanoma, all of which are uncommon in children. Warts, following infection with HPV, are common in both groups and rarely become malignant (warty dysplasia), particularly on sun-exposed skin areas [24].

Early detection and treatment of skin malignancies is necessary to avoid metastatic spread. Management strategies should focus on regular full-skin check and lymph node examination, aggressive treatment of established malignancies (including reduction in immunosuppression), and prophylactic measures to reduce the risk of additional photodamage and malignant transformation [25].

Preventative measures include minimizing immunosuppression and avoiding excessive sun exposure, and this advice should be routinely provided to parents and children undergoing transplantation. Children should be advised to use adequate sun block and wear protective clothing.

As it may be difficult to differentiate between benign and dysplastic lesions, any skin lesion that suddenly appears, changes color or size, becomes itchy or painful, or starts bleeding, oozing or crusting, should be seen by a dermatologist and a skin biopsy performed to confirm the diagnosis and commence appropriate treatment.

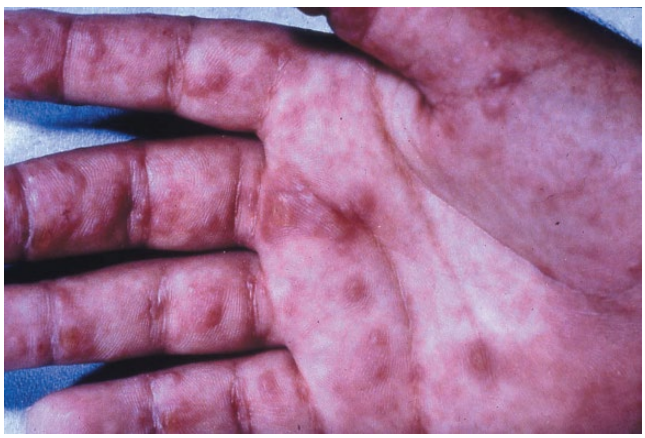
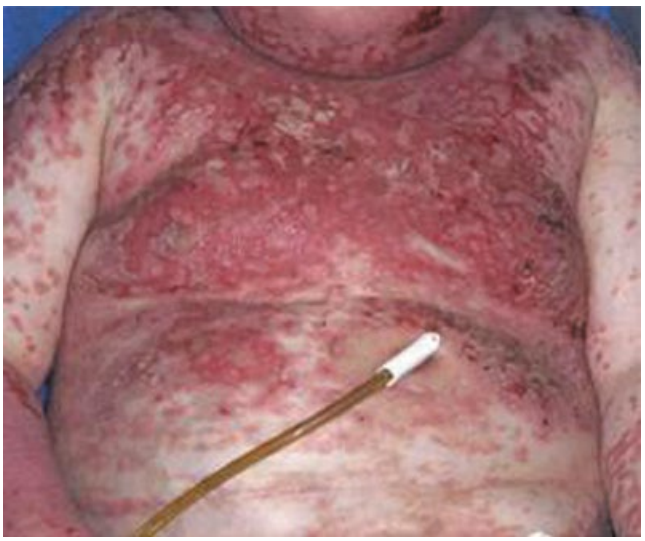


Figure 23.14 This papular skin rash was secondary to T-cell infiltration of the skin, which resolved on reduction of immunosuppression.

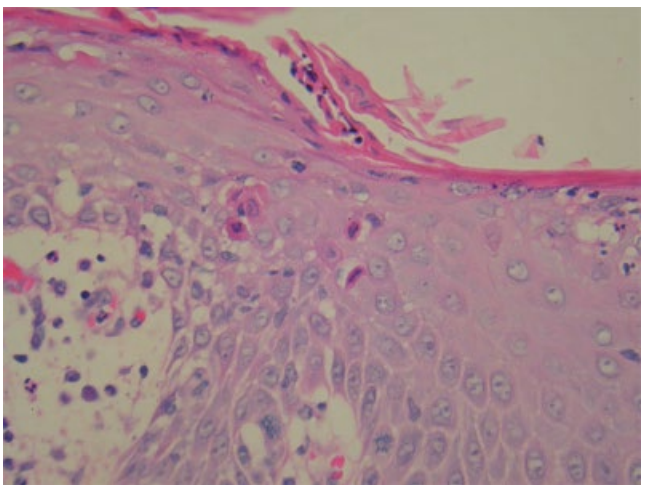
Graft-versus-host disease

Acute graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality following bone marrow transplantation, affecting the liver, skin, and gut (see Chapter 22). It is very rare following liver transplantation but common after intestinal transplantation [26].

Cutaneous acute GVHD presents between 7 days and 7 weeks after transplantation, with a maculopapular rash, which may look like measles, accompanied by palmar and plantar erythema and sometimes edema (Figure 23.15). The rash may be pruritic or feel like sunburn. The lesions coalesce and spread to the trunk, face, and extremities.



(A)



(B)

Figure 23.15 Cutaneous graft-versus-host disease (GVHD) presents with a maculopapular rash, which may be pruritic. (A) The lesions coalesce and spread to the trunk, face, and extremities. (B) A skin biopsy, which showed inflammatory changes and apoptotic keratinocytes centrally on hematoxylin–eosin stains, typical of severe GVHD. (Original magnification × 400.)

Sometimes the rash becomes bullous and progresses to toxic epidermal necrolysis.

The diagnosis can be confirmed by skin biopsy, which shows dermoepidermal clefts, necrosis of keratinocytes surrounded by lymphocytes ("satellite cell necrosis"), vacuolization of basal epidermal cells, and edema.

Cutaneous lesions in chronic GVHD are itchy, flat-topped, red-purple shiny papules resembling lichen planus. Later, the skin may thicken and look like scleroderma, with hyperpigmentation and hypopigmentation.

Non-specific skin rashes

Rashes in immunosuppressed patients vary enormously in their presentation and represent a wide variety of underlying diagnoses. Histopathological interpretation is difficult without an informed dermatological differential diagnosis. In some cases, the diagnosis may become more obvious at a later stage, and careful documentation, including medical illustration and review by a dermatologist, are therefore essential.

Liver disease induced by treatment of dermatological conditions

A number of frequently used treatments for common skin conditions may potentially induce liver dysfunction.

Chinese herbal medicine for atopic eczema

Chinese herbal mixtures have been used to treat atopic eczema for many years. Their efficacy has attracted public attention, and clinical trials in children and adults have shown marked benefit, although other reporters have not been able to replicate these results [27]. Hepatotoxicity, ranging from mild to acute liver failure, has been a problem with some Chinese herbal remedies, although the results are heterogeneous [28].

Methotrexate treatment of psoriasis

Methotrexate is a first-line systemic therapy for psoriasis, as it is highly efficacious for severe disease. However, chronic administration of methotrexate is associated with a risk of hepatic damage and hepatic fibrosis. Liver function tests (LFTs) are indicated before and during methotrexate therapy. If there are persistent elevations (two to three times normal) in LFTs, methotrexate should be discontinued until they normalize; if elevations persist for more than 2 months, a liver biopsy is indicated. Measurement of serum aminoterminal propeptide of type III procollagen (PIIINP) and/or a FibroScan®, might reduce the need for a liver biopsy, as they are markers of fibrosis [29].

Approach to diagnosis of dermatological lesions

A practical approach to the diagnosis of skin disorders in immunosuppressed children after transplantation is outlined in Figure 23.16. A dermatological history is essential, including the patient's own history and family history, as well as a detailed review of systemic and topical therapy, as previous treatment can be just as important in determining the cause of a rash. This, in combination with a careful documentation of the site and/or distribution of lesions and a description of the characteristics of individual lesions, may

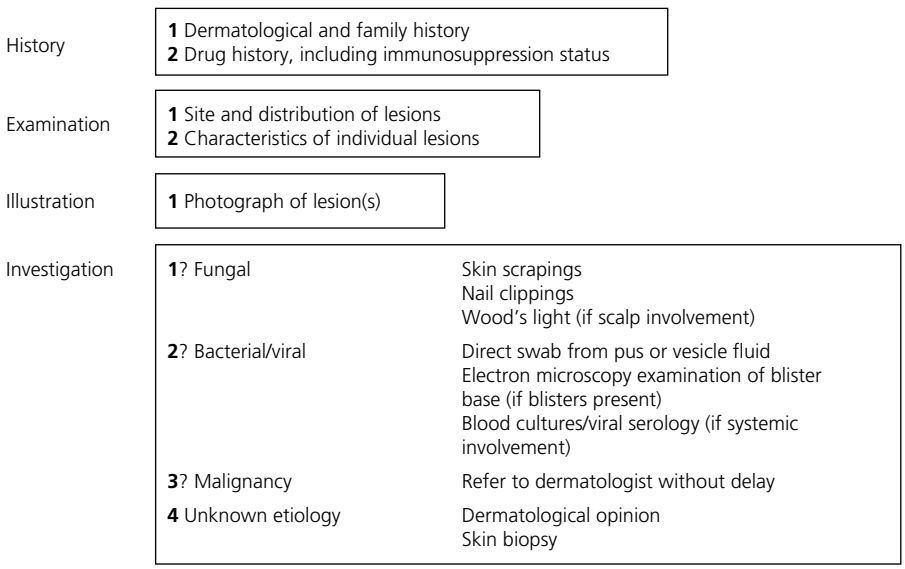


Figure 23.16 A practical approach to the diagnosis of skin lesions and rashes.

in many cases offer sufficient information for a correct diagnosis to be made. Further investigations and/or referral to a dermatologist are indicated if the patient is systemically unwell, if the diagnosis is not clear, or if there is suspicion of skin malignancy [30].

Techniques

Skin scrapings for mycology

The scaly edge of the lesion should be carefully scraped with a scalpel blade onto black paper and sent to the microbiology laboratory for microscopic examination and mycological culture.

Nail clippings

Nail clippings should be taken as proximally as possible, to optimize the chances of identifying fungus on microscopy or culture.

Scalp lesions

Scalp lesions should be examined under ultraviolet light (Wood's light) to detect fluorescence, although some ringworm species do not fluoresce. Plucked hairs from the edge of a lesion can be sent for microscopic examination and culture.

Skin cultures

Pus or vesicle fluid can be swabbed directly. Samples for bacteriological examination should be sent either dry or in a transport medium, while virological samples should be sent in a special viral transport medium. In the case of blisters, the base should be scraped for electron-microscopic examination.

Skin biopsies

A punch or incisional biopsy should be performed, preferably under local anesthesia. A fresh lesion should be selected for biopsy, as older lesions may show secondary changes, which make histological interpretation more difficult.

It should be established beforehand from the laboratory what transport medium is required. Formaldehyde is routine for light microscopy, but is unsuitable for fat-containing lesions – for example, xanthomas. Glutaraldehyde is usual for electron microscopy. If there is any doubt, and for skin to be cultured for microorganisms, the specimen should be placed on a saline-soaked gauze swab and conveyed directly to the laboratory.

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CHAPTER 24

Dental Care of Children with Liver Disease

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Key points

- Children with liver disease and liver transplantation require specialist pediatric dental care.
- They are more likely to have: enamel defects on their teeth; delayed eruption of primary teeth; intrinsic green staining of teeth and of the oral soft tissues from hyperbilirubinemia; tooth decay (dental caries); and drug-induced gingival overgrowth.
- Dental treatment is complex due to a bleeding tendency or immunosuppression post-transplant.
- Patients need lifelong oral surveillance, since they are at risk of oral cancer.

Formation and composition of enamel and dentine

Mature enamel is a crystalline material, in the form of hydroxyapatite, that is 96% by weight mineral and as such is the hardest calcified tissue in the human body. Enamel is laid down in an incremental fashion from the tip of the cusp to the gum margin, and it is possible to see a microscopic neonatal line marking the border between the enamel matrix that is formed before birth and that formed after. Hard tissue formation in the crowns of primary incisor teeth starts between the 13th and 16th weeks of intrauterine life and continues until enamel calcification is completed 3 months after birth.

Dental disease

Dental disease is common in children with liver disease or post-transplant. Families of children with liver disease are busy with hospital appointments and may not have had time to attend for regular dental check-ups. Also, the balance between poor fat metabolism and the nutritional needs of the child is usually such that a high concentration of sugar is required to provide the necessary calories.

The medical team can assist with caries prevention by encouraging attendance at the dentist, maintaining good oral hygiene, and the use of fluoride toothpaste. Of recent interest, is the possible beneficial effect of sonic-rotating toothbrushes in children with gingival overgrowth [1–3].

Children who have had invasive dental or medical procedures are more likely to be afraid of dental treatment and either avoid attending the dentist altogether or cooperate poorly. As such, they usually need specialist pediatric dentistry care that includes behavior management and sedation or general anesthesia.

Dental caries (tooth decay)

Tooth decay (caries) is the commonest disease of childhood in the world today [4] and is more prevalent in ethnic minority groups, and in children from low-income and single-parent families [5]. Caries occurs when bacteria in the mouth metabolize dietary sugars, they use the glycolytic biochemical pathway, resulting in (Krebs cycle) acids. These acids then cause demineralization and breakdown of the hydroxyapatite molecule and subsequent cavitation of the enamel and dentine (Figure 24.1). To prevent caries, sugar frequency needs to be reduced to only three or four times a day. So, limiting sugars to mealtime only is part of routine dental advice. Fluoride has a topical action on the tooth by being incorporated into the molecular structure as fluorapatite. Fluorapatite is less soluble in acid and so is more resistant to caries than hydroxyapatite.

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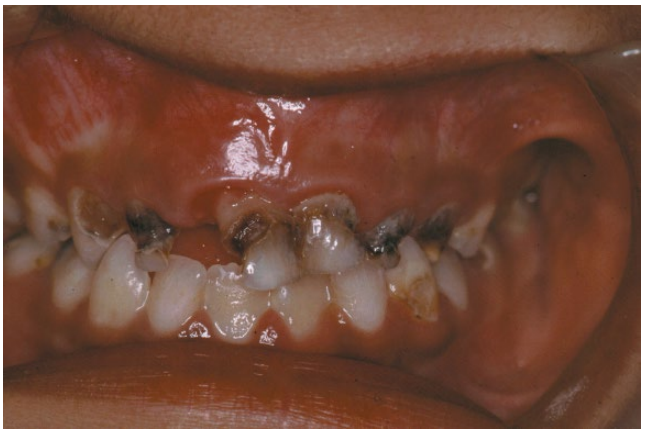


Figure 24.1 Anterior view of a 6-year-old child with rampant dental caries of the primary dentition.

Frequent administration of liquid medicines sweetened with sucrose, or dietary supplements containing sugars has significantly contributed to dental caries in children with chronic medical disorders. Unfortunately, there is also evidence to suggest that medically compromised children have a much higher proportion of untreated caries than the average child, brush their teeth less often, and seldom use fluoride supplements.

Children with liver disease often have sore mouths, gingivitis and tooth decay. Untreated tooth decay can lead to pulpal inflammation with subsequent loss of vitality and dental abscess. Therefore, children with liver disease may be more likely to require extraction of their teeth of poor prognosis if the caries is advanced.

Behavioral management techniques, even when conscious sedation is used, require patience and time. The progress of treatment has to be at the child's own pace but for many children with liver disease the time available for completion of the dental treatment is limited by the overriding urgency for transplant surgery. Therefore, dental treatment under general anesthesia is often the only option, but this does little to foster a future positive attitude towards dentistry and postoperative morbidity, especially oral pain when extractions are performed, is common. To avoid this, these children are best referred to a specialist pediatric dentist before problems arise so that their dental disease can be stabilized and preventive care initiated.

Dentists need to use high-speed suction during operative dental procedures to guard against ingestion of blood for children with liver disease and the dose of amide local anesthetic solutions (lidocaine/lignocaine) needs to be reduced. These children might also require antifibrinolytic agents, such as tranexamic acid to aid hemostasis following dental surgery or tooth extraction. Packing the sockets with a clot-inducing material such as oxidized cellulose polymer [6] is necessary. General anesthesia is best provided by an anesthetist suitably experienced in managing children with compromised liver function and children will have to be admitted as an inpatient so that the dental, anesthetic, and liver teams can liaise.

Periodontal disease

Diseases of the periodontal tissues begin in childhood. Dental plaque initiates an inflammatory reaction, modulated by the immune response, local microbial species, and genetic predisposition. Periodontal disease progresses from gingivitis to gradual loss of the attachment of the tooth to the surrounding connective tissues and bone, causing tooth mobility, bleeding, pocketing, and eventual abscess and tooth loss.

Prevention is by removing the dental plaque by tooth brushing and flossing. In children with gingival overgrowth caused by drugs such as ciclosporin and nifedipine, the tooth brushing is more difficult to perform since the pockets around the gum margin are deeper, so can be more difficult to clean. Parents are advised to supervise and to assist with tooth brushing for children up to at least 7 years of age. For children, with liver grafts, regular appointments with a dentist or a hygienist may be needed, especially if there is gingival overgrowth.

Prevention of dental disease

Children with liver disease should be labelled as "high-caries-risk". This is due to their enamel defects and highly cariogenic diet. The UK Department of Health Oral Health Improvement Toolkit contains evidence-based measures including: parental supervision of twice daily tooth brushing; regular dental check-ups; and reducing sugar frequency [7]. It is important that the parents are not confused by contradictory messages about diet; this requires a degree of pragmatism by the dental team and places a greater burden upon them to deliver stringent preventive care. It is helpful if the dietitian and physician can, where possible, limit the frequency of sugar and dietary supplements to mealtimes and prescribe sugar-free medications in order to reduce the incidence of dental caries.

Key points: caries prevention in a high caries risk child

This includes:

- Limit sugar frequency to 3 or 4 times daily.
- Use 1450 p.p.m. fluoride toothpaste twice daily.
- Fissure seal permanent teeth as soon as possible after they erupt.
- Visit the dentist every 4 months: this enables advice, scaling, and application of topical fluoride varnish.

The evidence for topical fluoride in caries prevention, either as toothpaste or varnish is overwhelming. A child who is a high caries risk should have their teeth brushed twice daily with a 1450 p.p.m. fluoride toothpaste. This should be spat out rather than rinsed out. Fluoride varnish should be professionally applied three times a year. Chlorhexidine delivered as a gel, varnish, or rinse reduces the bacterial load, and

possibly also the risk of bacteremia as well as preventing periodontal disease. All susceptible fissures, especially on adult molar teeth should be sealed using fissure sealants.

Managing active oral infection

When a child with liver disease or transplant presents with a dental abscess, antibiotics should be prescribed as part of the dental treatment to prevent systemic infection. The choice of antibiotics should be discussed with the patient's physician. A dental examination should be carried out as part of a pre-liver transplant assessment. Any active decay should be treated and any active oral infection must be eliminated.

The patient's physician should be consulted as to whether antifibrinolytic drugs, vitamin K, fresh frozen plasma, or other interventions are necessary. Because of bleeding risk, these children should ideally be treated in a hospital dental setting.

Key points: treating children with dental disease

This includes:

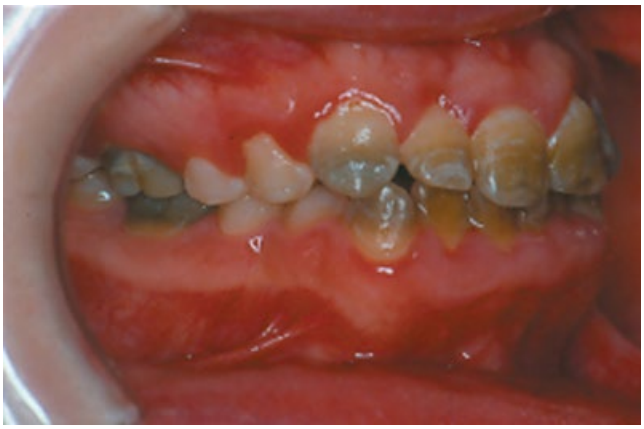
- Use of high-speed suction during operative dental procedures to guard against ingestion of blood.
- Limit the dose of amide local anesthetic solutions (lidocaine/lignocaine).
- Pack sockets and use an antifibrinolytic agent, such as tranexamic acid.
- General anesthesia needs to be in a hospital and in liaison with the liver team and by an anesthetist experienced in managing these children.

Intrinsic dental pigmentation

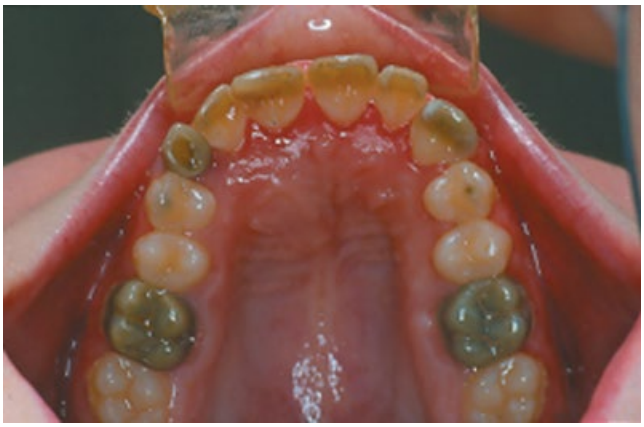
Dental discoloration can arise from extrinsic sources such as chromogenic bacteria, food pigment, dye, and tobacco. Intrinsic discoloration is found in many conditions, including: fluorosis, tetracycline ingestion, lepromatous leprosy, and hemolytic diseases of the newborn and congenital erythropoietic porphyria. The earliest reports of intrinsic green pigmented primary teeth were published over 50 years ago in children with hyperbilirubinemia caused by rhesus incompatibility.

Intrinsically pigmented green primary and permanent teeth, alveolar bone, and oral mucosa have been widely reported in children with liver disease. It is thought that this pigment is deposited in the enamel organ during tooth formation as biliverdin, the oxidation product of the bilirubin molecule, in those areas of dentine and enamel that were undergoing calcification during the period when the child was jaundiced and stops at the stage of root development that corresponded to the time when the child receives a liver graft.

However, owing to the very high mineral content of enamel and dentine, biochemical and histological examination is difficult and there are no radiographic or histological abnormalities associated with the pigmentation. The deposition of intrinsic stain may occur at any time during hard tissue formation but it is likely that it is mainly deposited after birth (Figure 24.2).



(A)



(B)

Figure 24.2 (A) Buccal view of the permanent dentition. (B) Maxillary view of the permanent dentition. This is the dentition of a 16-year-old girl with partial green intrinsic staining. Pigments were deposited during the formation of the incisors and first permanent molars at the time of birth. Subsequently developing premolar teeth are unaffected after transplantation.

Treatment of discolored teeth

There are a variety of treatment choices for improving the esthetics of discolored permanent anterior teeth. In many cases, there is a hierarchy of treatment options and these should be pursued in a logical, stepwise order. Vital bleaching is one of the simplest techniques, involving the external application of either 0.1–6% hydrogen peroxide or 10% carbamide peroxide gel. The application can either be applied by a dentist in the dental setting or by the use of custom-fitted trays by the patient at home [8]. The results have been shown to be variable, and are both untried in green-stained

teeth and unlikely to improve the appearance when the dentine is stained.

More advanced techniques involve the use of adhesive dental materials. These materials are now widely used to restore the esthetics of a child's discolored permanent incisor teeth. Composite restorations (white fillings) and porcelain veneers can easily be applied to the labial surface of the incisor teeth to camouflage unsightly intrinsic discoloration. There are many color-modifying adhesive materials which, used singly or in combination, can restore a child's smile throughout the formative years. These techniques are nondestructive of tooth tissue and the technique does not require local anesthesia. These veneers extend into the space between the teeth and underneath the gingival margin; it is therefore imperative that the child should have scrupulous oral hygiene, otherwise there will be increased plaque retention, leading to caries and periodontal disease. In adulthood, porcelain crowns may be the treatment of choice to mask the discoloration fully, but these cannot be provided sooner than the third decade of life, since not only is the immature dental pulp too large, risking exposure during crown preparation, but also the gingival contour shrinks back during adolescence, leaving unsightly crown margins visible if the definitive restoration is provided too early.

Enamel hypoplasia in children with liver disease

The mineralized enamel contains a history of the child's early life. Enamel does not remodel, and so disturbances during development remain in the tooth as a permanent record. Enamel hypoplasia is a quantitative defect of enamel, visually and morphologically identified as involving the surface of the enamel and associated with a reduced thickness. The defective enamel may have shallow or deep pits or wide or narrow grooves, arranged horizontally in a linear fashion or generally distributed around all or part of the enamel surface. Enamel defects can be hereditary or acquired and can be associated with numerous systemic disorders and low birth weight [9]. The presence of enamel defects in permanent teeth has been reported to be between 30% and 49%. These developmental defects of enamel may be classified as either hypoplasia or hypomineralization. Hypoplasia is a result of disturbance to the ameloblasts during matrix secretion. Hypomineralization occurs once the full thickness of enamel matrix is laid down and is a result of a disturbance during calcification or maturation. Both types of defect can exist together and it may be difficult to distinguish between them.

Enamel defects are much less common in primary teeth than their permanent successors. They have been attributed to various factors such as fever, local infection, nutritional deficiency, prolonged hypocalcemia, and steroid therapy. Trauma to the mineralizing primary teeth caused by pressure of the laryngoscope on the alveolar ridge during intubation has also been suggested as an etiological factor. Enamel defects may be more prevalent in children with liver disease. These hypoplastic primary teeth are more susceptible to caries, necessitating stringent preventive therapy [1].

Dental treatment of teeth with enamel defects

First permanent molars that have defective enamel are more susceptible to caries, and so dental management focusses on caries prevention by limiting sugar frequency and providing motivation toward good oral hygiene and the use of topical fluorides and fissure sealants. These teeth are sometimes extracted as part of an elective orthodontic treatment plan. Children with permanent incisor teeth that have an enamel defect usually complain about poor esthetics and this can also be something that they are being teased about in school. These teeth can be restored using the same bleaching and adhesive techniques as previously described.

Key points: children with liver disease and liver grafts

These children are more likely to have:

- Enamel defects on their teeth.
- Delayed eruption, especially of the primary teeth.
- Intrinsic green staining of teeth and of the oral soft tissues.
- Tooth decay (dental caries), especially those who have biliary atresia, due to their high-sugar diet.
- Drug-induced gingival overgrowth.

Guidelines for the dental management of children with liver disease

The following are suggested guidelines for the dental management of children with liver disease.

- Parents need active encouragement to ensure that their child receives lifelong dental care. Parents can become so overwhelmed by the medical treatment that they simply forget that continued dental care is vital to the health of the child. Early involvement of the dental team helps overcome this problem and enables parents and children to benefit fully from preventive care and acclimatization therapy.
- Children with liver disease are at risk of dental caries. They need early referral to a specialist pediatric dental service for coordination of their preventive therapy. Shared dental care may be appropriate with the primary dental care provider and the specialist pediatric dental service, to reduce the burden of care for these children. Preventive management will include dietary control, fluoride toothpaste and varnish,

fissure sealants, and oral hygiene instruction, perhaps using a sonic toothbrush.

- When nutritional support is required, it is helpful if where possible, dietary supplements are given at meal times and sugar-free medications should be prescribed.
- Dental treatment such as extractions and oral surgical procedures need to be carried out by a pediatric dentist in consultation with the liver physician, to ensure that the appropriate medical precautions are taken, e.g., antifibrinolytic agents. Antibiotic prophylaxis should only be given to those patients who have a cardiac condition and are at risk of infective endocarditis as per the UK National Institute for Health and Care Excellence (NICE) guidelines of 2008.
- Children who require treatment under general anesthesia need to be treated in a hospital in liaison with the liver team. Consideration should be given toward combining medical and dental treatments under the same general anesthetic.
- High-speed suction during operative dental procedures should be used to guard against ingestion of blood, since this may increase the risk for hepatic coma.
- The pediatric dentist needs to eliminate or stabilize oral infection. Active dental disease should be stabilized using temporary restorations in the first instance, especially if the child is very unwell. Unrestorable and pulpally involved teeth should be removed.
- Gingival overgrowth can be reduced with good plaque control, and removing the orthodontic bands may make it easier to maintain good oral hygiene.
- The dose of amide local anesthetic solutions (lidocaine/lignocaine) should be reduced.
- Parents should be made aware of the likelihood of the occurrence of intrinsic green discoloration and enamel defects, but should be reassured that there are techniques available to improve their smile.

Oral care of transplant recipients

Oral complications of immunosuppression include bacterial infection, oral candidiasis, aphthous ulcers, and reactivation of herpes simplex virus. The children might also have periodontal disease, delayed wound healing, and excessive bleeding.

Delayed eruption in pediatric liver transplantation

Delayed eruption of the primary dentition has been reported in 29–40% of pediatric liver-graft recipients [1], and as high as 48% in malnourished children. Figure 24.3 shows delayed emergence of upper primary incisors in a 22-month-old liver-graft recipient. Primary tooth eruption times do not vary greatly between populations or between genders. There are also other local causes such as ectopic crypt position, supernumerary teeth, and lack of space.

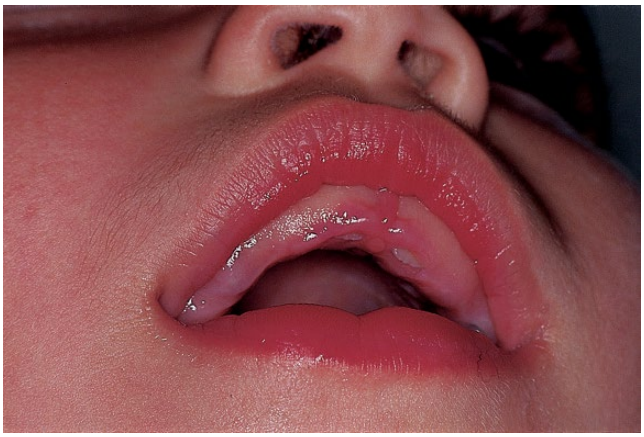


Figure 24.3 Delayed emergence of upper primary incisors in a 22-month-old child after liver transplantation. The upper central incisors are partially erupted, but there is no sign of the lateral incisors. This may be related to gingival overgrowth.

Tooth eruption ultimately depends upon the imbalance of forces acting on the tooth and the forces resisting its movement. Therefore, the resilience, texture, or thickness of the overlying mucosa may counteract the forces of eruption and impede the emergence of the tooth. Primary tooth emergence is related to general somatic growth, nutritional status, prematurity, and birth weight [10, 11]. Children with delayed eruption can catch up with their peers provided the nutritional difficulties have been resolved.

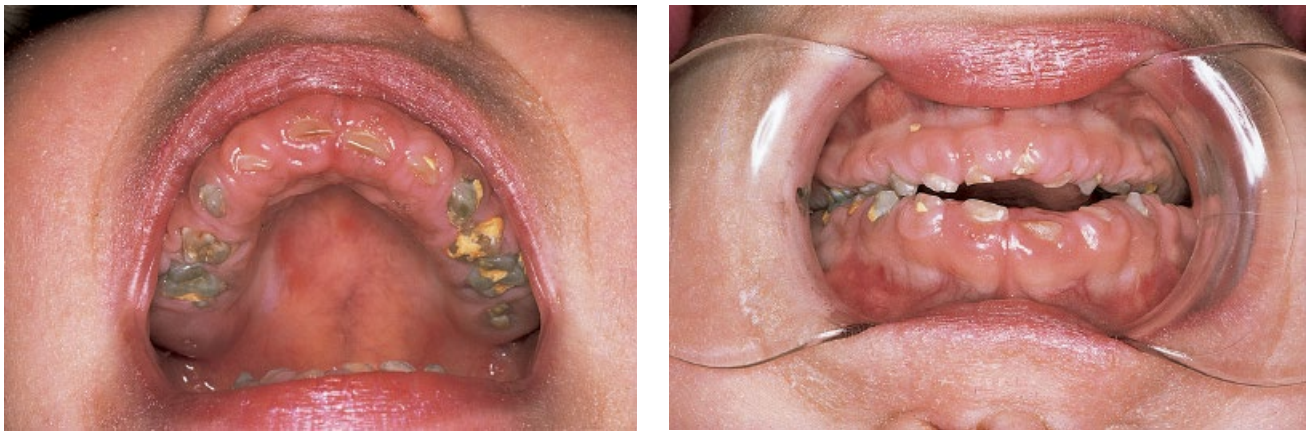
In some infants with liver grafts, teeth might "erupt" as normal through the bone but not 'emerge' into the mouth through the gum.

Immunosuppressive therapy and delayed eruption

Corticosteroid therapy is known to slow growth and skeletal maturation and possibly also to cause delayed eruption of adult teeth but there have been few reports of delayed eruption in primary teeth. Ciclosporin, although rarely used now, causes gingival overgrowth, and is implicated in tooth delay because it toughens the overlying mucosa but there is little research evidence to support this theory. Also, the delay could be due to the underlying poor nutrition.

Teething troubles and the management of delayed eruption

Teething rings, hard and fibrous foods to chew on, teething gels, and systemic analgesia are all useful to manage the pain that children experience whilst they are teething. Forewarning



(A) (B)

Figure 24.4 Severe gingival overgrowth after liver transplantation in a child receiving both ciclosporin and nifedipine. (A) Note the poor oral hygiene and calculus deposits. (B) The teeth are hypoplastic and have severe intrinsic green pigmentation from prolonged jaundice.

parents – especially parents of children who have been severely malnourished – of the likelihood of delayed eruption helps parents to manage their child better. Correcting the underlying medical condition, for example, discontinuing corticosteroid medication and providing nutritional support, is often all that is needed for the teeth to come through. Surgical intervention may be necessary in some cases, but a "wait-and see" approach might avoid an unnecessary and traumatic admission.

Ciclosporin-induced gingival overgrowth in transplant recipients

The supporting tissues of the teeth consist of the marginal and attached gingiva, the periodontal ligament, cementum covering the dentine on the root, and the alveolar bone. Periodontal diseases are a group of acute and chronic infections that affect these tissues. Such conditions often begin during childhood as inflammation of the gum margin, with redness, swelling, and bleeding on brushing. This is gingivitis. Children often worry when their gums bleed and this makes them brush less, rather than brush more thoroughly. Gingivitis increases between the ages of 5 and 9 years and is linked to plaque, debris, and calculus accumulation.

Gingivitis is worse when oral hygiene is poor, but it can be reversed with effective plaque control, namely tooth brushing. Drug-induced gingival overgrowth deepens the pocket between the gingiva and the tooth, so it increases the likelihood of plaque retention. This then makes plaque removal more difficult and leads to worsened gingivitis and pocketing. Gingival overgrowth is the only known oral side effect of ciclosporin therapy. The prevalence of ciclosporin-induced gingival overgrowth in the pediatric liver transplant population has been found to be between 51% and 100% [12]. This variation might be due to individual sensitivity and may be genetic. However, irrespective of the etiology, effective plaque removal by tooth brushing and flossing to get between the teeth are key [13–17] (Figure 24.4).

Key points: children with liver disease and transplants

These children need to:

- Use 1450 p.p.m. fluoride toothpaste to combat tooth decay.
- Brush well to combat gingivitis, especially when they have drug-induced gingival overgrowth.
- Be reassured that healthy gums (even those with overgrowth) *don't* bleed.

Pathogenesis of gingival overgrowth

Ciclosporin-induced gingival overgrowth is firm and pink, with focal lobulations and a stippled surface consisting primarily of a highly vascularized connective tissue with an overlying irregular, multilayered, parakeratinized epithelium of variable thickness. The predominant feature is a proliferation of collagen fibers in the corium, which are lightly distributed, in a foamy basophilic ground substance. There is a marked plasma cell, macrophage, and T-cell infiltrate, and modification of the appearance of gingival fibroblasts. Gingival overgrowth may be caused by a subcellular imbalance between tissue formation and degradation, leading to an accumulation or inhibition of fibroblast matrix that may be modulated by inflammation and growth. No clear relationship has yet been found between ciclosporin dosage/serum trough level and gingival overgrowth.

The risk of ciclosporin-induced gingival overgrowth is greatest in adolescents, which may be due to the added effect of growth hormone, estrogen, and progesterone. The effect of the duration of ciclosporin therapy on gingival overgrowth in humans has not been established, although in young

children this might be the most critical factor. The overgrowth reduces once ciclosporin is discontinued.

Plaque and ciclosporin-induced gingival overgrowth

The severity of ciclosporin-induced gingival overgrowth is related to gingival irritants, such as dental plaque, calculus, imperfections in dental restorations, orthodontic appliances, and the effects of mouth breathing. Certainly, there is an association between poor oral hygiene and gingival overgrowth but this might not be the only etiological factor [17]. It is possible that the overgrowth is due to genetic predisposition and that the plaque is an environmental factor that modulates the response. Clearly, more studies are required in order to clarify these relationships.

Gingival overgrowth, Neoral®, tacrolimus, and nifedipine

The Neoral preparation of ciclosporin has improved bioavailability but might lead to increased gingival overgrowth. Tacrolimus causes less gingival overgrowth but, not in every case, and can cause oral ulcers and reports of oral "numbness or tingling." Nifedipine is a known cause of gingival overgrowth, and it also acts synergistically with ciclosporin (see Figure 24.4). This may be due to similarities in the mechanism of action of the two drugs at the cellular level [12, 18, 19].

Management of gingival overgrowth

Children with liver grafts need to have excellent oral hygiene. The health of their periodontal tissues needs to be monitored throughout their lives, and they need constant encouragement to keep up the best possible standard of oral cleanliness. Poor oral hygiene and periodontal disease predispose to transient bacteremia that can harm the solid tissue graft. Chlorhexidine oral gel may be helpful, especially if the gingivae are already so swollen, bleeding, and painful that the child finds it difficult to brush the affected area. The chlorhexidine oral gel could be used on a toothbrush instead of toothpaste or applied using a finger or soft cloth. However, since it is the fluoride in toothpaste that prevents tooth decay, it is important that tooth brushing with a fluoride toothpaste still continues.

Metronidazole and azithromycin have been shown to reduce gingival overgrowth. Azathioprine may exert a protective effect directly through its anti-inflammatory properties or perhaps there is an improvement because it reduces the ciclosporin dosage [18]. For a few, surgical excision of the overgrown tissue (gingivectomy) or gingival recontouring (gingivoplasty) may be required. This improves esthetics and reduces plaque retention since it allows more effective plaque removal. Alternatively, a change of immunosuppression to tacrolimus or mycophenolate mofetil (see Chapter 31) may be beneficial but this does not always happen and persistent overgrowth can occur.

Prophylactic antibiotic therapy

Prophylactic antibiotic therapy is no longer indicated for dental surgery pre- and post-liver transplant. This is following changes to the guidelines on prophylaxis in patients undergoing dental treatment with cardiac conditions [20].

Key points: children with liver disease and liver grafts

These children are more likely to need the following:

- Extra time to treat them.
- Prevention of oral disease: 1450 p.p.m. fluoride toothpaste twice daily brushing. Fluoride rinses, chlorhexidine gel, and reduced sugar frequency whenever feasible.
- Antibiotic prophylaxis.
- Coordination regarding immunosuppressant and dietary advice.
- General anesthesia in a hospital and combining the pediatric dentistry and liver teams.
- Lifelong oral surveillance, since they are at risk of cancer.

Guidelines for the dental management of pediatric liver-graft recipients

The dental management of pediatric recipients of liver transplants should be based on the following guidelines:

Before liver transplantation

- Good oral hygiene is the most important factor in reducing the risk of significant infection in susceptible individuals. Intensive oral hygiene therapy, preventive care, and careful parental counseling are of paramount importance if good oral health is to be maintained. This is best achieved when liver transplantation is first contemplated, since dental care is then seen by the family as being an integral part of the child's treatment.
- It is important that each child who is likely to be scheduled for liver grafting has a full dental examination, so that potential sources of infection can be treated and preventive therapy commenced. Where possible, active treatment should be completed before transplantation; this includes the removal of any teeth that are of dubious prognosis, since these may become a source of infection in the immunocompromised child.
- Children who already attend their local general dental practice regularly should be advised to continue, in order

to "normalize" their lives as much as possible. Nevertheless, oral care still needs to be coordinated by a pediatric dentist who is not only skilled in the management of medically compromised children but is also capable of coordinating the various medical and dental specialties into a framework for the better oral care of the child.

After liver transplantation

- Following liver transplantation, patients require routine dental check-ups, topical fluoride varnish application, and reinforcement of preventive advice. Tooth brushing can be complemented with a chlorhexidine oral rinse or gel, and the use of a sonic toothbrush might be suggested. A 1450 p.p.m. fluoride toothpaste should be recommended, especially if the child lives in a non-fluoridated area, to reduce the risk of tooth decay.
- Parents should be made aware that delayed eruption of the primary dentition sometimes occurs, and palliative treatment – for example, the use of teething rings – should be recommended. The development of the dentition should be monitored, but parents can be reassured that these teeth generally do erupt eventually and often without recourse to surgical intervention.
- Gingival overgrowth in liver-graft recipients is most common and severe in adolescents. Good oral hygiene is vital to reduce the risk of periodontal disease. A sonic toothbrush may be helpful in this cohort.
- The long-term use of immunosuppressive drugs and other treatments puts transplant patients at risk of developing cancers, including cancers of the oral cavity. Regular surveillance of the head and neck region should be performed and biopsy new oral lesions that could be suspicious.

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SECTION 11

Surgical Disorders of the Liver and Bile Ducts

CHAPTER 25

Biliary Atresia and Other Causes of Surgical Jaundice in Infancy

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Key points

- The commonest cause of surgical jaundice in the newborn is biliary atresia.
- The key to its recognition in the community is to demonstrate that prolonged jaundice beyond 14 days is conjugated in nature.
- Definitive diagnosis needs to be expeditious and timely and may involve a number of tests of exclusion with confirmation only occurring at surgical exploration.
- Kasai portoenterostomy is potentially beneficial in >95% of infants.
- Clearance of jaundice should be achieved in >50% with corresponding long-term native liver survival.
- Other surgical causes (in the newborn) include cystic choledochal malformation, inspissated bile syndrome, and spontaneous perforation of the bile duct.

Biliary atresia

Biliary atresia (BA) is one of the key defining diseases dealt with by the pediatric hepatologist and hepatobiliary surgeon. Its origins are mysterious, its diagnosis contentious, and its outcome uncertain.

Modern management of this disease has evolved but is essentially unchanged since the 1980s involving an initial attempt at restoration of bile flow and salvage of the native liver with the Kasai portoenterostomy (KPE) and if that fails or complications ensue then liver transplantation offers the best chance of survival. Long-term survival with a normal life is possible with KPE or a functioning transplant.

Background

The first clear description of BA in the English language literature was by an Edinburgh physician, John Thompson, writing in 1891. His patient, a girl, was born and swiftly developed pale white stools, dark urine, and became increasingly jaundiced. She died at about 4 months of age and the postmortem showed a cirrhotic liver, a normal but collapsed gallbladder attached to a patent common bile duct, and two small bile-containing cysts in the porta hepatis, with effectively absence of the common hepatic duct.

William Ladd from Boston, USA, described operating on 10 infants with "surgical" jaundice, some of which were attributable to BA, in 1928. This sparked the first phase of management involving surgical exploration of the biliary tact to identify patent biliary remnants containing bile and hence suitable for a relatively straightforward bilio-enteric reconstruction. Unfortunately such "correctable" cases were few in number and the remainder were considered inoperable and left to die. However during the 1950s, a Japanese surgeon Morio Kasai (1922–2008) (Figure 25.1) developed a more aggressive approach to dissection higher in the porta hepatis simply removing all visible extrahepatic parts and anastomosing a Roux loop to an apparently solid transected portal plate [1]. As it turned out even that which looked apparently solid would still contain microscopic biliary ductules retaining a connection to the intrahepatic biliary tree. If enough of these were uncovered then bile flow could be restored. Results were still unpredictable and in a significant proportion of infants a complete failure but eventually it became widely adopted worldwide by the 1970s even in initially sceptical North American and European institutions.

Liver transplantation, the other pillar of current treatment programs, came into reality with the first transplant performed by Thomas Starzl (Figure 25.2) in Denver, Colorado,

Diseases of the Liver and Biliary System in Children, Fourth Edition. Edited by Deirdre A. Kelly.

in 1963 in a 3-year-old girl with BA and end-stage liver failure. This too was actually a failure with his patient dying on the operating table but lead to the development of transplant programs in the 1960s which then receded due to lack of effective immunosuppression protocols. The identification and usage of ciclosporin as a truly effective immunosuppressive in the early 1980s enabled further transplant programs and led to our current "seamless" approach to management [2].

Figure 25.1 Morio Kasai (1922–2008).

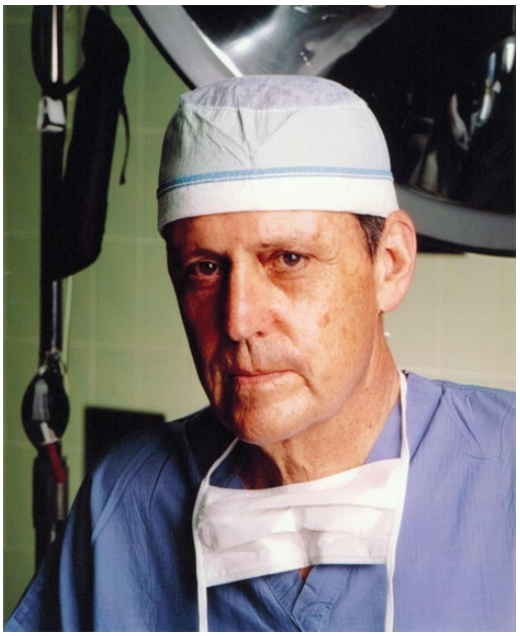

Figure 25.2 Thomas Starzl (born 1926).

Classification and etiology

BA is best described as an *occlusive pan-ductular cholangiopathy* affecting both intra- and extrahepatic bile ducts. There are two ways to classify this complex disease.

Pathological classification

This is the commonest and most traditional classification whereby BA is divided into three types based on the most proximal level of occlusion of the extrahepatic biliary tree (Figure 25.3). In types 1 and 2, there is a degree of preservation of structure in the intrahepatic bile ducts but they are still irregular, deformed, and pruned – and do not dilate, even when obstructed. Type 3, the commonest variant, is typically a solid dense fibro-inflammatory proximal remnant at the porta hepatis. The distal duct may be atrophic, absent or relatively well preserved – typically as a mucocele. Type 3 intrahepatic bile ducts are usually grossly abnormal with myriad small ductules coalescing at the porta hepatis. Sometimes this can be visualized radiographically as a "cloud" (Figure 25.4).

Extrahepatic cyst formation may be evident and contains clear mucus or bile (depending on preservation with intrahepatic bile ductules). This is termed cystic biliary atresia (CBA) and it is important to distinguish it from simple obstruction in a cystic choledochal malformation (CM) [3]. In order to make this distinction conclusively, a cholangiogram is required which will show a distorted, deformed non-dilated intrahepatic duct system (if anything) in the former

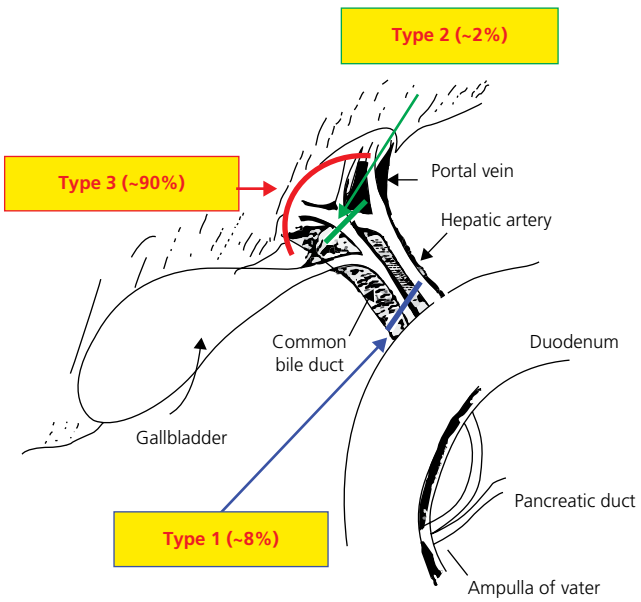

Figure 25.3 Classification of biliary atresia.

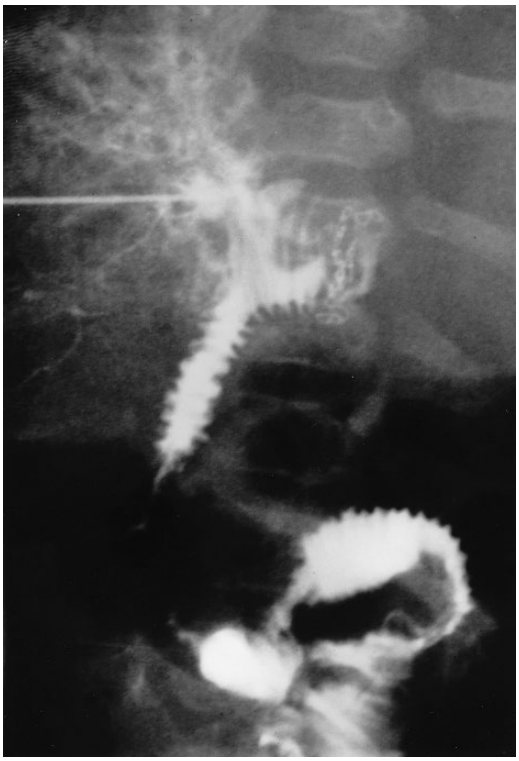

Figure 25.4 A percutaneous cholangiogram in a child who had previously undergone a Kasai portoenterostomy. There is free flow of contrast into the Roux loop. The bile ducts are very abnormal and show the typical cloud-like appearance of biliary atresia.

and a well-preserved and "tree-like" dilated intrahepatic duct system in the latter.

Phenotypic classification

Traditionally BA was described as occurring in two forms – perinatal and embryonic – but these terms are confusing and as more is discovered about the underlying etiology, the classification of BA is based on phenotypic characteristics and perceived etiology [4, 5]. Four variants are now recognized, and there may be more.

Isolated biliary atresia

This is the largest group for which there is no clear etiology. It appears to have a late onset in bile duct development as no other system/organ is affected. One hypothesis is that as the intra- and extrahepatic bile ducts develop from different sources with differing timescales, linkage must occur to provide for luminal bile duct patency from canalicular membrane to duodenum. This is achieved at 10–12 weeks of gestation and if disturbed perhaps BA is one consequence. Alternatively, bile ducts may form completely normally in the first trimester but obliteration occurs at some later stage, possibly even postnatally. This may be associated with a possible "neonatal hepatitis" type picture, or postnatal destruction of the extrahepatic biliary tree could occur secondary to ischemic insults affecting the bile ducts or following biliary duct perforations. However, in these cases the intrahepatic ductules dilate which does not occur in "true" BA.

Other circumstantial evidence of the prenatal origins of isolated BA has been provided by studies which found low levels of the hepatic specific enzyme γ-glutamyl transpeptidase (GGT) in amniotic fluid in the second trimester in infants who later turned out to have BA.

Table 25.1 Biliary atresia splenic malformation (BASM) syndrome.

Components	Feature
Splenic	Polysplenia, double spleen
	Asplenia
Abdominal situs	Situs inversus
Cardiac	For example, atrial septal defect, ventricular septal defect, tetralogy of Fallot, atrial isomerism
Vascular	Preduodenal portal vein
	Absence of vena cava
	Absence of portal vein
Gastrointestinal	Malrotation, annular pancreas

Biliary atresia splenic malformation syndrome

This syndrome encompasses BA, splenic anomalies (usually polysplenia), vascular anomalies (usually preduodenal portal vein and absence of vena cava), visceral asymmetry (usually situs inversus), and cardiac anomalies [6] (Table 25.1). There is a female preponderance and it is linked to maternal diabetes and several other early embryonic events such as in vitro fertilization [6]. The formation of the extrahepatic duct occurs from 20 to 38 days, in tandem with key events in the formation of the heart, spleen, determination of situs, etc. Postnatally these infants typically have no common bile duct, a tiny atrophic gallbladder, and no evidence of inflammatory response. The liver is also usually symmetrical, whatever the nature of the abdominal situs. Furthermore, despite the protracted timeline the liver parenchyma appears entirely normal at birth [7].

Biliary atresia splenic malformation (BASM) syndrome is the variant that is most likely to have an underlying genetic defect. Mutations in the *CFC-1* gene (Ch2q 21.1 loci) have been found in 50% of infants with BASM in one French study [8]. The gene encodes for CRYPTIC protein which is also related to disorders of heterotaxy and cardiac anomalies (transposition of the great vessels/double outlet right ventricle). It may also have a role in mesoderm and neural patterning during gastrulation.

A smaller number of infants with BASM have immotile cilia syndrome (Kartagener syndrome) which provides an interesting speculation about mechanism. Dysfunctional cilia could be incriminated in determination of visceral situs, but how cilial dysfunction interacts with the developing biliary tree is not known. Normally, only rats and squirrel monkeys have ciliated intrahepatic bile ducts, although there may be chemosensory cilia on cholangiocytes in humans.

There are other syndromic associations which are not within the BASM spectrum. Thus cat-eye syndrome characterized by coloboma, anorectal atresia, and chromosome 22 aneuploidy has been described.

Finally, other more common congenital abnormalities such as esophageal atresia, jejunal atresia, and anorectal malformations arise more commonly than would be expected by chance in infants with otherwise isolated BA.

Cystic biliary atresia

This subtype accounts for about 10% of all BA cases and is caused by extrahepatic cystic formation in an otherwise obliterated biliary tract. The cyst may be filled with bile or mucus depending on the degree of preservation of the connection with the intrahepatic ducts. The largest can be detected antenatally. In our series [3], 50% were detected between 18 and 20 weeks' gestation at maternal antenatal ultrasound screening. It is important that this type of BA is not confused postnatally with an early obstructing CM and precise diagnosis can only be confirmed at surgery with a cholangiogram. Various patterns of intrahepatic ducts can be recognized including that of a grossly abnormal, irregular, pruned tree or it can have a cloud-like appearance caused by multiple interconnections of the filamentous intrahepatic biliary ductules. There is no ethnic or genetic preponderance and there is a better outcome following KPE.

Cytomegalovirus-associated biliary atresia

The proposal that viruses may be responsible for numerous infantile cholestatic conditions is attributed to an American pediatrician, Benjamin Landing. Since then a number of candidate viruses have been suggested that may trigger bile duct injury. These include human papillomavirus, Epstein–Barr virus, cytomegalovirus (CMV), rotavirus, and reovirus. The last three viruses have been the most frequently studied in BA.

Rauschenfels *et al.* looked at 74 infants with BA and screened for a panel of 11 viruses using RNA/DNA profiling. A minority of BA infants (42%) showed some evidence of intrahepatic viral RNA or DNA (33% reovirus, 11% CMV). This group, however, concluded that viruses were not "main players" in the etiology of BA, but were merely a secondary phenomenon [9].

CMV is a double-stranded DNA virus of the Herpesviridae family that can infect biliary epithelial cells and hepatocytes and occasionally but not commonly CMV inclusion bodies can be seen. This has been the subject of most recent studies on the perceived relationship between viruses and BA. Interestingly while there are many animal models of BA the vast majority of these are focussed on rotavirus rather than CMV [5].

In a group of 210 infants with BA, 20 (9.5%) of the patients were CMV IgM positive at the time of presentation. CMV-positive infants were characterized by late presentation, more deranged biochemical derangement, and a non-white origin. They had a higher degree of inflammation and fibrosis on liver histology, even when corrected for age and a different T-helper cell profile compared to those who were CMV IgM negative [10, 11].

It seems likely that viral pathogens are present in up to 60% of BA livers. It is possible that if the virus is cleared quickly from the liver, perhaps within the first few weeks of life as occurs in animal models then the actual role of viruses in BA may be even more significant. The potential for antiviral therapy exists but no convincing studies have been published to date.

It is possible that the virus acts as a trigger, activating an immune-mediated destructive process. This inflammatory process would be expected to continue post-KPE and there is some evidence for this. Levels of cellular adhesion molecules and proinflammatory cytokines remain higher and continue to rise up until at least 4 months after surgery [12].

A novel mechanism of immune damage has been recently suggested based on the observation that male BA infants have a three-fold increase in maternal origin cells in their livers. These were later shown to be maternal origin chimeric $CD8^+$ T cells and CD45 natural killer (NK) cells and appear capable of initiating immune cholangiolar damage. This phenomenon has been termed *maternal microchimerism* and it may be the reason that the destructive process is time limited.

Genetics

A number of genes are important in both bile duct development (e.g., *JAG1, HNF-6*) and visceral and somatic symmetry (e.g., *INV*, *CFC-1*) although the animal work does not correlate well with mutations in humans. A possible genetic link with mutations in the *CFC-1* gene has been mentioned previously in infants with BASM [8]. Transforming growth factor β (TGF-β) pathways have been shown to play an important role in liver fibrosis in experimental BA models and analysis of TGF-β-related gene transcripts confirm dysregulation of the upstream regulators of TGF-β function and downstream end products.

A large study from China genotyped 500,000 single nucleotide polymorphisms (SNPs) in 200 BA patients and 400 controls [13]. The top 10 candidate SNPs were further analyzed. They discovered a strong association of BA with a SNP located on chromosome 10q24. There are two genes located near this SNP which could be influenced. Firstly, *XPNPEP1* (X-prolyl aminopeptidase) which is expressed on biliary epithelia and is involved in the metabolism of inflammatory mediators. The second gene which is appearing to be more active in BA is *ADD3* (adducin 3) which is expressed in hepatocytes and biliary epithelia. Defects in the gene may lead to excess deposition of actin and myosin and resultant biliary fibrosis. The association of *ADD3* and BA in the white, North American/European population has also been shown.

Cellular kinetics and inflammation in biliary atresia

BA is not simply a mechanical obstruction of the biliary tree as there is a marked inflammatory process in most types (possibly with the exception of BASM) with an obvious mononuclear infiltrate and expression of a variety of adhesion molecules on intrahepatic biliary and vascular epithelial surfaces. It is not clear whether this is a primary or secondary [14].

There is abnormal expression of cell adhesion molecules (proteins involved in cell–cell binding) with both intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 (but not E-selectin) identifiable on epithelial structures in both liver, and to a lesser extent the biliary remnant. The mononuclear infiltrate itself is largely composed of $CD4^+$ T lymphocytes (specifically Th1 and Th17), and $CD56^+$ NK cells, which exhibit markers for proliferation ($CD71^+$) and activation (particularly LFA-1^+ but also $CD25^+$) [20, 28]. There is a distinct subset of $CD8^+$ cells but many studies suggest these may be less important as they lack markers of activation such as perforin, granzyme B, and Fas ligand.

Increased levels of the soluble adhesion molecules ICAM-1 and VCAM-1 were detected in the circulation at the time of Kasai together with rising levels of inflammatory cytokines (e.g., interleukin (IL)-2, tumor necrosis factor (TNF)-α) postoperatively [12]. After about 6–9 months post-Kasai, these return to more normal values (unpublished observations).

The resident (Kupffer cells) or recruited macrophages/monocytes have a crucial role in the development of fibrosis in established BA. They may be involved as both the presenters of antigenic material in the first place or the initiating driver for fibrosis. Increased levels of both $CD68^+$ cells and its circulating markers (TNF-α and IL-18) impair the prognosis post-Kasai [12].

Epidemiology

As BA is a diverse disease it is not surprising that its epidemiology also varies. Infants with *developmental BA* (i.e., cystic BA and BASM) have a marked female predominance not seen in the *isolated BA* group [3, 6, 15]. Historically there has been a suggestion of a seasonal variation in incidence, though this has not been confirmed in large national studies [15, 16]. The implication is that if more infants with BA were born during the winter months this could be related to the winter prevalence of viruses.

The incidence of BA varies according to geography. Highest incidences are reported from Asia, particularly from Taiwan (1 in 5000 live births), Japan, and mainland China. In the UK, Ireland, and Europe the incidence is about 1 in 17,000–20,000 [15, 16] which is similar to the incidence in North America although national studies are lacking. There are significant regional differences within England and Wales with some areas having unexpectedly high incidences which may be a reflection of the multiracial nature of the UK. Developmental BA was more common in infants of white origin, for instance [15].

Clinical features

Antenatal diagnosis is possible in the small number with cystic BA (<10%). Another subset of the developmental group can present within the first week of life, but the diagnosis is usually made when being investigated/treated for an associated anomaly, e.g., at laparotomy for jejunal atresia/malrotation.

Birthweight is normal in most infants with BA (either developmental or non-developmental origin) [15]. However, they may then fail to thrive due to reduced fat absorption. Deficiencies in the fat-soluble vitamins A, D, E, and most importantly K may lead to a bleeding tendency (occasionally catastrophic) with an elevated international normalized ratio (INR) or prothrombin time.

Otherwise the key features are conjugated jaundice together with pale, unpigmented stools, and dark urine (bilirubinuria) in an otherwise healthy neonate. Liver fibrosis and cirrhosis develop later even in infants with developmental BA. Ascites and marked hepatosplenomegaly are uncommon and not usually seen until after about 3 months.

Differential diagnosis

The differential diagnosis of conjugated jaundice is discussed in Chapter 8. It includes TORCH infections (e.g., toxoplasma, rubella, cytomegalovirus, hepatitis, etc.); genetic conditions (e.g., α1-antitrypsin deficiency, Alagille syndrome, progressive familial intrahepatic cholestasis (PFIC) disorders), metabolic conditions (e.g., cystic fibrosis, galactosemia), and parenteral nutrition induced liver disease.

The surgical differential diagnosis includes obstructed CM, inspissated bile syndrome (usually affecting preterm infants), and spontaneous perforation of the bile duct [17] (Figure 25.5). The key feature in these disorders is that the intrahepatic bile ducts dilate when obstructed and should be distinguishable on ultrasound.

Laboratory findings

Liver biochemistry is non-specific and demonstrates conjugated jaundice (variable, but rarely >250 μmol/L), modestly raised transaminases (aspartate aminotransferase (AST) >100 IU/L), and significantly raised GGT (>200 IU/L). A recent study that looked retrospectively at 31 infants with

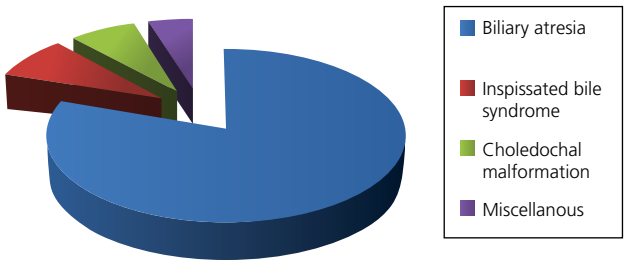

Figure 25.5 Causes of surgical jaundice (n = 171) based on King's College Hospital series (1992–1999). (Values taken from Davenport *et al.* 2003 [17].)

BA who had their split bilirubin levels analyzed at <48 h of age found that most had elevated conjugated bilirubin at this early age suggesting established biliary pathology at the time of birth [18].

Bile acids assayed on the screening heel prick Guthrie cards were elevated in 77% of infants with BA. These results show potential for a screening tool if proved sensitive enough and cost effective.

The AST/platelet ratio index (APRi) is a surrogate marker for liver fibrosis. In a cohort of 260 infants with BA [19], APRi correlated with age at surgery, bilirubin, and spleen size, and was significantly raised in CMV-positive immunoglobulin M (IgM) infants. Native liver survival was significantly improved but only in those with the lowest quartile of APRi values (<0.43). Its use in defining "cirrhosis" at the time of laparotomy showed that using a cut-off of >1.2 there was a sensitivity of 75% and specificity of 84%. Whether this helps the decision to offer a KPE or go straight to transplantation is debatable.

Screening for biliary atresia

The most established screening program for BA is in Taiwan where the incidence is 1 in 5000 live births. This involves giving a stool color chart to each new mother for comparison with their infant's stool. Early referral and investigations are arranged if pale stools are recognized. This has reduced the average time to surgery in Taiwan to below 50 days of age and improved outcomes.

Radiology

Ultrasound is the primary investigation typically showing a shrunken, atrophic gallbladder with no evidence of filling between feeds. About 20% will show a "normal" gallbladder – which turn out to be a mucocele of the gallbladder in continuity with a relatively preserved common bile duct and often absence of common hepatic duct. Ultrasound usually excludes other causes of surgical jaundice (CM, inspissated bile) as these invariably demonstrate a dilated intrahepatic duct system or CBD. A more specific but controversial ultrasound finding is that of the "triangular cord sign". This was first described by Park *et al*. in 1996 and is supposed to represent a solid proximal biliary remnant in front of the bifurcation of the portal vein. An accuracy rate of up to 80% is reported, but this has not been replicated in most centers and may be a late sign.

Additional diagnostic techniques

Liver biopsy is the mainstay for diagnosis for most centers in North America and Europe with an accuracy of up to 90% but it does rely on skilled histopathological interpretation [20]. The characteristics which confirm "large duct obstruction" on biopsy are bile duct proliferation, portal fibrosis, and the absence of sinusoidal fibrosis. If the biopsy is performed early (<4 weeks) then it may be difficult to give a conclusive diagnosis on biopsy alone.

Direct cholangiography is possible either by endoscopic retrograde cholangiopancreatography (ERCP) but does rely on a skilled operator and failure to cannulate the common bile duct may be operator error or confirmatory of BA [21]. Alternatively a cholangiogram can be performed laparoscopically by cannulation of the gallbladder which may confirm the presence of bile and delineate the biliary tree. If an atrophic gallbladder is found with no lumen then this confirms the diagnosis of BA.

Other investigations include: radioisotope scans (technetium (Tc) labelled iminodiacetic acid derivatives) which have a role in some centers by confirming the need for laparotomy by demonstrating the absence of biliary excretion. There may be overlap with severe forms of medical cholestasis and it is not specific. A simpler test is placement of a nasoduodenal tube and aspiration over 24 h which is used in many Asian centers with undeniable accuracy.

Management

Most infants with BA will have an attempt at restoration of bile flow and preservation of their native liver with a KPE. Only <5% of the England and Wales cohort post-centralization were considered for primary liver transplant on the grounds of perceived futility of a KPE. These were usually infants with missed diagnosis and over 100 days old. Age should not be the only factor used in this decision, as there was a 40% 5-year native liver survival in infants who were >100 days at the time of KPE [22]. Currently if there are features of cirrhosis such as gross ascites, then listing for transplant is a better option.

Surgery: the Kasai portoenterostomy

This is performed electively on a well-prepared stable infant with normal coagulation and platelet count. The small number with severe cardiac anomalies should have these corrected (if possible) before KPE.

The key part of the operation is the dissection within the porta hepatis and many centers completely mobilize the liver by dividing its ligaments and eviscerating it to lie on the anterior abdominal wall to improve access. This impedes caval venous return so needs volume support and anesthetic awareness. Other surgeons leave the liver in situ but will have to sling right and left portal veins to provide the necessary biliary exposure.

Surgery. The gallbladder is mobilized from its bed and the distal common bile duct divided and then dissected back towards the porta hepatis (Figure 25.6). On the left side, there is often an isthmus of liver parenchyma (from segment III to IV) which may need division by coagulation diathermy to open up the recessus of Rex (where the umbilical vein joins the left portal vein). On the right side, the division of right vascular pedicle

into anterior and posterior should be visualized. The "width" of the transected portal plate should extend from this bifurcation and a small innominate fossa on the extreme right to the point where the umbilical vein joins the left portal vein. This is not the operation Kasai described – his line of transection was within the "U" of the portal vein confluence.

A retrocolic Roux loop measuring 40–45 cm should now be constructed. The jejunojejunostomy lies about 10 cm from the ligament of Trietz and can be stapled or sutured. The proximal anastomosis must be wide (~2 cm), end-to-side and completed by fine precise, suturing (e.g. 6/0 PDS®) at the edge of the portal plate.

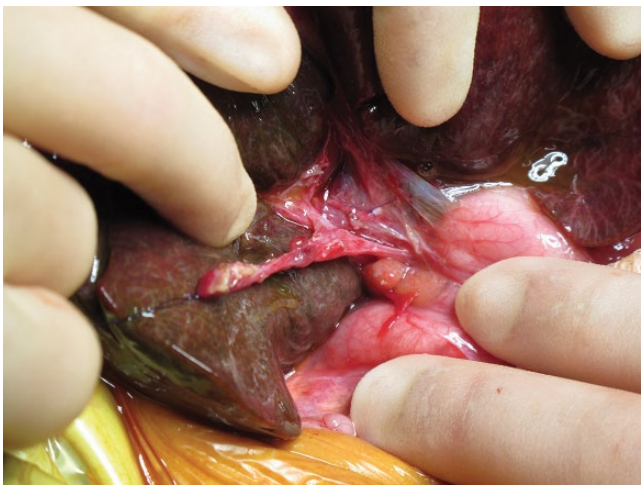

Figure 25.6 Operative appearance of isolated type 3 biliary atresia showing intact but obliterated bile ducts. Note shrunken, atrophic nature of the gallbladder. (Reproduced courtesy of Dr Enrico La Pergola, Department of Pediatric Surgery, Padova, Italy.)

Laparoscopic KPE has been reported from a number of centers but it is rarely performed outside the major centers in North America, Europe, and Japan [23] and two large centers (Hannover, Germany, and Hong Kong) have reverted to the open operation with restoration of their previous results [24, 25].

Postoperatively these infants are nursed in the ward and usually are discharged home within a week of surgery. Table 25.2 illustrates the pre- and postoperative management schedule of an infant undergoing KPE.

Adjuvant therapy for biliary atresia

The success of the operation is determined by clearance of jaundice within 6 months. A number of drugs have been suggested to improve the outcome of KPE. These include the following.

Corticosteroids

Steroids have been used for at least the last 20 years albeit delivered in an uncontrolled pragmatic fashion. The rationale behind the use of steroids is two-fold. Steroids may reduce the inflammatory element in a proportion of infants with BA, or they may increase bile flow and keep open the primitive bile ductule–Roux loop connection in the early postoperative phase.

There have been two prospective, double-blind, randomized, placebo-controlled trials. The first one used a low dose of prednisolone (2 mg/kg/day) in two English high-volume centers [26] in 73 infants. This showed a statistically significant improvement in early bilirubin levels (especially in the "younger" liver) in the steroid group but did not reduce the need for transplant or improve overall

Table 25.2 Management of biliary atresia.

Preoperative work-up (24–48 h preop.)	Laboratory investigations	Full blood count, renal profile, clotting screen (if abnormal correct preop.) Cross-match – packed cells (fresh frozen plasma and platelets if required)
	Medications	Antibiotics – e.g. cephalosporin/piptazobactam/gentamicin Bowel preparation – lactulose
	Other	ECHO/cardiac opinion if associated cardiac anomalies
Postoperative (Day 1–7)	Analgesia	Epidural analgesia (up to 72 h) IV paracetamol Oral morphine
	Fluid/enteral intake	IV maintenance crystalloid fluid Replace drain and/or nasogastric losses Enteral – clear fluids once nasogastric aspirates low/bowels open Milk – breast milk or medium-chain triglyceride based formula, e.g., Heparon®
	Antibiotics	Treatment dose cephalosporin/piptazobactam/gentamicin 6 days
	Steroids	High-dose steroid (e.g., oral prednisolone starting at 5 mg/kg/day)
	Antivirals*	Cytomegalovirus IgM + ve infants, e.g., ganciclovir, valganciclovir
Long term	Medications	Vitamins ADEK (fat soluble) Ursodeoxycholic acid Prophylactic antibiotics (e.g., cephalexin)
	Immunizations	Childhood immunization schedule accelerated to be complete by 18 months of age due to possibility of transplantation

* Antivirals are not routinely used, but may be considered in infants who are cytomegalovirus IgM + ve to improve outcome.

survival. The STeroids in biliary Atresia Randomized Trial (START) trial [27] randomized 70 infants from 14 North American centers to a steroid arm using initially i.v. methylprednisolone 4 mg/kg/day for the first 3 days followed by oral prednisolone (4 mg/kg/day until the second week, 2 mg/kg × 2 weeks, followed by a tapering protocol over the next 9-week period). Although there was a difference of 10% in the main primary end point (clearance of jaundice) from 49%, in the placebo group to 59% in the steroid group this was not statistically significant. A subgroup analysis of infants <70 days at KPE (n = 76) showed that 72% (28/39) in the steroid group cleared their jaundice compared to 57% (21/37) in the placebo group which was not statistically different ($P = 0.36$).

A follow-up study to the original UK trial examined the use of a high-dose prednisolone cohort (starting at 5 mg/kg/day) [28] and again showed the same beneficial biochemical effects as before (including a reduction in AST and APRi levels) with a significant 15% difference in jaundice clearance in the high-dose steroid group (67% (41/62) versus 52% (47/91)).

The most recent meta-analysis of previous published trials suggested a positive effect of postoperative steroids [29] and many centers still use a variety of post-KPE steroid regimens.

Ursodeoxycholic acid

This may be beneficial if surgery has restored bile flow to reasonable levels. UDCA "enriches" bile and has a choleretic effect, increasing hepatic clearance of toxic endogenous bile acids and may confer a cytoprotective effect on hepatocytes. There is a single study which demonstrated a beneficial effect of UDCA on liver function in 16 children >1 year post-KPE [30].

Postoperative complications

The most common problem is that the operation is not effective in restoring bile flow. Jaundice increases with the development of end-stage liver disease and cirrhosis.

Reoperation is futile unless the operation was performed inadequately or by an inexperienced surgeon. The key management in these infants is to prepare them for liver transplant by strict attention to nutrition, vitamin deficiencies, and fluid management.

Cholangitis

KPE creates a link between the intrahepatic biliary ductules and the intestine and therefore predisposes to ascending cholangitis. The organisms are gut derived and include various Gram-negative pathogens such as *Escherichia coli* and *Klebsiella* spp.

A single episode is relatively common and was seen in up to 50% of older series, though it seems less common today. Prophylactic antibiotics (e.g., cephalosporin/piptazobactam/gentamicin) should be used as part of the postoperative protocol (see Table 25.2); however its value beyond a month or so is questionable. The risk is most apparent in the first 2 years postsurgery and then it declines, perhaps due to immunological tolerance and improvement in host immunity.

The key clinical features are pyrexia, worsening jaundice, and a change in liver biochemistry. All children with pyrexia post-KPE should be suspected of having cholangitis until proved otherwise. Episodes should be treated aggressively with broad-spectrum intravenous antibiotics effective against Gram-negative organisms (e.g., gentamicin, meropenem, Tazocin® (piperacillin/tazobactam)) for 10–14 days.

Late-presenting cholangitis in an older child should be rigorously investigated with radioisotope biliary excretion scans as mechanical Roux loop obstruction may be the cause and can be surgically corrected.

Nutrition and growth failure

Maintenance of good nutrition and restoration of normal growth are vital to the infant's well-being. This is particularly so in those who have failed to clear their jaundice. All infants require good nutritional intake, and fat-soluble vitamins (see Chapter 8). Infants who become progressively malnourished should receive additional calories and protein by overnight nasogastric feeding.

Growth failure may be defined as height or weight <2 standard deviations below the population mean and is a major risk factor for poor outcome and a recent report from the Biliary Atresia Research Consortium (BARC) identified this as a key measure of outcome associated with transplantation or death by 24 months of age [31].

Similarly, Studies in Pediatric Liver Transplantation (SPLIT) looked at 775 children with BA awaiting transplantation and again identified growth failure as an independent risk factor for pre- and post-transplant mortality and graft failure. There is a clear correlation between improvements in nutrition and better outcome following transplantation.

Portal hypertension and esophageal varices

Abnormally raised portal venous pressure (i.e., portal hypertension or PHT) is seen at the time of KPE in about 70% of BA infants and is caused by intrinsic liver fibrosis [32]. This correlates with the age at KPE, the level of jaundice, and ultrasound-measured spleen size [32]. It is not a good predictor of outcome either in terms of response to KPE or in predicting the development of varices.

Upper gastrointestinal varices take time to develop requiring sustained PHT for a period of time and so gastrointestinal bleeding is unusual before 9–12 months of age and usually occurs from about 2–3 years. About 50% of children who survive with their native liver beyond 2 years will have definite varices at endoscopy. Overall 20% will have some form of bleeding episode such as hematemesis or melena [33].

Emergency variceal treatment and reduction of PHT should be administered in those children with BA who bleed

by the use of vasopressin (e.g., terlipressin) or somatostatin analogues (e.g. octreotide). Endoscopic treatment should be definitive with banding in the older child or in the very young, the more traditional injection sclerotherapy using ethanolamine (see also Chapter 21).

Ascites

Occasionally ascites is an isolated finding in those infants who have cleared their jaundice, but is more usually a consequence of PHT, hypoalbuminemia, and hyponatremia. Ascites is conventionally treated by salt restriction, fluid restriction, and the use of diuretics particularly spironolactone. If gross then the ascites can be tapped with judicious use of volume and albumin replacement (see Chapter 21).

Hepatopulmonary syndrome

Intrapulmonary shunting and hypoxia may develop even in anicteric children following the KPE. The mechanism for this is unknown although it is more common in those children with BASM, and may be a manifestation of a pre-existing congenital vascular anomaly. It can be diagnosed using arterial blood gas estimation with and without inspired oxygen and typically hypoxia is worse in the standing position (platypnea). Specific ventilation/perfusion radionuclide lung scans quantify the degree of shunting. HPS is resistant to conventional therapy and liver transplantation appears to be the only specific treatment. Even then following a successful transplant recovery can be slow over many months before oxygen supplementation can be discarded (see also Chapters 21, 22, and 27).

Malignant transformation

Malignant change involving both cholangiocarcinoma and hepatocellular carcinoma (HCC) has been reported in children post-KPE [34] even in those who have cleared their jaundice. The series from King's College Hospital, UK, identified four cases of HCC, which suggested an incidence of about 1% overall [34]. The median age at presentation of the HCC was just over 2 years of age and all were cured by transplantation. Surveillance using regular ultrasonography and serial α-fetoprotein levels is recommended but may not be sensitive enough. Suspicious nodules should be thoroughly investigated with cross-sectional magnetic resonance imaging (MRI) or computed tomography (CT) imaging and biopsy.

Outcome and results

It is important to use a combination of reproducible outcome measures which accurately reflect performance and effective management of BA to allow comparison between centers. These should include:

- Clearance of jaundice to a pre-set level (typically ≤20 μmol/L in Europe and <1.5 mg/dL in North America). A clearance rate of >50% should be expected in experienced surgeons operating on infants <70 days of age.

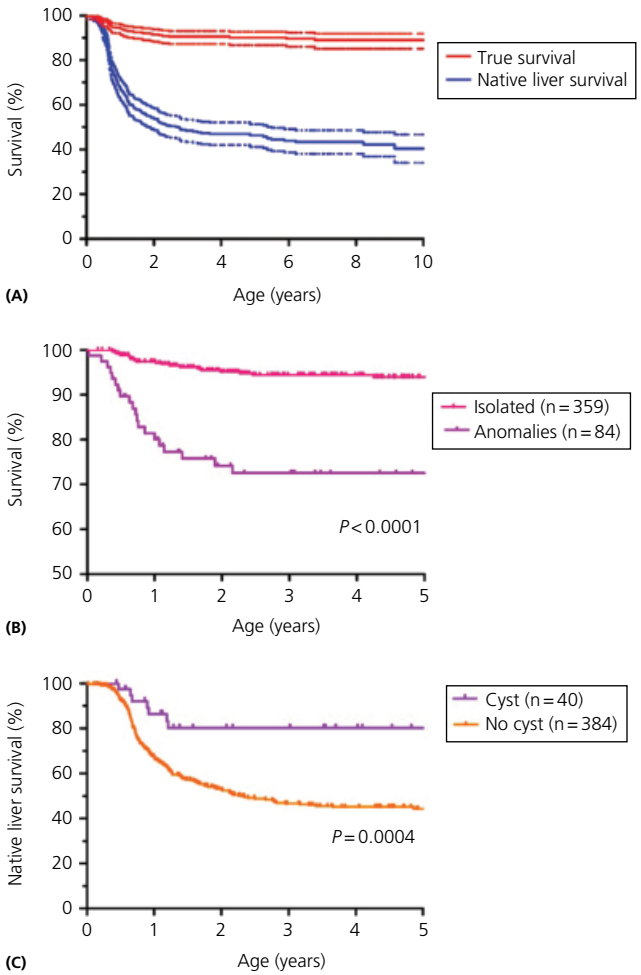

Figure 25.7 (A) Actuarial true and native liver survival curves (median (±95% CI)) for biliary atresia (BA) (n = 443) in England and Wales (1999–2009). (Reproduced with permission from Davenport *et al.* 2011 [35]. Reproduced with permission from Elsevier.) (B) Effect of anomalies in BA. True survival in BA and other anomalies (n = 84) versus isolated BA (n = 359). Curves are significantly different ($\chi^2 = 33$; $P = 0.0001$). (C) Effect of cystic change in BA. Native liver survival of cystic change (n = 40) versus obliterated BA (n = 384). Curves are significantly different ($\chi^2 = 12.35$; $P = 0.0004$).

- Actuarial native liver survival (end points of death and liver transplant). Although the single most important factor is clearance of jaundice this can be affected by post-operative complications and quality of medical follow-up.
- Actuarial true survival (end points death). This reflects the success of the original operation, the associated abnormalities (chiefly cardiac), and the access and safety of the transplant procedure.

The benchmark study of outcome following centralization of BA management in the UK from 1999 has allowed publication of key national statistics up to 10 years of age (Figure 25.7A–C). This includes a cohort of 424 infants who underwent KPE in England and Wales (1999–2010). Clearance of jaundice occurred in 55%, with a 5- and 10-year native liver survival estimate of 47% and 43%, respectively;

with the overall survival estimate at 10 years being 90% [35]. There is some evidence that these statistics have improved in the most recent 5-year period, possibly due to increasing use of high-dose adjuvant steroids (personal observation). There was a positive effect on survival in children with CBA, a negative effect on survival of other anomalies, and the lack of any real statistical effect of age on outcome.

Centralization of resources is the single most important public health measure that can change the outcome of this unpredictable disease.

Conclusion

Although the cause of BA remains elusive a complementary system of surgical and medical treatment has evolved, which has improved the overall survival to adulthood in affected infants from around 10% in the 1970s to about 90%.

Choledochal malformation

Definition

CMs may be characterized as an inherent dilatation of the biliary tree without evidence of mechanical obstruction such as the dilatation secondary to a stone impacted in the distal common bile duct.

The term choledochal *malformation* is preferred to choledochal *cyst* because the latter should be restricted to those that look like a cyst. Most of these malformations are not rounded or spherical, hence the need for a less "descriptive" term.

Epidemiology

CM can present at any point from the antenatal scan to an incidental finding at postmortem and this makes the true incidence difficult to define. Children presenting early in life with obstruction are much less common than those with BA and an estimate of 1 in 100,000 live births would be reasonable. The incidence is much higher in Asian countries and there is a marked female predominance of about 4 : 1 [36].

Classification of congenital choledochal malformation

The original classification was based upon a literature review of 94 cases by Alonso-Lej *et al.* [37] published up until the 1950s and included:

- Type 1: the classical cystic dilatation of the common bile duct.
- Type 2: diverticulum from the common bile duct.
- Type 3: localized dilatation of the distal common bile duct within the wall of duodenum – the so-called choledochocele.

The Japanese surgeon Takuji Todani defined three variants of type I (A, B, and C); two variants of type IV (A and B); and adding isolated intrahepatic dilatation as type V [38]. It is complex and many authors continue to misquote the original.

The King's College Hospital classification is illustrated in Figure 25.8 simplifies the Todani classification into types IC and IF (depending on the predominant appearance as cystic or fusiform) as well as limiting type 4 to the combination of intra- and extra-hepatic dilatation [39, 40].

Type 4 CM is found in about 20% of series and may be the natural history of untreated type 1F and type 1C. Some authors have distinguished cystic intrahepatic from a more fusiform intrahepatic dilatation implying that the latter diminishes considerably following effective surgery. Our follow-up study shows considerable resolution in intrahepatic duct dilatation in the first year post-surgery with the relief of biliary pressure [39].

Isolated type 5 intrahepatic dilatation does occur and is distinct from what we recognize as Caroli syndrome or disease. This is an obvious genetic abnormality, stemming from a basement membrane defect in the small bile ducts and is associated with similar pathology affecting the kidney. Intrinsic liver fibrosis in the absence of any particular bile duct obstruction is also a feature (see Chapter 14).

A common pancreatobiliary channel is a key diagnostic and typical clinical feature in types 1C, 1F, and 4 where the junction of the bile and pancreatic duct is outside the wall of the duodenum and not under sphincteric control.

Etiology

There are two hypotheses to explain the common types of CM. The Babbitt hypothesis suggests that there is reflux of activated pancreatic enzymes via the common channel into the biliary tract. As a result there is enzymatic induced biliary epithelial damage and weakening of the bile duct wall with secondary dilatation.

An older hypothesis suggests dilatation is simply the result of a narrow stenotic portion of common bile duct proximal to the common channel generating high intraductal pressures upstream as is seen in many other congenital scenarios (e.g., esophageal and jejunal atresia).

We have published a series of reports trying to distinguish these alternatives clinically [40, 41]. Firstly, we established that there was an inverse relationship between bile amylase (a surrogate of pancreatic juice reflux) and measured choledochal pressure [40]; secondly, we observed that there was a stepwise increase in pressures from type 1F to type 1C to type 4 accompanied by a reduction in their bile amylases. Finally, we related these variables to a histological score reflecting the biliary epithelial damage and showed that the most abnormal histology was in those with the highest pressures (and therefore the lowest amylase levels) [41]. These observations based on clinical observations and practice do seem to negate the central hypothesis of Babbitt.

Clinical features

The classical presentation of a cystic CM is with abdominal pain, jaundice, and a mass in the right upper quadrant but nowadays is rarely seen in most large series [40]. Most infants tend to present with obstructive jaundice. In later childhood and adult life, the presentation is usually

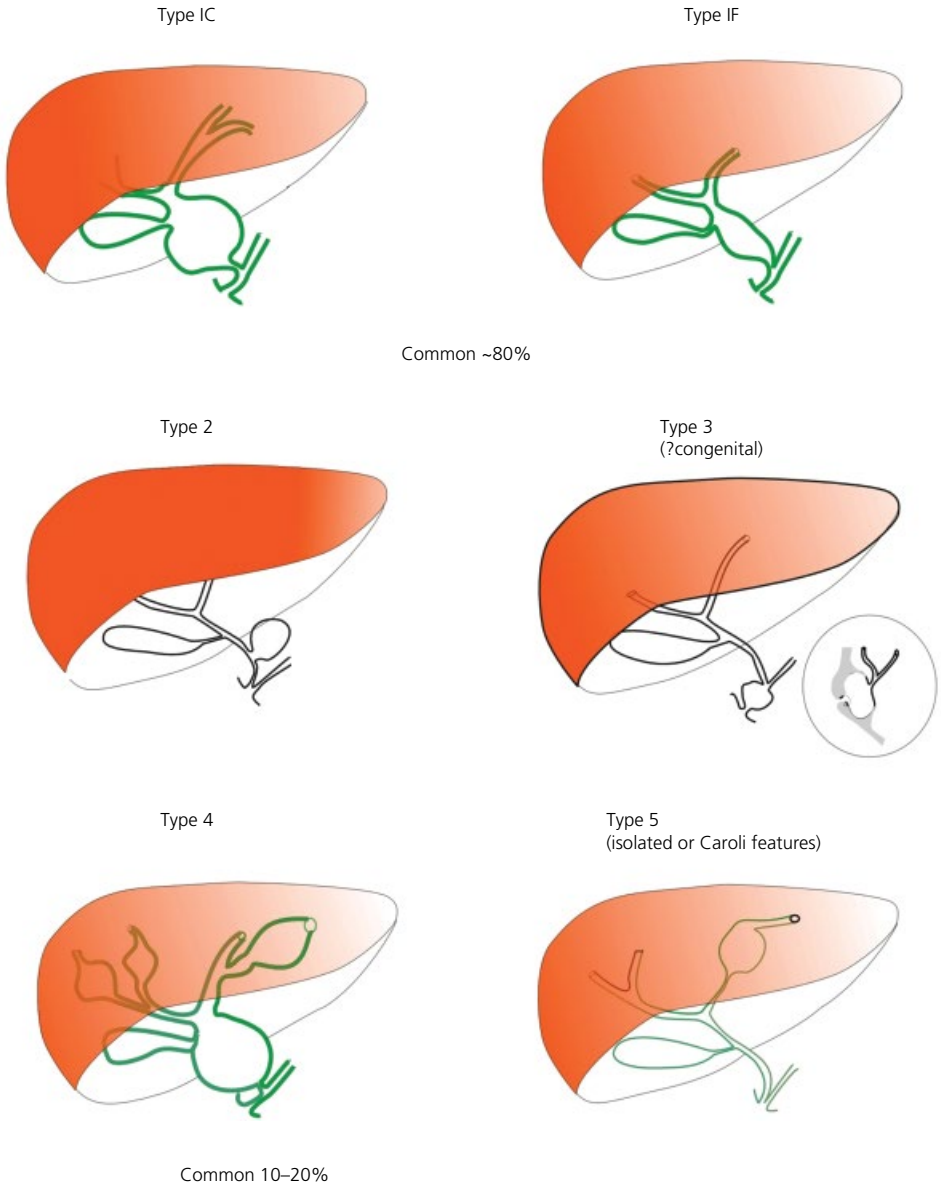

Figure 25.8 Classification of choledochal malformation. (From King's College Hospital, London, UK. Reproduced with permission of King's College Hospital NHS Foundation Trust.)

recurrent abdominal pain with or without features of acute pancreatitis. If untreated or disregarded biliary cirrhosis is a possible sequel and in adulthood a small proportion will present with malignant transformation. A closed high-pressure biliary tract predisposes to superadded bacterial infection and cholangitis but that is fortunately rare.

Abnormal liver biochemistry is characterized by a high conjugated bilirubin and raised GT with only modest elevation in other liver enzymes such as AST. Albumin and globulin levels should be normal. Plasma amylase levels should also be ascertained to assess the possible diagnosis of pancreatitis but this is rare in infancy.

The key diagnosis is based on the abdominal ultrasound which should demonstrate abnormal dilatation of the common bile duct in all the common types. Variation in choledochal size does not happen in practice, so if it is observed then an intraluminal obstruction may be more likely. Cross-sectional imaging using CT or MRI is important to confirm the diagnosis and 3D reconstruction eliminates most structural uncertainty. ERCP is rarely needed to confirm the diagnosis but may have a role in the older child with a marginally dilated biliary tract and a possible common channel as a cause of recurrent pancreatitis.

Antenatal presentation

This mode of presentation makes up about 15–20% of large clinical series of routine maternal ultrasound screening (Figure 25.9). Typically a cyst is detected from around 20–22

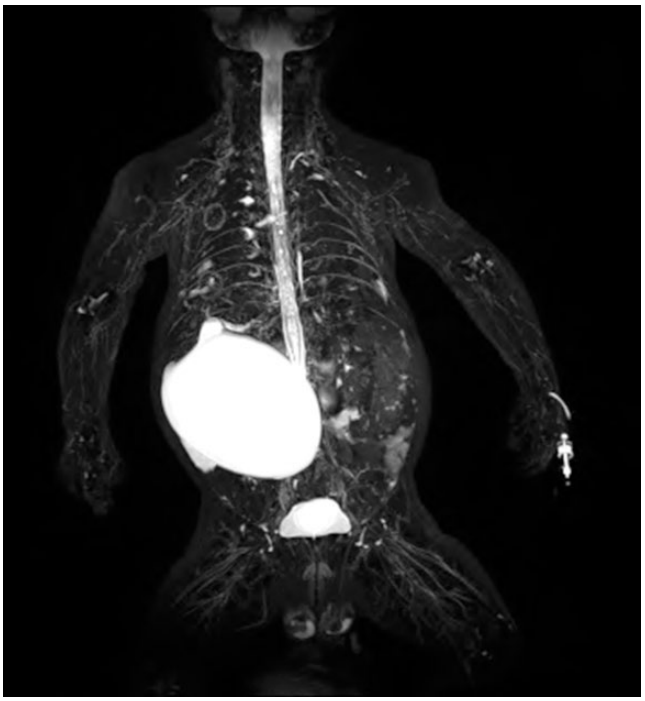

Figure 25.9 Antenatally diagnosed type 1C choledochal malformation. MRI scan reconstruction showing huge cyst extending from the liver to the pelvis. Intraoperative pressure measured at 25 mmHg with bile amylase of 10 IU/L. (Reproduced courtesy of Mr Brice Antao, Department of Pediatric Surgery, Crumlin Hospital, Dublin, Ireland.)

weeks' gestation. Although its actual origin may not be confirmed it usually grows in line with fetal growth.

The differential diagnosis includes CBA and benign simple parenchymal liver cysts. No formal antenatal intervention is required but the newly born infant will need radiological imaging. Clinical observation of pale stools, a rising conjugated bilirubin fraction, and ultrasound confirmation of a dilated biliary tract are all required to make a diagnosis. If there is doubt a radioisotope study may reassure that bile drainage is unaffected.

Some children, usually those with type 1C, do drain bile satisfactorily and their surgery can be deferred until 3–6 months of age. Children with persisting jaundice with obstruction require surgical intervention.

Management

Surgery is the only effective treatment for CM, although the timing perhaps can be negotiated.

The standard surgical approach to a CM is a laparotomy, resection of the dilated extrahepatic bile duct, and an hepaticojejunostomy–Roux-en-Y as the reconstruction method (Figure 25.10). Most published refinements have concentrated on details such as on-table choledochoscopy to ensure effective clearance of the residual intrahepatic ducts and clearance of the pancreatic duct and common channel to forestall the development of recurrent pancreatitis.

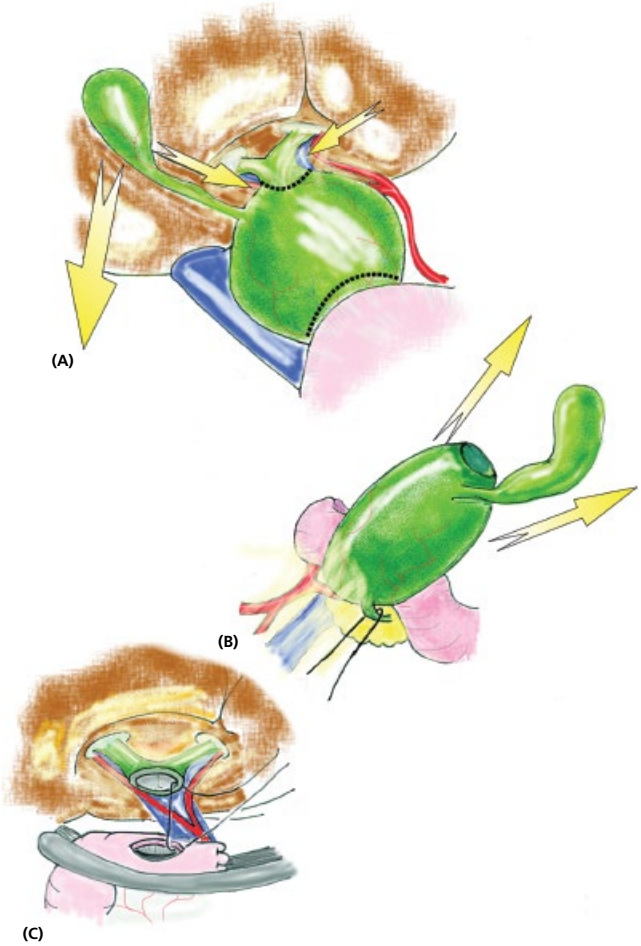

Figure 25.10 Schematic illustration of steps during dissection of choledochal cyst. (A) Following confirmation of the anatomy with cholangiography the gallbladder is mobilized from the bed of the liver. The junction of the cyst with the common hepatic duct is dissected free and divided. (B) The cyst is then separated from the medially placed hepatic artery and then posteriorly placed portal vein towards the duodenum. The distal part is dissected within the head of pancreas down to just above the junction with the pancreatic duct. This is then divided to remove the cyst completely. (C) The reconstruction involves a 40–50-cm Roux loop and hepaticojejunostomy. The posterior wall is completed with untied sutures before approximating bowel and common hepatic duct.

The main change in surgical practice has been the advent of laparoscopic surgery although it remains a challenging undertaking and not for the occasional operator. The first laparoscopic resection of choledochal cyst in a 6-year-old child was performed in 1995 in Schio, Italy, by Farello *et al.* Since then increasing numbers of specialist centers have taken this approach using minimally invasive techniques. By comparison with the open operation, operative times are longer and in the smaller series there was a higher incidence of complications such as anastomotic leaks without any real diminution in hospital stay. More recently, series of more than 200 cases have been published from Bejing (China) and Hanoi (Vietnam) with much lower complication rates which

testifies to the increased centralization of expertise in those countries and the effect of high-volume surgery [36, 42].

To shorten the time for surgery, other ancillary devices such as robot-assisted surgery have been used. This is limited to the older child because of port size constraints and because the technology is expensive. Extracorporeal jejunojeunostomy through a widened umbilical incision is also a time-saving method but it is the other anastomosis – the hepaticojejunostomy – which is difficult. Some surgeons have reverted to a more old-fashioned technique – hepaticoduodenostomy – to shorten operative times. Here the transected hepatic duct and the mobilized first and second parts of the duodenum can be in close proximity and it avoids a further intestinal anastomosis. However, there are some fundamental problems with this technique as it causes bile gastritis in about 10–15% and exposes the dilated biliary tree directly to duodenal contents, which may be a problem in the long term.

The long (40–50 cm) Roux loop and hepaticojejunostomy have been the mainstay of biliary reconstructive surgery for around 50 years and seems to be a reasonable clinical choice. In over 100 operated CMs, there was a significant incidence of failure to thrive with lower preoperative weights compared to the age-corrected normal population. There was satisfactory catch-up weight gain evident at 1 year after surgery, which was sustained [43].

Conclusion

Outcome in CM has improved dramatically over the last 60 years led by earlier diagnosis, prompt surgical excision, and effective biliary drainage. There are still unresolved issues around the nature and style of surgical reconstruction but long-term success with normal liver function is unequivocal.

Spontaneous biliary perforation

Spontaneous biliary perforation (SBP) is a distinct disease of unknown origin presenting with bile ascites during the first few weeks of life. It is a rare condition with less than 170 cases reported in the literature [44, 45]. Nonetheless it is the fourth commonest cause of surgical jaundice in infancy after BA, inspissated bile syndrome, and CM [46].

The largest case series remains that of Chardot *et al.* [45] who described nine cases [*sic*] of bile duct perforations in infants – two from the cystic duct, one common hepatic, one common bile duct, and four at the typical junctional site. Two other cases with biliary stenosis were attributed to those that had spontaneously sealed.

Although it does occur in older children, the condition predominantly affects infants from 2 to 20 weeks of age with the perforation occurring at the junction of the cystic and common hepatic duct.

Etiology

The most common site of perforation is anteriorly at the junction of the cystic duct with the common hepatic duct. Posterior perforation is also described and, because subsequent bile leakage is into the lesser sac, there may be diagnostic delay.

It is suggested that the etiology may be a congenital mural weakness, or ischemia due to an abnormal arterial supply at this site. It has been reported in association with distal bile duct obstruction such as inspissated bile or stones and attributed to a sudden rise in biliary pressure. CMs may also perforate, again probably due to elevated intrabiliary pressure but usually this occurs in older children [44].

Clinical features

Most anterior perforations are initially constrained by adjacent structures but then leak into the general peritoneal cavity becoming evident as bile ascites. This causes abdominal distension and vomiting, and discoloration of hydroceles or hernia sacs. Bilirubin is absorbed across the peritoneum and little bile is excreted into the gastrointestinal tract resulting in a conjugated jaundice and acholic stool. In the absence of secondary infection, infants are usually relatively well despite the presence of up to 500 ml of bile in their abdomens.

Posterior perforations are much less obvious with recurrent vomiting the only consistent sign. Less commonly, there is an acute deterioration associated with sudden abdominal distension, fever, vomiting, and pain from biliary peritonitis and hemodynamic instability.

Ultrasound is the key investigation and defines both the volume of ascites and nature of loculated collections inferior to the liver. Radioisotope imaging (e.g., HIDA) should be diagnostic of bile leak and should also assess the degree of any distal obstruction.

ERCP can be diagnostic though few centers possess the relevant endoscopes and expertise to perform it. MRCP may become a more useful diagnostic tool particularly with respect to defining loculations and the periperforation inflammatory mass due to technology advancements.

Management

Laparotomy and intraoperative cholangiogram are always required to facilitate bile drainage and provide definitive treatment. Although there are reports of "simple" external bile drainage alone, this will not establish the degree of integrity or obstruction of the biliary tree.

Intraoperative cholangiogram via the gallbladder will show the site of the leak and outline the biliary tract. If there is distal bile duct obstruction, with inspissated bile for instance, then flushing with saline may relieve the problem. Obviously if the site of perforation is in the gallbladder or cystic duct then a cholecystectomy will be curative.

There may be significant inflammation around the porta hepatis with pseudocyst formation, making definitive surgery a risky prospect. In this case, it may be advisable to place a peritoneal drain and bring out the fundus of the gallbladder as a cholecystostomy (for later cholangiography).

If the perforation can be identified, it can be sutured or patched directly as long as there is no distal obstruction. A small "T-tube" can be placed (6 FG is usually the smallest available) [44] which should be left to drain freely as a controlled external biliary fistula for 2–3 weeks and then removed, if contrast shows free onward passage into the duodenum.

Occasionally there is little that is left of a bile duct structure, particularly if it is a posterior perforation, and then reconstruction using a Roux-en-Y hepaticojejunostomy is advised [47].

In the older child, ERCP and insertion of a transampullary biliary stent is a more definitive therapeutic device as it is for adult practice, particular when traumatic in origin.

Spontaneous, or otherwise, perforation can lead to several complications. The commonest complication is stenosis due to fibrosis and inflammation at the site of perforation and obstructive jaundice may be the first presentation after a silent perforation. Some cases of so-called "acquired" BA may arise in this way [48]. These infants are usually older than those with typical BA, without the cirrhotic livers of those with comparatively older "congenital" BA. They may have had a complicated neonatal history with prematurity or necrotizing enterocolitis. In contrast to standard BA they will have dilated proximal bile ducts.

Portal vein thrombosis is a specific complication of bile duct perforation, particularly in those arising from the posterior aspect of the duct [47]. Its onset may be delayed by several months and only recognized by the development of PHT and variceal formation. Therefore it is advisable to follow these infants with serial ultrasound scans to monitor portal venous flow. Anticoagulation is advised in those at particular risk [47].

Inspissated bile syndrome

Inspissated bile or "sludge" is most commonly found in infants and children with:

- Systemic infections.
- Increased turnover of red cell recycling and hemolysis, such as due to ABO or rhesus incompatibility in infants and sickle cell disease in adolescents.
- Parenteral nutrition – due to inhibition of the normal contractility of the gallbladder. One study suggested this may form within 3 weeks in ~6% of patients.
- Rapid weight loss – perhaps after bariatric surgery,
- Biliary stasis – due to structural problems such as a CM.
- Metabolic derangements of bile formation – typically PFIC and citrin deficiency.
- Drug therapy – particularly involving third-generation cephalosporins (e.g., ceftriaxone).

The sludge is formed of precipitates of cholesterol monohydrate crystals, calcium bilirubinate, calcium phosphate, calcium carbonate, and calcium salts of fatty acids which embed into biliary mucin to form "sludge".

Sludge may be transient, lasting only a few days, resolving with restoration of better health or gallbladder motility. However it may become impacted in the distal common bile duct causing dilatation and conjugated jaundice. The diagnosis is obvious on ultrasound with both the intraluminal impacted content and the resultant dilatation evident.

The management is medical involving administration of UDCA (10–20 mg/kg) if there is minor biliary dilatation and a short history; endoscopic involving ERCP ± sphincterotomy or percutaneous transhepatic cholangiogram (PTC) and duct flushing. If these measures fail, then open or laparoscopic surgical intervention is possible. Typically this simply involves a cholangiogram via the gallbladder and manual flushing of the ducts.

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CHAPTER 26

Liver Trauma in Children

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Key points

- Liver injury is a very common injury in childhood, often as part of a polytrauma.
- Treatment has changed over the last decades from mandatory surgical to non-operative in most cases.
- Non-operative management of blunt hepatic injuries carries a complication rate of approximately 7%, and associated mortality is usually related to severe head injury.
- Penetrating injuries require surgical exploration in most cases.
- Surgical management (when indicated) can be taxing and should preferably only be performed in centers of expertise.
- Interventional radiological techniques are an important component of management.
- The prognosis is excellent nowadays.

The liver is more often injured than any other solid abdominal organ [1]. Hemorrhage resulting from injury to the liver remains the major cause of morbidity and mortality following blunt abdominal trauma either from an open wound or blunt force. Pedestrian traffic crashes are responsible for more than 80% of these injuries, with falls, assault, and child abuse accounting for the remainder. Management of such injuries in a patient with polytrauma presents a diagnostic and therapeutic challenge to the attending surgeon [2]. Penetrating injuries, although occurring less frequently, are challenging for the surgeon with regard to operative intervention, depending on the nature of the injury [3]. Injuries to the extrahepatic biliary tree constitute less than 2% of all intra-abdominal injuries. Iatrogenic injury to the liver and biliary system occurs particularly in the sick neonate during a laparotomy; in the older child undergoing liver resection or cholecystectomy; as a result of needle biopsy; in invasive imaging techniques; during inappropriately low insertion of chest drains; and in external cardiac massage during resuscitation [4]. Fortunately, long-term consequences are rare, largely owing to the regenerative capacity of the liver [5]. In this chapter, the history of the development of liver trauma management, pathophysiology, current perspectives in management, and long-term outcomes are discussed.

History

The major problem in the management of liver trauma was and remains control of hemorrhage. In the 19th century, German experience indicated that resectional debridement of devitalized tissue was possible, that hepatic wounds were significant only when larger vessels were involved, and that hemorrhage could be controlled and the liver repaired at laparotomy. Further recognition that suture ligation of intrahepatic blood vessels, as well as hemostatic mattress suturing of liver tissue was effective, paved the way for further advances. Historical landmarks in the management of liver trauma include inflow vascular occlusion (Pringle maneuver) with packing, total vascular exclusion, inferior vena cava bypass, a return to a more conservative approach, and transplantation [6]. In the 20th century, the management of children with blunt injury to the liver and other solid viscera swung from conservatism through aggressive intervention and back to a selective non-operative approach [2, 7]. Beckman, in the 1920s, advocated "intelligent conservatism" on the basis of 40% negative findings at laparotomy and a nearly 50% mortality after laparotomy for significant injury [8]. Most centers now accept the proven efficacy of non-operative management for 75–85% of children with blunt hepatic

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injuries, as current imaging modalities allow for accurate non-invasive visualization of solid organ injuries [1, 2, 7]. Penetrating hepatobiliary injuries are generally managed according to set protocols similar to those developed for adults, but with recent advocacy of a more aggressive approach in children because of the high incidence of injury to adjacent intra-abdominal viscera [3]. The early diagnosis of bile duct injuries remains difficult as symptoms and signs may develop insidiously. A high index of suspicion is necessary and specific investigations such as an excretion radionuclide scan (diisopropyl or hepatobiliary iminodiacetic acid (DISIDA or HIDA)) or endoscopic retrograde cholangiography (ERCP) may be needed [9]. A raised serum bilirubin in the trauma patient is an early warning of biliary pathology. Early diagnostic laparoscopy may be of value, but the exact place of this procedure in the injured child has yet to be ascertained [10].

Pathophysiology of liver injury

The classification of liver injury adopted by the American Association of Trauma (Table 26.1) is helpful in defining optimal treatment [11].

Violent compression of the right chest wall and upper abdomen is the most frequent mechanism of blunt injury and in most cases is due to a pedestrian traffic accident [12]. This has a two-fold effect: a compression force of varying intensity causes parenchyma, portal triad structures, and hepatic veins to be torn or disrupted; and a decelerating injury with traction results in tearing of the liver along its points of fixation, namely the peritoneal attachments to the diaphragm, venous drainage into the inferior vena cava and porta hepatis, and at the distal end of the common bile duct [13]. The fractures may be shallow or deep and typically radiate away from the point of impact. Hemorrhage from the portal vein, hepatic artery, and hepatic veins is initially brisk, and bile leaks from the ruptured ducts. Varying amounts of liver tissue may be devitalized. The right lobe, particularly the posterosuperior aspect, is most often involved.

Bleeding may be confined to the retroperitoneal area where tamponade may occur. If Glisson's capsule remains intact, a subcapsular hematoma may strip the capsule off the liver, thus propagating its expansion. Hematomas may also be contained deep within the liver substance. Initial expansion and liquefaction is often observed and can be monitored both clinically and by ultrasound scanning or computed tomography (CT). Most resolve over a period of 2–3 months. A few become infected by a variety of Gram-negative or -positive organisms and go on to abscess formation [14, 15]. When arterial bleeding is the cause, continued expansion and early rupture may occur with later development of a pseudoaneurysm and hemobilia [16, 17]. Delayed rupture can occur but is rare [8, 18]. Occasionally hematomas may become encysted [19]. This could be due to bile leakage into the hematoma, which is known to inhibit liver healing [20].

Extrahepatic bile duct injury may initially go unrecognized, resulting in biliary ascites and peritonitis, or a localized intra-abdominal biliary collection [9]. Late biliary stricture may develop.

Penetrating wounds – mainly gunshot and stab injuries – have a more random pattern. The extent of injury depends on the velocity and size of the missile and its trajectory. Extrahepatic structures are more frequently damaged than occurs with blunt trauma, high-velocity bullets and shotgun injuries being particularly destructive. Hepatic vein injury at the cavovenous junction, despite being a low-pressure system, is the major cause of mortality. Embolization of blood clot or devitalized hepatic substance may occur.

Table 26.1 Classification of liver injury according to the American Association of Trauma Management of Acute Liver Trauma. (From Sandblom [20]. Reproduced with permission of Walters Kluwer Health.)

Grade	Injury description	Extent
I	Hematoma Laceration	Subcapsular, not expanding, <10% surface area Capsular tear, not bleeding, <1 cm deep
II	Hematoma Laceration	Subcapsular, not expanding, 10–50% surface area Capsular tear, active bleeding; 1–3 cm parenchymal depth, <10 cm length
III	Hematoma Laceration	Subcapsular, >50% surface area or expanding; ruptured subcapsular hematomas with active bleeding; intraparenchymal hematomas >2 cm or expanding >3 cm parenchymal depth
IV	Hematoma Laceration	Ruptured intraparenchymal hematomas with active bleeding Parenchymal disruption involving 25–50% of hepatic lobe
V	Hematoma Laceration	Parenchymal disruption involving >50% of hepatic lobe Juxtahepatic venous injuries; i.e., retrohepatic vena cava/major hepatic veins
VI	Vascular	Hepatic avulsion

Presentation and investigation

Liver injuries have been graded in adults on a scale of I to VI. Hepatic injury must be suspected in all children with trauma to the right chest and abdomen. Almost invariably other injuries are present [1].

Those children who respond to adequate fluid resuscitation and remain stable should have confirmation of their injuries with CT (Figure 26.1) or radioisotope studies. However, the severity of injury as assessed by CT does not necessarily correlate with a need for surgery [11]. Ultrasonography scanning has not proved to be a good investigation for early documentation of injury as picture

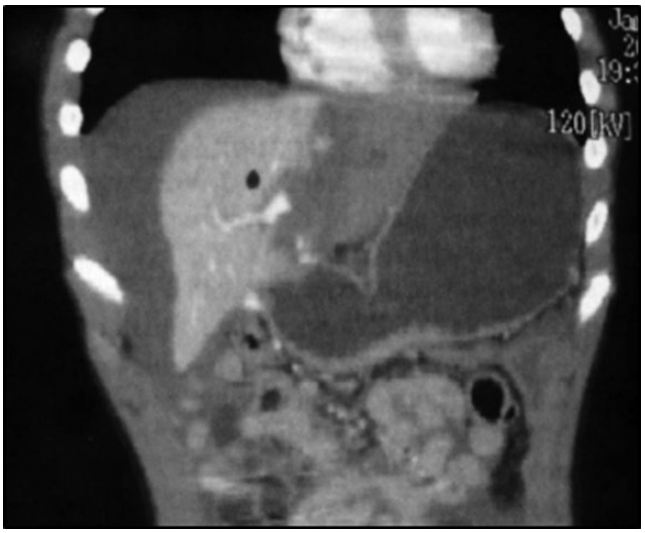

Figure 26.1 Coronal CT of a child after blunt abdominal trauma, showing hypoperfusion of the left liver, a "blush" of contrast indicating ongoing hemorrhage, and a large amount of fluid (blood) surrounding the liver. The stomach also appears to be dilated. Urgent laparotomy and resection of the almost completely avulsed left lateral segment was required in this case.

resolution may be suboptimal owing to rib fractures, abdominal tenderness, and gaseous distension, but it is excellent for monitoring the healing of the hepatic lesion.

Focussed abdominal sonography for trauma (FAST) has recently been identified as a useful screening modality for blunt abdominal trauma in children, when used in combination with physical examination. A combination of negative clinical examination and FAST rules out a clinically significant injury, while a positive FAST prompts further imaging.

Radionuclide technetium scanning was useful, but CT with contrast is currently the investigation of choice in patients with clinical evidence of hemoperitoneum associated with shock or multiple injuries [21].

Acute management

Non-operative management

Liver injuries can be complex and patients with a blunt liver injury should be preferably treated in a specialist center by experienced pediatric surgeons. The management protocol for blunt hepatic trauma has changed over the last 30 years, from an early mandatory operative approach to a more conservative, non-operative management approach. Improved pediatric resuscitative measures together with better imaging and intensive care facilities have facilitated the effectiveness of non-operative management. The number of liver injuries successfully managed non-operatively has increased exponentially over the years such that non-operative management is now standard management for most pediatric liver injuries.

The number of patients requiring surgery for isolated liver injuries has significantly decreased, with fewer complications, less transfusion requirements, and shorter duration of in-hospital stay. Even though most patients can be treated without surgery, the challenge is to identify the severely injured child early and institute aggressive resuscitation and expedite laparotomy [22].

Operative management

If surgery is indicated, immediate control of hemorrhage is a priority. After evacuation of the intraperitoneal blood and hematoma this can be achieved by bimanual compression, compression against the right diaphragm by sponge forceps, compression of the aorta against the spine just posterior and to the right of the esophageal hiatus, and cross-clamping of the free edge of the lesser omentum (Pringle maneuver). Continued bleeding indicates hepatic vein or vena cava injury [13]. If inadequate technical facilities are available, perihepatic packing with the aim of restoring the liver anatomy has been advocated in adults and can be similarly utilized (with appropriate-sized packs) in children (Figure 26.2) [23].

As the ribs are far more pliable in small children and most of the liver is located beyond the costal margin, the tamponade effect of packing may be less secure. It is also important not to create an abdominal compartment syndrome by increasing pressure in the abdomen from bleeding or packing. These factors, combined with the greater exposure and easier access to the retrohepatic cava, favor more active measures to control hemorrhage with an attempt at suture repair of the vascular injury [13]. Once vascular control of the liver has been achieved, the injuries can be assessed and dealt with [4].

Principles of surgical management include exposure of actively bleeding areas and control with clips or suture ligation. If bleeding arteries have been sutured or ligated close to the hilum, cholangiography is indicated to ensure that patency of a major bile duct has not been compromised. Devitalized liver tissue should be removed. This may require resection of a large segment of liver, the most frequent situation being when the liver is split along the plane of the right hepatic vein with virtual sequestration of segments VI und VII. Dead space can be filled with omentum.

Hemorrhage from oozing surfaces can be controlled with electrocautery and fibrin glue or another hemostatic agent. It is unwise to attempt mass mattress sutures of bleeding lacerations. Superficial penetrating wounds should be enlarged to secure hemostasis using finger fracture to expose the surface of traumatized liver. Selective hepatic artery ligation is indicated only when intrahepatic suture ligation has failed to control arterial hemorrhage, and should be accompanied by cholecystectomy if performed proximal to the common hepatic artery bifurcation.

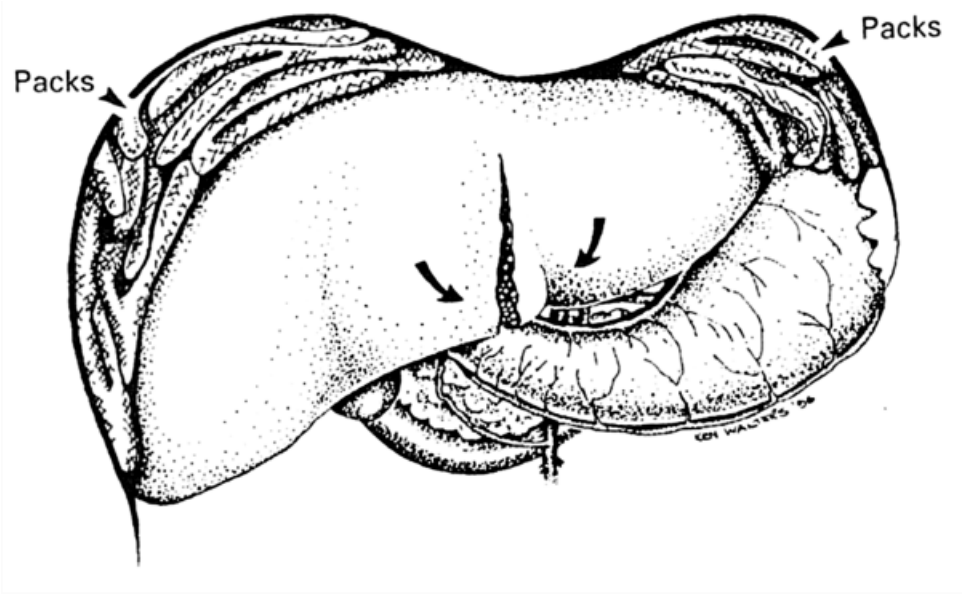

Figure 26.2 Technique of perihepatic packing, the purpose of which should be restoring the hepatic anatomy and not putting packs on the bleeding surface within the liver.

Major hepatic resection is undertaken only when the area to be resected is very severely traumatized. If bleeding persists and varies with respiration it must be assumed that the vena cava or hepatic veins are the source [13]. Stable retrohepatic hematomas, where the origin is likely to be low-pressure bleeding, should be left undisturbed. Hepatic vein and vena cava repair requires prior control of hemorrhage.

Total vascular exclusion of the liver has its advocates but may impair venous return to the heart in an already precarious hemodynamic situation. Also, veins from the adrenal gland and diaphragm may cause persistent bleeding. Unfortunately, the expertise is often not to hand, but in the appropriate setting venovenous bypass, as used in liver transplantation, utilizing the Biomedicus centrifugal pump (Minnesota, MN, USA) may be effective [1].

Intraoperative cholangiography via the gallbladder with the distal bile duct clamped will detect leaking or occluded ducts. Ducts with lacerations but in continuity can be repaired or left if located deep in liver tissue. Drainage of all these injuries was once thought to be essential but is probably unnecessary with grade II injuries that have ceased bleeding. Where there is bile leakage, closed suction drains of adequate size should be placed, as open drainage has been associated with an increased incidence of sepsis.

Interventional radiology

Unfortunately, radiological intervention is not available in all pediatric trauma centers and requires highly specialized and dedicated radiological personnel. In general, only hemodynamically stable patients should undergo angiography, unless it is convenient to do it in situ, as transport to and from the suite may lead to a detrimental outcome if there is a sudden deterioration. In centers where this service is available, sophisticated treatment protocols have been developed. It has been suggested that angiography should be performed in all cases of liver injury grade III and higher. In the first instance, a venoportal injection will detect parenchymal damage, followed by an arterial phase.

If active bleeding is detected, angiography provides the opportunity of embolization using polyvinyl alcohol particles, Gelfoam, coils, or a combination of these. Angiographic embolization may arrest hemorrhage and thus avoid laparotomy, but has a high morbidity [14].

Early and late complications and treatment

Figures 26.3 and 26.4 outline the management algorithms for acute and post-acute liver trauma.

Extrahepatic biliary tract injury

The development of extrahepatic bile duct and gallbladder complications after extrahepatic biliary tract injury depend on the effectiveness of early management and the timing of diagnosis. If treatment is delayed, these injuries may lead to biliary ascites or biliary stricture formation [9].

Injury to the gallbladder is usually the result of penetrating trauma and is managed by simple suture or cholecystectomy. Following blunt trauma, contusion or perforation may be seen.

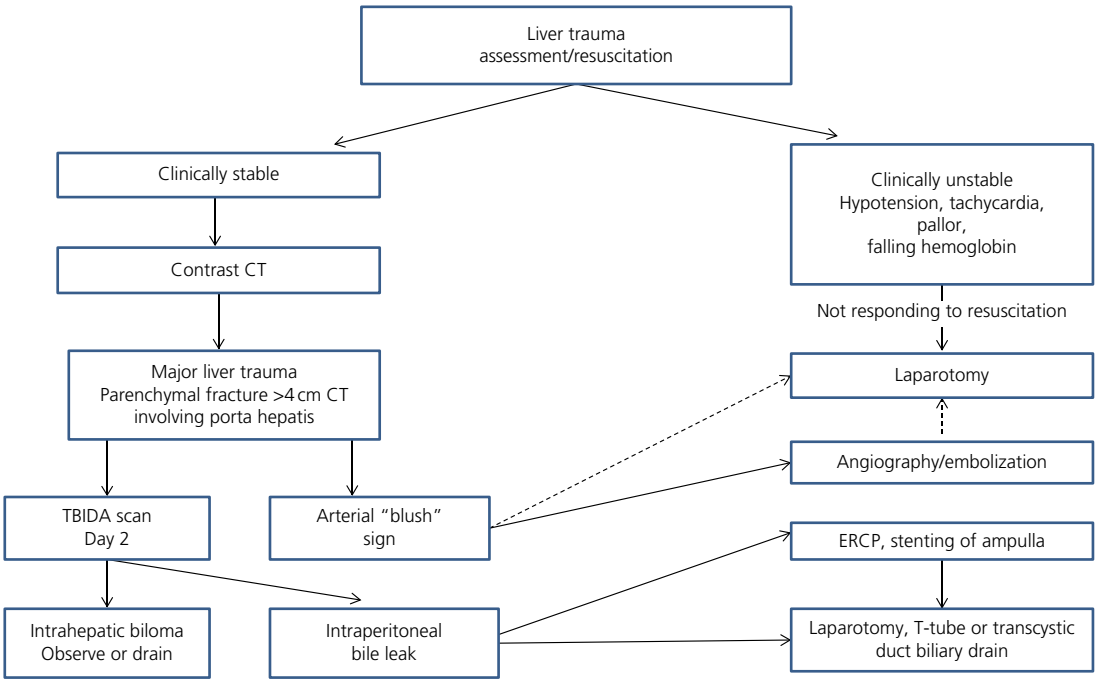

Figure 26.3 Algorithm for the management of acute liver trauma. CT, computed tomography; ERCP, endoscopic retrograde cholangiography; TBIDA, technetium-99 trimethylbromo-im-indolacetic acid.

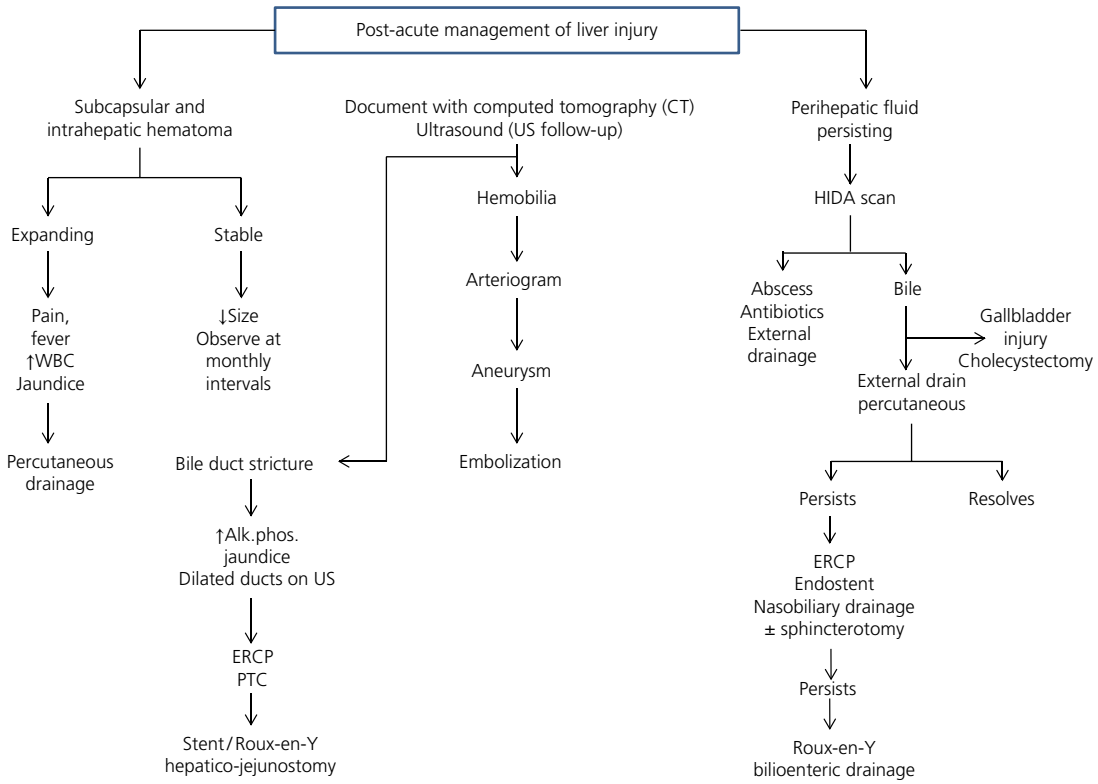

Figure 26.4 Algorithm for the management of post-acute liver trauma. ERCP, endoscopic retrograde cholangiography; US, ultrasound; HIDA, hepatobiliary iminodiacetic acid; PTC, percutaneous transhepatic cholangiography; WBC, whole blood cell count.

In most cases cholecystectomy will be performed, but reports of successful repair or cholecystostomy drainage exist [3].

Post-traumatic cholecystitis has been described following hemorrhage into the gallbladder with subsequent cystic duct obstruction [8]. Patients present with clinical evidence of acute cholecystitis accompanied by jaundice, melena stools, and hematemesis.

After injury to the biliary tree, bile leakage may localize around the area of damage as a bile lake or pseudocyst. Depending on the extent of leakage, the patient may present with ascites, nausea, low-grade fever, jaundice, or localizing signs of abdominal pain and a mass.

The majority of extrahepatic bile duct injuries are due to penetrating trauma and occur in only 0.5% of patients requiring laparotomy for blunt abdominal trauma. Injury following blunt trauma is predominantly at the distal end of the common bile duct (55%) or proximal to the bifurcation (25%), both being points of relative fixity. Total avulsion may occur. Also, post-traumatic ischemic injury of the bile ducts may occur with delay in presentation of up to several months. Persistent abdominal pain, low-grade fever, jaundice, prolonged ileus and ascites are suggestive clinical features.

Diagnosis

- Ultrasound examination is the least invasive investigation and will identify fluid collection, hematomas, and gallbladder and portal triad structures [4].
- Radionuclide excretion scanning or ultrasound-guided needle aspiration can be used to confirm the biliary nature of the fluid collection.
- Paracentesis to identify blood or bile is helpful.
- Imaging with percutaneous transhepatic cholangiography (PTC) or ERCP are used to identify the suspected injury.

Treatment

Minor lacerations can be treated with suture repair and external drainage. Major duct injuries (those involving >50% circumference) are best managed with biliary enteric anastomosis via a long Roux-en-Y choledocho-jejunostomy with a very low incidence of long-term complication [9]. Attempts at primary duct repair have led to an unacceptably high stricture rate. Biliary stents placed endoscopically may be particularly useful in proximal hepatic duct or intrahepatic segmental duct injuries, and may act by both reducing physiological intrabiliary pressure and by bridging the site of injury. However, stent migration and occlusion are recognized complications. Delayed presentation with obstructive jaundice may result in the development of biliary cirrhosis.

Pseudoaneurysms

Pseudoaneurysms develop between 2 and 4 weeks post injury. CT angiography should be performed at 2 weeks to detect their formation and embolization is the current treatment of choice (Figure 26.5) [8, 17].

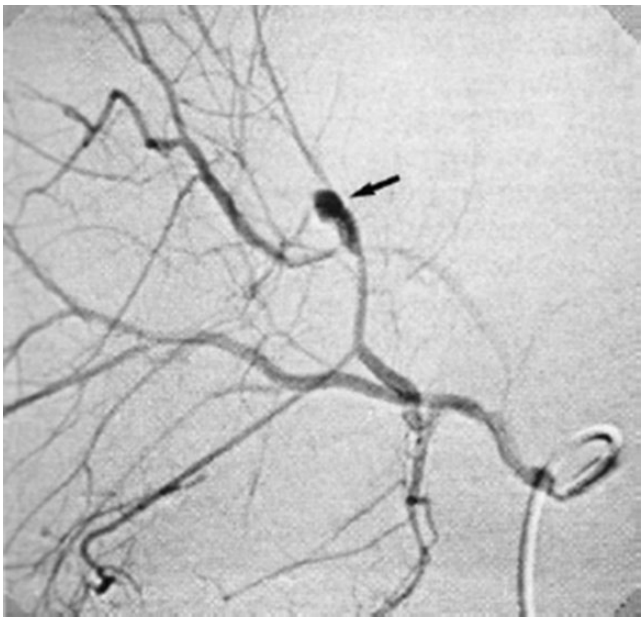

Figure 26.5 Angiography demonstrating a pseudoaneurysm of a branch of the right hepatic artery (arrow) in a patient presenting with biliary fistula and hemobilia 2 weeks after injury.

Hemobilia

Bleeding into the gastrointestinal tract from the hepatobiliary system may develop up to months after injury and presents with biliary colic, hematemesis and melena, and localized pain and tenderness, along with jaundice and fever. Most cases result from blunt trauma but some are iatrogenic from needle biopsy [8, 17]. Although bile has a significant fibrinolytic effect, depending on the extent of hemorrhage, the biliary system may become occluded with blood clot leading to cholestasis.

Diagnosis

- Technetium-99 m albumin scintigraphy may detect active bleeding or small amounts of hemorrhage.
- Upper gastrointestinal endoscopy may visualize blood at the ampulla of Vater.

Treatment

Historically, open operation and hepatotomy with oversewing or segmental resection of the area involved was used. As most cases arise from a pseudoaneurysm, the current preferred management is angiographic embolization.

Intrahepatic bile duct rupture

Intrahepatic biliary leaks resulting from minor and segmental duct disruption may lead to biliary ascites or peritonitis, a localized perihepatic collection, or an intrahepatic biloma. Most leaks resolve with adequate internal drainage [1, 4, 17]. A few persist and may require further attempts at internal drainage via either endoscopic placement of a biliary endoprosthesis or nasobiliary drainage tube with or without

endoscopic sphincterotomy. When these attempts fail, formal Roux-en-Y bilioenteric drainage into a Roux loop sutured over the source of the leak is a simple and effective solution [17].

Bilemia

Biliovenous fistulae with leakage of bile into the systemic circulation have been described after blunt trauma, and if untreated carry a mortality of up to 60% [24]. These usually result from massive trauma or attempts at direct suture of the liver. Ruptured bile ducts and hepatic veins may communicate within a cavity of damaged and necrotic parenchymal tissue. Leakage of bile into the venous system may be facilitated by the secretion pressure of the bile rather than the negative pressure in the hepatic venous system. This is especially true if bile is being secreted into a contained space via a ruptured duct.

Clinical presentation is that of rapidly increasing jaundice following injury or liver biopsy, or sudden deterioration after a period of stability. Management options include simple drainage, open or percutaneous drainage, segmental resection, and endoscopic stent drainage with balloon catheter tamponade.

Intrahepatic hematomas and post-traumatic hepatic cysts

In most cases, post-traumatic hematomas resolve within 2–3 months of injury [1, 2, 7]. Occasionally, infection may supervene with abscess formation [16]. Rarely, hematomas develop into mature cysts lined by fibrous tissue with calcification and hemosiderin deposition containing a mixture of bile and serous fluid [25]. Treatment with simple drainage usually suffices, but longstanding cysts may require limited resection, decortication, or de-roofing with plugging of the defect using omentum.

Vascular outflow obstruction

Post-traumatic hepatic vein obstruction may be segmental or complete and may arise as a result of an expanding hematoma, attempts at suture repair, or from hepatic vein thrombosis. Presentation is with pain, hepatomegaly, and ascites, and liver histology is typical of the Budd–Chiari syndrome, showing centrilobular congestion [26]. Spontaneous resolution may occur if hematoma or thrombosis is the cause. Thrombolytic therapy is probably contraindicated in the post-trauma setting. Other surgical options are stenting, mesocaval or mesoatrial shunt, or even liver transplantation [4, 26]. Long-term follow-up is required as cirrhosis could develop.

Gallstones and cholecystitis

Cholelithiasis and cholecystitis occurring several months after injury, without evidence of prior biliary disease or gallstones, has been described. Stones are thought to result from blood clots at the time of biliary hemorrhage. However, patients with severe injuries are hospitalized, starved, and perhaps fed intravenously during convalescence, thus other predisposing factors are often present.

Iatrogenic complications

The most frequent procedure carried out on the liver is percutaneous needle biopsy, and in most such cases the liver is diseased or a transplant. Although morbidity is low, especially with the disposable Menghini-type needles (Hepafix® set), late complications include hematoma, abscess, arteriovenous fistula, and hemobilia [6].

Extensive surgical resections for liver tumors are now commonplace, but it is important to be aware of variations in arterial anatomy. In approximately 25% of patients, a major portion of the arterial supply to the left lobe comes from the right hepatic artery. Disturbance of this blood supply may result in ischemic necrosis of the left extrahepatic bile ducts, requiring Roux-en-Y hepatico-jejunostomy.

Portacaval shunting is occasionally used for massive liver trauma in association with major hepatectomy, and by itself does not result in inhibition of liver regeneration in the short term. Long-term effects of portal diversion depend on the degree of diversion and the extent of collateral blood supply, but some liver atrophy and eventually encephalopathy are likely to develop.

The increased incidence of gallstones recently reported in children has resulted in a <0.5% incidence of bile duct injuries following laparoscopic cholecystectomy [22, 27].

The Red Cross War Memorial Children's Hospital experience

A group of 409 patients were seen over a 32-year period. The age of patients ranged between 3 weeks and 13 years (mean 7 years). Overall, most injuries were motor vehicle related – 303 pedestrians and 47 passengers – followed by 26 falls, 17 non-accidental injury cases, 3 bicycle injuries, 3 crush injuries, and 1 unknown cause. An isolated hepatic injury was sustained in 163 patients and 246 had multiple injuries. Associated injuries included 160 head injuries, 163 fractures, 102 thoracic injuries, and 191 intra-abdominal (including 96 spleen (Figure 26.6), 70 renal, 5 pancreatic, and 5 hollow viscus injuries). A total of 368 patients were managed non-operatively, while 30 underwent laparotomy and 2 died very soon after arrival. The total number of fatalities was three, one due to severe head injury and two due to injuries sustained to the liver. A total of 146 patients required a blood transfusion: 31% of the non-operative group (given a mean of 17 mL/kg) and 100% of the operative group (given a mean of 30.4 mL/kg). There were 13 complications in the non-operative group; in addition to the aforementioned avulsion, these included two ruptured subcapsular hematomas (Figure 26.7), requiring delayed surgery to evacuate

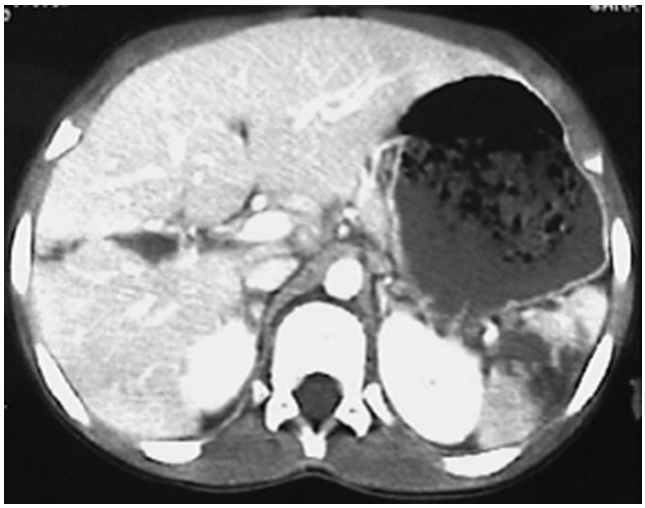

Figure 26.6 Abdominal contrast CT scan of a 7-year-old boy with a liver rupture grade II and an associated splenic rupture.

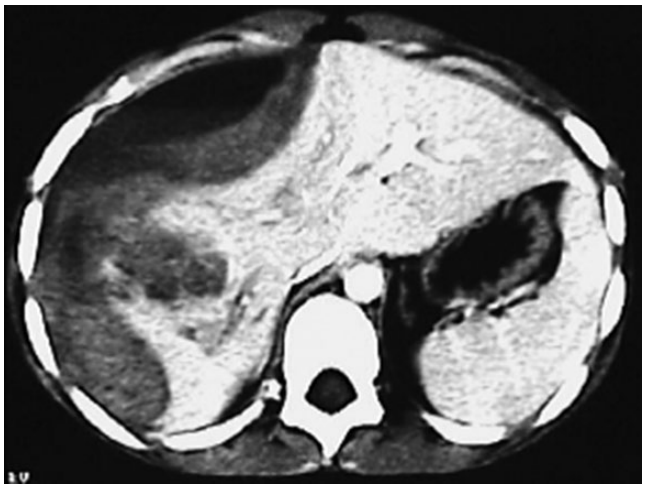

Figure 26.7 Abdominal contrast CT scan of a 10-year-old girl with massive subcapsular hematoma, partially liquefied.

the hematomas, seven abscesses, one pancreatic pseudocyst, and one fat embolism syndrome. Over the last 5 years of this period, 15 children under the age of 13 years were admitted directly to the mortuary after a fatal injury to the liver.

Outcome and prognosis

The efficacy of non-operative management for 75–85% of children with blunt hepatic injuries is now established [28], although penetrating injuries require exploration in most cases. Non-operative management of blunt hepatic injuries carries a complication rate of approximately 7%, and associated mortality is usually related to severe head injury. Delayed or recurrent hemorrhage from the lacerated liver is most often a consequence of inappropriate management selection.

Intrahepatic or subcapsular hematomas may rupture up to a month after injury and these patients require vigilant follow-up and periodic reimaging with CT or ultrasound to monitor resolution of the hematomas [3].

The relatively high morbidity in the few children who require surgical exploration is attributable to the extent of associated injuries, as well as to the liver injury itself, and sometimes to poor surgical technique. Infection in the liver during the convalescent phase is rare and usually follows surgical exploration and attempts at mass suture of the liver with open as opposed to closed drainage.

Hepatobiliary trauma encompasses a wide spectrum of injuries, so management after the acute phase is difficult to standardize. Children with grade I or II injuries who respond to conservative treatment are unlikely to suffer late complications and observation in hospital beyond 5–7 days is of little benefit. It is also difficult to assess whether routine abdominal ultrasound is cost effective in the weeks or months following injury, although it would be wise to document resolution of the lesion for medico-legal purposes.

Children who have had a hepatic injury are advised to abstain from contact sport for 3–6 months after discharge from hospital, but compliance with such advice is likely to be low.

Of special concern are children who have sustained major injuries. Although long-term clinical sequelae are rare, lobar or segmental atrophy with compensatory hypertrophy may result in axial rotation and vascular displacement. This may have relevance should further hepatobiliary surgery be contemplated.

Fortunately, complications following liver injuries can often be managed by using interventional radiological techniques. Subphrenic or subhepatic pus or bile collections can be drained percutaneously under ultrasound guidance. Hemobilia may resolve spontaneously or can be dealt with by angiographic embolization. Biliary fistulae that persist require placement of a temporary biliary endoprosthesis with or without endoscopic sphincterotomy. Persistent cases respond to Roux-en-Y enteric drainage. Likewise, extrahepatic biliary problems can ultimately be managed in a similar fashion (see Figure 26.4).

Conclusion

Modern management of liver trauma has improved outcomes and reduced the complication rate. The ongoing challenge is to eliminate the small but tragic early mortality from hemorrhage as evidenced by the number of children dying before admission to an adequate trauma center.

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CHAPTER 27

Surgical Management of Portal Hypertension

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Key points

- Complex high-flow arterioportal fistula hepatic malformations are best treated early with surgical resection/devascularization procedures as they do not respond well to embolization.
- For children with extrahepatic portal hypertension, a normal liver, and a patent recessus of Rex, a bypass to the Rex recessus is an adequate intervention as it cures the disease and all related side effects. It is now considered that a pre-emptive operation can be performed in children with a favorable anatomy with good success.
- Portosystemic shunt surgery has been replaced in the last two decades by liver transplantation in cirrhotic patients, or by interventional radiological intervention (transjugular intrahepatic portosystemic shunt), for well-selected cases with compensated liver disease and a patent portal system.
- The role and the timing of using portosystemic shunts in patients with a normal liver and a thrombosed portal vein (cavernoma) remains controversial.

During the last few decades, the efficacy of endoscopic therapy for variceal bleeds and of interventional radiological procedures has reduced the need for emergency surgical interventions in patients with portal hypertension. In parallel, the successful development of liver transplantation in the pediatric age group, with the excellent outcomes and survival rates that are now achieved, when a transplant is performed under elective conditions, has made liver replacement the gold standard – being the complete cure for chronic portal hypertension caused by irreversible hepatic damage.

For patients with a normal liver and portal cavernoma (prehepatic portal hypertension), the meso-Rex bypass shunt is now used worldwide, but its feasibility is limited to those with favorable anatomy. Portosystemic shunt procedures are still electively indicated for managing chronic portal hypertension in some specific conditions: (i) complicated prehepatic portal hypertension when a meso-Rex bypass cannot be considered; (ii) diseases that cause progressive damage/fibrosis of the liver parenchyma; and (iii) failure of conservative management when no alternative procedure can be proposed. Portosystemic shunt procedures are rarely considered as a bridge to transplant, except for rare indications.

The classification of portal hypertension types is typically based on the anatomical–pathological site of the primary or dominant lesion (pre-, post-, or intrahepatic/pre-, post-, or sinusoidal level), with many different diseases proceeding through a similar lesion pattern (portal vein thrombosis, cirrhotic changes, portal tract fibrosis, hepatic vein obstruction) (Box 27.1). The primary cause and the anatomical lesional levels (pre-, post-, or sinusoidal) have a key role in defining the strategy and adequate treatment protocols. It is important to consider those patients who have a normal hepatic parenchyma (or who have reversible damage with preserved hepatic function) at diagnosis; these cases may benefit from different surgical strategies when compared with those who have severe or irreversible parenchymal changes and hepatic dysfunction. As secondary damage and parenchymal fibrosis can develop in patients with an initially normal parenchyma, as a result of chronic blood or biliary stasis (cardiomyopathy, Budd–Chiari syndrome, cavernoma, arterioportal hypertension), timing is important for decision making.

Diseases of the Liver and Biliary System in Children, Fourth Edition. Edited by Deirdre A. Kelly.

Box 27.1 Anatomical–physiopathological classification of portal hypertension causes.

Prehepatic

- Extrahepatic:
  - Portal cavernoma – due to thrombosis of the portal vein
  - Arterioportal fistula – due to the flow at high pressure from an artery into a vein of the portal system in the splanchnic area
- Intrahepatic:
  - Thrombosis of the intrahepatic branches of the portal vein
  - Arterioportal fistula – due to the flow at high pressure from an artery into a major portal vein (major trunks or segmental radicals):
  - Simple arterioportal fistula
  - Complex vascular malformation with arterioportal fistula

Intrahepatic

- Presinusoidal: due to hepatic portal tract fibrosis (congenital hepatic fibrosis, schistosomiasis)
- Sinusoidal: due to cirrhosis (biliary atresia, post-hepatitis)
- Postsinusoidal: due to fibrosis or an obstruction of the sus-hepatic venules (veno-occlusive disease)

Posthepatic

- Due to stricture or thrombosis of the main sus-hepatic or caval vein (Budd–Chiari syndrome)

The prevention of predictable hepatic damage, by a timely procedure, is important for managing children and avoiding prolonged conservative management. The excellent outcomes of elective surgery should encourage teams to revise their strategies, therapeutic algorithms, and timing and choice of interventions.

Surgically relevant pathophysiological aspects and clinical presentations of portal hypertension

Portal system physiology and "hydraulics"

The portal system is a unique venous architecture in the body, a system that is independent of the systemic system of all of the other veins that run from any tissue straight to the heart. It is a "closed" system of veins that is composed of two capillary systems – one being the inflow (the capillary network of the gut, spleen, and pancreas), and the other, the only outflow (the hepatic sinusoidal capillary system) – connected by a network of larger veins (splanchnic veins) (Figure 27.1). This can be compared to a tree with its radicular system, where the water and the nutrients flow from the peripheral radices to the root branches, then through the trunk and the branches, to end up in the leaves.

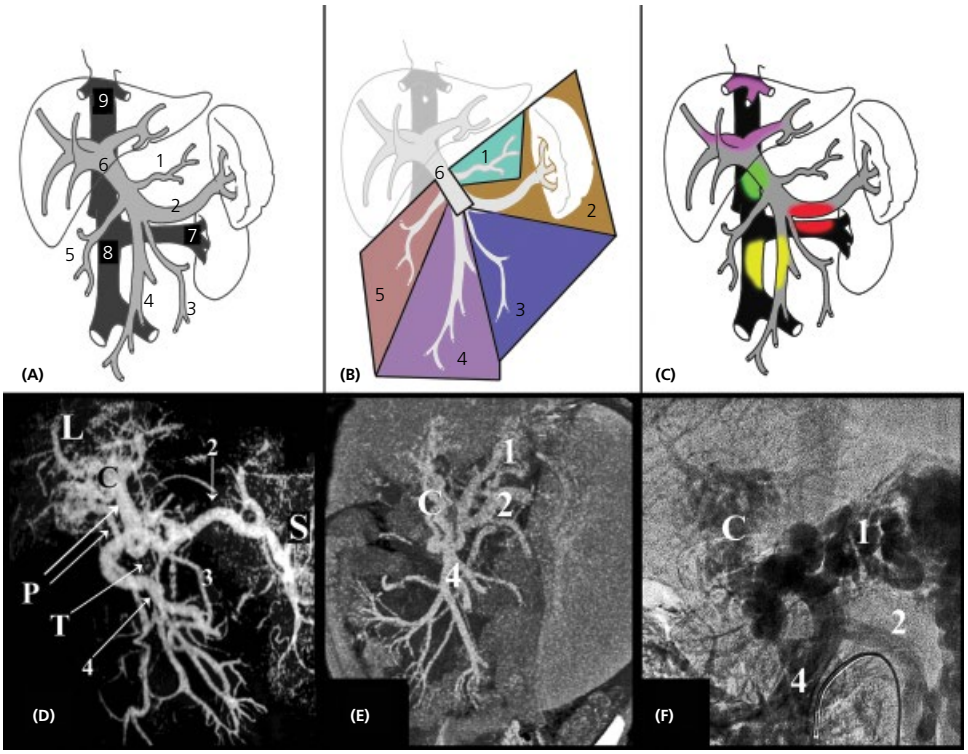

Figure 27.1 Anatomy of the portal system. (A) Schematic view of the splanchnic veins (1, gastric; 2, splenic; 3, inferior mesenteric; 4, superior mesenteric; 5, Henle trunk) and portal vein (6), and of the inferior caval system (7, left renal vein; 8, infrahepatic inferior vena cava; 9, retrohepatic vena cava and hepatic veins). (B) Areas of drainage (labeled as in A). (C) Areas of portal and caval systems with a close spatial relationship (green, portocaval; red, splenorenal; yellow, mesentericocaval; pink, portohepatic). (D–F) Remodeled splanchnic venous anatomy in patients with extrahepatic portal hypertension and a cavernoma ("C") (labeled as in A). L, liver; P, pericholedochal veins; T, thrombosed splenomesenteric junction.

In the portal system, the blood flow varies physiologically in relation to the variations of the functioning of the gut system, but the high conductance of the hepatic sinusoidal vasculature allows for the maintenance of a low pressure with a low central venous pressure gradient (only a few mmHg difference). However, because it is a closed system, any modification of its conductance at the level of the liver, or a major increase of blood inflow per se, or both phenomena in association, will have a direct effect resulting in a rise of pressure as a simple hemodynamic consequence.

The hepatic sinusoidal vasculature consists of myriads of capillaries with exceptional and unique properties, i.e., a fenestrated endothelium and double arterial and venous inflows. This means that the liver is a vascular system, in which the walls of the vasculature are composed of hepatocytes. Portal venules and capillaries both have a low resistance to blood flow, and thus a high conductance. These flow characteristics are reinforced by the structure and hemodynamic properties of the venous hepatic outflow, with a drainage system consisting of large veins that work under the effect of the thoracoabdominal pressure gradient, and of the right atrium. These both contribute to a suction-pumping effect – a negative pressure created by inspiration while a positive pressure develops in the abdomen, doubled by a low pressure in the right atrium at a diastole initiation (see Chapter 1).

Surgical relevance

1 The hepatic sinusoidal vasculature is very sensitive to inflammation, pressure, or blood stasis, all of which may remodel the endothelial wall. This leads to progressive sinusoidal and portal tract fibrosis; an extension of the portal fibrosis and of the portohepatic bridging; and ends in typical cirrhotic changes. These damages develop progressively – they are often subclinical for a long time and independent of liver function, which typically remains normal – and worsen with time. Although these changes are not usually reversible, progression may be halted if the underlying cause is managed adequately. In consequence, the timing of any intervention is crucial, in order to halt or reverse the fibrotic changes earlier.
2 Children with extrahepatic portal hypertension due to thrombosis of the portal vein, and a secondary cavernomatous transformation, have specific pathophysiological features. Typically, the vascular lesion is extrahepatic (portal trunk) (Figure 27.1) with the liver parenchyma and the sinusoidal architecture remaining normal, and the intrahepatic portal venous branches remaining patent [1]. The arterial/venous flow balance patterns change with the venous flows being randomly redistributed. In some portions of the liver, the arteries feed the sinusoids, in both an antero- and a retrograde direction, resulting in a reversed flow in the portal veins of the corresponding segment. The flow of these veins, in turn, feeds other veins of other segments in an anterograde (hepatopetal) mode. This mechanism maintains patent intrahepatic portal veins, although the flows are quite low. More importantly, the sinusoidal network remains normal and this keeps a low pressure within the system with a high conductance capacity. This situation is highly favorable for the rerouting of the flow in and through the liver with a meso-Rex bypass [2, 3], as the latter allows connection of the superior mesenteric vein (high-pressure compartment) to the low-pressure intrahepatic system (at the Rex vein) [3].

Clinical presentations and investigations

Clinical features

Children may present with a gastrointestinal bleed, isolated splenomegaly, or growth failure. An upper respiratory tract infection and, historically, aspirin therapy may be a precipitant. Anecdotal reports suggest that air transport is an additional trigger.

Splenomegaly may be associated with evidence of hypersplenism. However, unlike children with cirrhosis, humoral immunity is preserved. Ascites usually denotes the presence of chronic liver disease but may occur transiently after a major variceal bleed in those with extrahepatic portal hypertension.

Portal hypertension may cause mucosal edema in the small intestine leading to malabsorption, protein loss, and failure to thrive. Growth failure may also be present in children with extrahepatic portal hypertension. In established portal hypertension, dilated cutaneous collateral veins carry blood away from the umbilicus towards the tributaries of the vena cava (caput medusae). In longstanding disease, varices around the common bile duct may cause bile duct dilation and rarely obstructive jaundice. Rarely, pulmonary hypertension may coexist with extrahepatic portal hypertension. Children may also have neurocognitive dysfunction or subclinical encephalopathy.

Children with Budd–Chiari syndrome due to a congenital web or thrombosis may present with ascites, hepatomegaly. or acute liver failure.

Investigations

Hematology and coagulation profile

A full blood count may show anemia, leukopenia, and/or thrombocytopenia from hypersplenism. The prothrombin time and international normalized ratio (INR) are commonly prolonged in patients with intrinsic liver disease or Budd–Chiari syndrome. In extrahepatic portal hypertension, the prothrombin time is often slightly prolonged in association with a reduced level of protein S and C, and factor VII concentration. The presence of reduced procoagulant and anticoagulant protein concentrations is probably due to reduced hepatic portal blood flow and/or portosystemic shunting or even consumption within the cavernoma.

An underlying myeloproliferative disorder or thrombophilic state should be excluded by bone marrow aspirate and

estimations of protein C and S, factor V Leiden, and lupus anticoagulant.

Biochemical liver function tests

Plasma albumin may be reduced following a variceal bleed but biochemical liver function is essentially normal in extrahepatic portal hypertension; it can be abnormal in patients with primary liver disease.

Abdominal ultrasound scan and Doppler study

This confirms non-specific features of portal hypertension, such as large collateral veins and splenomegaly, and allows analysis of the cause as underlying liver disease or the presence of arterioportal fistula/malformation. Color Doppler flow studies are essential to provide information on the direction and velocity of flow in the portal vein, hepatic veins, and vena cava. Hepatic vein obstruction may be noted in Budd–Chiari syndrome

Gastrointestinal endoscopy

Endoscopy can be used to evaluate gastroesophageal and anorectal varices and mucosal features of portal hypertension at all ages. Esophageal varices are graded according to severity. Large varices may show "red signs" of recent or impending variceal hemorrhage; these stigmata include cherry-red spots (see Chapter 21). Endoscopic ultrasound assessment of submucosal and para-esophageal varices is a distinct advance with diagnostic accuracy (see Chapter 5). Portal gastropathy is characterized by mucosal hyperemia and dilated submucosal veins.

Computed tomography and magnetic resonance imaging

Both modalities are useful in evaluating focal liver lesions associated with portal hypertension. In extrahepatic portal hypertension, a variable degree of liver atrophy may be seen.

Angiography

- Angiography is particularly important when considering portosystemic shunt surgery, including meso-Rex surgery.
- Computed tomography (CT) or magnetic resonance angiography is being increasingly used as a non-invasive alternative to conventional angiography. It confirms the diagnosis of extrahepatic portal hypertension and assesses the patency and caliber of veins throughout the portomesenteric system.
- Conventional angiography can be performed by several routes, but the commonest is by indirect portography.
- Direct splenoportography after percutaneous needle puncture of the spleen also enables the measurement of splenic pulp pressure (an index of portal hypertension), which may be of value in assessing anastomotic portal vein strictures post transplant. Percutaneous transhepatic portography is occasionally used.
- Inferior vena cavography or magnetic resonance venography may be necessary to determine the patency of the inferior vena cava, hepatic veins, or intrahepatic portal and Rex veins.
- Transjugular retrograde portography should be considered for all patients with extrahepatic portal hypertension (cavernomatous transformation of the portal vein) as this is the most appropriate way of confirming patency of the recessus of Rex, and of the intrahepatic portal system anatomy.

Percutaneous liver biopsy

If there are no contraindications a biopsy is usually undertaken to diagnose any underlying liver disease. In extrahepatic portal hypertension, the liver architecture is normal but mild periportal fibrosis may be seen. In hepatic vein obstruction, liver biopsy typically shows marked venous congestion around the central venules with hepatocyte necrosis; in chronic cases, there is progression to hepatic fibrosis and cirrhosis.

Arterioportal hypertension

Arterioportal fistulae and arterioportal vascular malformations are rare. They may be congenital or acquired, intra- or extrahepatic (intrahepatic post-traumatic fistulae are the most frequent) (Figure 27.2A). Although it is rare, arterioportal fistulae may be present in a hemangiomatous mass (Figure 27.2B). Primarily, they cause arterial inflow into the portal system, and so there is an increase of pressure. The latter changes may either reach a high pressure level with acute complications (e.g., bleeding), or with chronic portal endothelial changes a progressively extending thrombosis. This makes it necessary to close the fistulous channel(s), in order to prevent, or treat, these severe complications. Although the flow can be moderated for some time in some cases (asymptomatic phase), it usually worsens at some (unpredictable) time in the life of the fistula, and then it behaves as a rapidly deteriorating clinical condition, as the arterial inflow has over-passed the hepatic sinusoidal system flow conductance. The high pressure that results from a direct arterial inflow causes a unique pressure on the endothelium of the capillaries and the venous walls, leading to progressive changes and damage within the liver and portal splanchnic system. These changes are characterized by endothelial thickening and hyperplasia, venous wall fibrosis, and, later, thrombosis. When severe, the portal system can be transformed to a point where the splanchnic venous drainage is altered and cannot be restored back to normal. The therapeutic options in these patients are limited, as the patients present a complex association of primary and secondary anatomical problems, and liver transplantation may be required. Even the latter option can be challenging due to the damaged venous wall and the reduced, or absence of, flow from the portal vein.

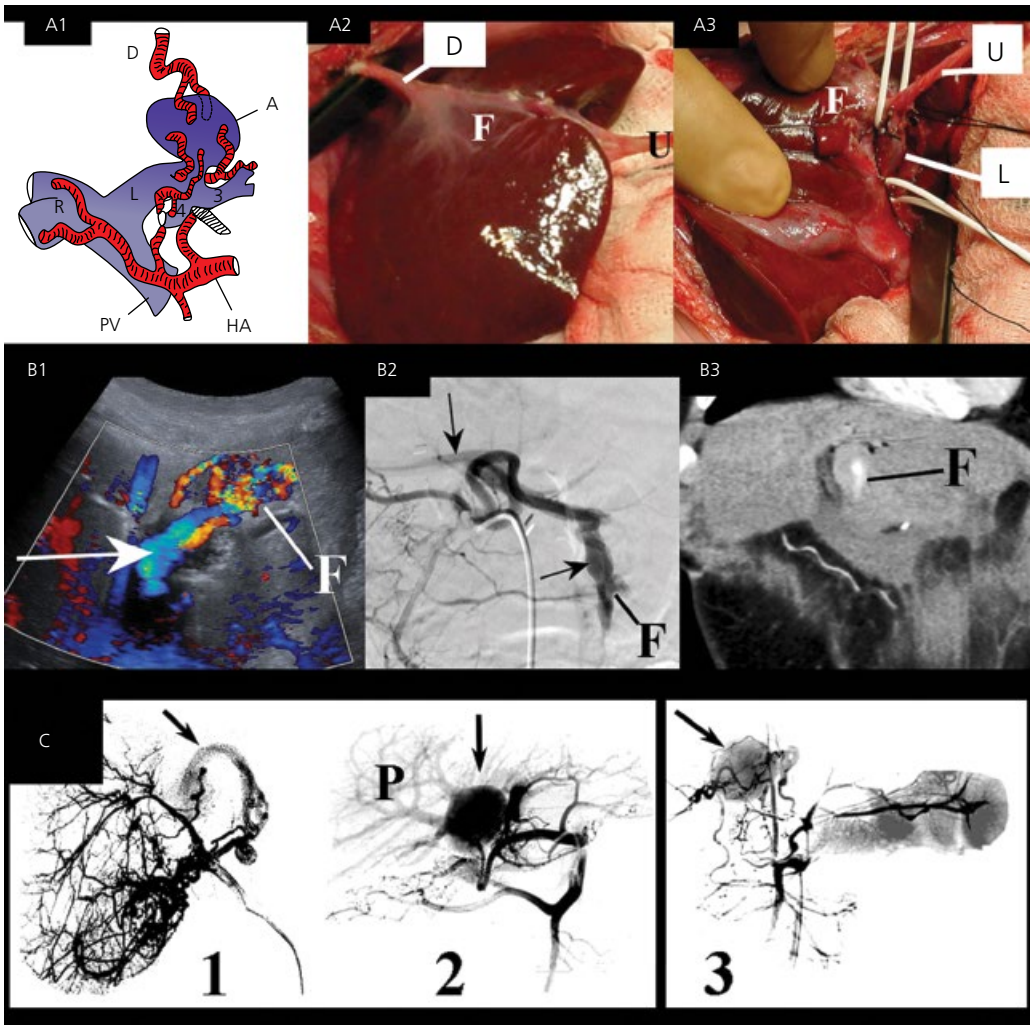

Figure 27.2 Arterioportal fistulae caused by vascular malformation (A,C) or biopsy trauma (B), managed either pre-emptively (A2,A3) or secondarily after failed recurrent attempts at embolizing (B2,B3, and C3). Management consisted of direct fistula resection/devascularization (A3,C3) or an atypical hepatectomy (B3). A, venous aneurysm (fistula); D, diaphragmatic artery; F, fistula; HA, hepatic artery; L, left portal vein; PV, portal vein; R, right portal vein; U, umbilical ligament. Arrows show contrast flowing into the venous part of the fistula or portal vein.

Surgical relevance

The timing of the intervention(s) and, most importantly, a suitable choice of procedure(s) is essential. While a simple one-channel arterioportal fistula benefits from a simple radiological procedure(s) [4], more complex malformations are challenging and difficult to manage. Most cases should first be considered for embolization, with repeated attempts at embolizing recurrent fistulous channels, as the malformation recruits new arterial sources for an inflow; at that point, the surgical options (even a transplant) are limited, as the arterial hepatic support is modified by the multiple previous embolizations. These complex cases must be approached in a multidisciplinary mode – from the diagnosis, through to a precise strategy and planning of the procedures – and should define the timing, taking into account that surgery may be compromised as time goes on. Surgery may be the best first option in some cases [5, 6].

Venous collateralization and collateral effects

Because the portal system is a closed venous system, it responds in two ways to a raised pressure. One is an expansion of the venous bed (i.e., by filling the system to its maximum capacity by using its natural elasticity, followed by a dilation/growth of the veins), possibly with diffusion of water if the oncotic pressure is low (edema of mesentery, ascites), and the second by finding alternative routes in order to bypass the high resistance of the liver. The latter phenomenon develops with time by exploiting the venous network at the margins of the splanchnic network and its natural connections with the systemic capillaries/veins. Under a positive pressure gradient, the splanchnic blood flows through these connections and they expand with time (Figure 27.3).

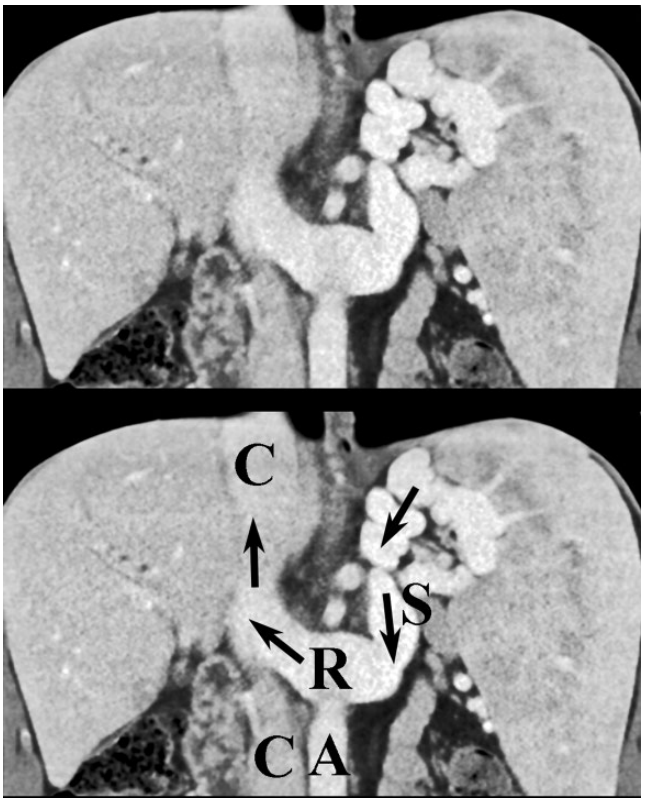

Figure 27.3 Huge splenorenal natural connections and portosystemic shunting in a patient with a cavernoma and chronic portal hypertension. The arrows show the direction of the venous flow from the retroperitoneal splenic vein collaterals (S) into the dilated left renal vein (R) and then into the vena cava (C). A, infrarenal aorta.

Venous collaterals can develop at three different levels and are associated with different risks and complications:

1 Superficially, very close to the mucosa, as typically seen in the esophagus (varices) (Figure 27.4) or the anal canal (hemorrhoids). The risk of trauma or irritation is high and the protection by the mucosa is limited. The major risk is rupture and bleeding.
2 Deeper in the digestive organ walls (typically all other parts of the digestive system, and the stomach in particular). Here there is less of a risk of rupture, but collaterals are associated with venous stasis (including a secondary development of mucosal congestion, petechiae, telangiectasia, and surface erosions) or dysfunction (malabsorption, pancreatitis).
3 Around the splanchnic organs and in the extraperitoneal space, and typically connecting into the azygos, adrenal, renal, gonadic, and caval veins (retroperitoneal), or into the abdominal wall, diaphragmatic, or iliac veins (preperitoneal). These veins are not at a risk of rupture (unless there is important trauma), but they can distend, carrying large amounts of blood and putting the patient at risk of developing portosystemic effects.

Although the above mechanisms aim at compensating for the increment of portal pressure, other physiopathological effects counteract and limit the effects of vein collateral development. These effects include the development of a hyperdynamic cardiocirculatory state and splanchnic vasodilation, or a progressive increase in the spleen mass with time, all resulting in an increment of venous inflow into the portal system. Taking all of these effects into account, portal hypertension remains and collateralization develops, and usually worsens rather than decreases with time (see Chapters 1 and 21).

Surgical relevance

1 Although this technique is no longer used, complete transection of the esophagus or the upper pole of the stomach was used to control gastroesophageal variceal bleeding (Figure 27.4B). It was only a palliative operation as there was recurrence of collaterals.
2 Even in absence of mucosal varices, occult bleeding and recurrent or chronic anemia can be the consequence of mucosal non-variceal changes, developing anywhere in the gut, but most commonly in the stomach and colon. In severe refractory cases, surgery may be indicated.
3 Large spontaneous portosystemic connections can develop with time (Figure 27.3), reducing the pressure and further development of varices. However, these natural shunts rarely achieve sufficient flow to reduce portal pressure into the normal range and should not be a contraindication for surgery.
4 The presence of large shunts in stable patients should be carefully considered because of the following points:
   - The patient is at a higher risk of developing portosystemic effects, even in a subclinical mode (encephalopathy, pulmonary arterial hypertension, hepatopulmonary syndrome).
   - These shunts may divert enough blood to compromise flow, either through the surgical shunt when fashioned, or through the portal vein at liver transplantation – thereby favoring a low flow and thrombosis. These natural shunts may require closure at surgery or transplantation.
   - Large natural retroperitoneal shunts are a possible route for non-surgical, non-transjugular intrahepatic portosystemic shunt (non-TIPSS) intervention aimed at controlling refractory bleeding (balloon-occluded retrograde transvenous obliteration procedures [7]). Although these procedures are not available everywhere, it is important to be aware of the technique.
   - The presence of large retroperitoneal splenorenal connections may be useful in cases with few surgical alternatives, such as in a patient with extensive mesenteric thrombosis and splenic vein cavernoma, in whom a splenectomy can be performed preserving all hilar vascular structures, and without touching the retroperitoneal structures.

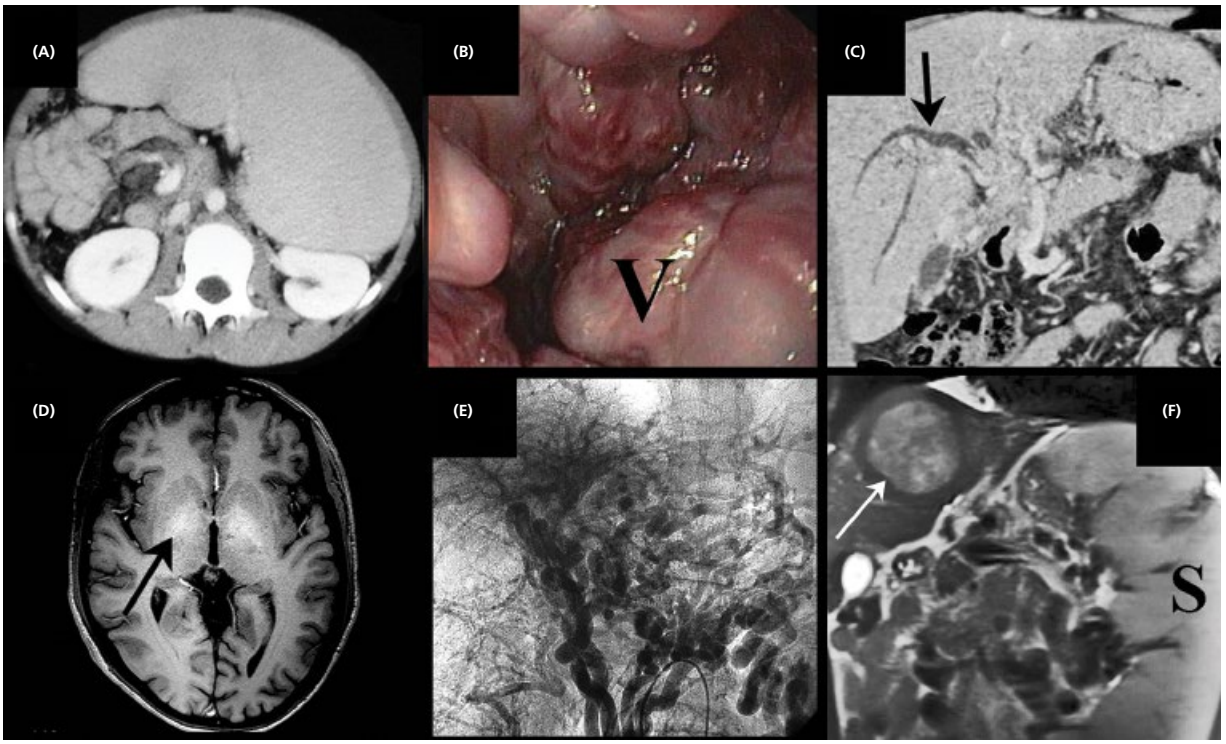

Figure 27.4 Complications of portal hypertension. (A) Splenomegaly. (B) Esophageal varices. V indicates a large, grade III varice. (C) Portal biliopathy in patients with portal cavernoma (arrow shows a dilate intrahepatic bile duct). (D) MRI T1-GRE hyperintense signal in globus pallidus (arrow) suggesting chronic encephalopathy. (E) Complete splanchnic vein system thrombosis. (F) Liver parenchymal atrophy and growth of regeneration node (arrow) in a patient with portal cavernoma and massive splenomegaly (S).

Systemic collateral effects of portosystemic connections

Systemic effects of portal hypertension are observed even if the liver function is normal because metabolites or vasoactive substances bypass the liver through the spontaneous collaterals and shunts. Although these collateral effects are rare, they should be considered in patients who are being followed up for chronic portal hypertension as they can develop subclinically. They are helpful in determining the choice of surgery and whether to proceed with surgery or not. There are two main groups of systemic effects.

Portopulmonary syndromes

Portopulmonary arterial hypertension

Portopulmonary arterial hypertension is rare in children, but is possibly more frequent in the second half of the pediatric age range, and it may be present subclinically in relatively asymptomatic patients [8, 9]. It is difficult to diagnose and may be the cause of sudden death after major surgery of either shunts or transplantation. If undiagnosed, it progresses to an advanced and irreversible state, and the prognosis is poor. If diagnosed before any irreversible anatomical changes develop in the lung tissue, portopulmonary arterial hypertension is a formal contraindication to a portosystemic shunt and is an indication to rapidly proceed to a liver transplantation [8]. In patients with extrahepatic portal hypertension, it may be possible to create a meso-Rex shunt (see Chapters 21 and 22).

Hepatopulmonary syndrome

Hepatopulmonary syndrome is characterized by oxygen desaturation, at first during effort, and then when resting [9]. This syndrome is more frequent than portopulmonary arterial hypertension in children and is easier to diagnose. Vasodilatative substances produced in the splanchnic area affect the lung microvasculature, causing insufficient oxygenation of the blood circulating through the alveolar wall capillaries as the capillaries are excessively dilated (to a diameter that does not allow any gas exchange to reach the center of the capillary). This condition is usually reversible following liver transplantation in most cases, although a meso-Rex bypass can be considered first in patients with extrahepatic portal hypertension (see Chapters 21 and 22).

Subclinical encephalopathy and encephalopathy

Hepatic encephalopathy is caused by a severe liver dysfunction or is due to portosystemic shunting. In patients with chronic portal hypertension caused by liver cirrhosis both of the former phenomena are present, but encephalopathy may also develop in patients with no

evident portosystemic shunts (fulminant liver failure on a normal liver), or in patients with spontaneous portosystemic neocollaterals and with a normal hepatic parenchyma and function (cavernomatous transformation of the portal vein). In the latter group of patients, although clinical encephalopathy is exceptional, subclinical encephalopathy may be identified by brain magnetic resonance imaging (MRI). A metabolite profile at 1H magnetic resonance spectroscopy allows for the detection of abnormal levels of glutamine and manganese, or the identification of white matter abnormalities [10, 11]. Psychometric testing (e.g., the trail-making test, the digit symbol, the block design, the psychometric hepatic encephalopathy score battery, computerized tests exploring attention, and the working memory) or an electroencephalograph(EEG) are also helpful [12]. In these patients, encephalopathy is often expressed as subtle neurological or psychiatric abnormalities, as a mild cognitive and psychomotor deficit, a lack of concentration and attention, and a lower school performance (see Chapter 21).

Surgical relevance

1 Subclinical encephalopathy should be excluded before the creation of a surgical or radiological portosystemic shunt, as this may transform subclinical encephalopathy into an overt encephalopathy.
Experience with using "calibrated" surgical shunts (limiting the diameter and thereby the flow, by using a small-diameter GoreTex®, for example) is anecdotal in children and is not recommended.
2 Clinical encephalopathy in patients with a large congenital (Abernethy malformation), acquired (surgical), or secondary to collateral circulation (chronic portal hypertension) shunt is an indication for a liver transplantation, except for those patients with an extrahepatic cavernoma and a normal liver.
3 In patients with a portal cavernoma, a normal liver, and a patent intrahepatic portal system, the meso-Rex bypass is a cure. It reverses the moderate neurocognitive disorders that are common in these patients, and performing a meso-Rex bypass pre-emptively has been recommended whenever feasible [13–15].

Splenomegaly

Splenomegaly is usually present in patients with cirrhosis and/or portal hypertension. Splenic enlargement can be massive in children with chronic extrahepatic portal hypertension and as their long-term survival is excellent, the spleen size grows progressively as they grow (see Figure 27.4A). Patients with a massive spleen may have abdominal discomfort and hypersplenism, which may lead to considering whether surgery should be performed. This is a relatively frequent request from teenager patients [5].

Surgical relevance

1 The absence of splenomegaly in patients with liver cirrhosis and hepatic dysfunction, or in patients with clinical encephalopathy and normal liver function, suggests a large natural or congenital portosystemic shunt. This may be relevant if surgery is considered, and if liver transplantation is proposed the shunt should be closed as part of a portal vein reconstruction.
2 Splenectomy is a palliative operation for portal hypertension and should never be proposed as the first operation because the inflow into the portal system is reduced by an ablation of the splenic component, and although there is an immediate drop in pressure and a temporary relief of portal hypertension, this is followed by an increase in pressureand a recurrence of complications, including variceal bleeding. A splenectomy should not be considered in the therapeutic algorithm of portal hypertension unless there is complete thrombosis of the splanchnic venous system.
3 If a splenectomy is performed, this should be done with the creation of a splenorenal shunt if the splenic vein is patent. Splenectomy alone increases the risk of thrombosis of the splenic vein, with a possible extension into the portal vein in the case of cirrhosis, or to the mesenteric veins in those patients with a cavernoma – both complications making further surgery hazardous.
4 In patients with a thrombosis of the splenic vein and a cavernoma of the pancreatic area, in whom a splenectomy cannot be associated with the creation of a shunt, a splenectomy should be performed while preserving all spontaneous splenorenal connections (resecting strictly along the splenic capsule, even at the hilum).
5 Although hypersplenism may be severe, with leukopenia and thrombopenia as low as <3000 and <50,000 cells/μL, respectively, these levels are rarely associated with clinical complications and should not be an indication for surgery.
6 Abdominal distension and discomfort may become distressing, especially in adolescence. This particular indication for a splenectomy should be considered seriously as teenagers tend to be non-adherent, while their lifestyle puts them at a higher risk (e.g., sport activities, driving, travelling), but reassurance is often sufficient.
7 In the case of major hypersplenism, a radical resection of the spleen is followed by a major increase in platelet numbers, often >1106, and this necessitates anti-aggregant management in order to prevent thrombosis, in particular, within the splanchnic venous system.
8 Post-splenectomy fulminant sepsis is rare, but may be fatal. Vaccinations against meningococcus, *Haemophilus influenzae*, and pneumococcus should be given a month in advance, and long-term postoperative antibiotic prophylaxis with penicillin V is recommended.
9 Splenic embolization is not effective in children and is a palliative intervention, with limited effects on portal

hypertension and hypersplenism. There are significant complications (pain, abscess) that create dense adhesions between the spleen and the surrounding organs or tissues, which makes further surgery difficult and possibly dangerous. This should be considered only as a last resort [16].

Splenic artery aneurysms

An aneurysm of the splenic artery is rare, but develops in patients with chronic portal hypertension, especially in those with a massive spleen. It is a life-threatening problem because the patient may exsanguinate if the aneurysm ruptures and pre-emptive exclusion is required. Typically, this condition is diagnosed in teenagers or young adults with extrahepatic portal hypertension, hepatic fibrosis, or cystic fibrosis (all conditions with longstanding portal hypertension and splenomegaly). Also, typically, the aneurysm is usually located very distally on the splenic artery course, often within the splenic hilum (in contrast to an isolated adult-type splenic artery aneurysm). It usually grows slowly, subclinically, and also "sub-radiologically" – as the aneurysm is difficult to identify, especially when it is lying surrounded by a network of circumvoluted dilated arteries, large veins, and huge retroperitoneal collaterals (Figure 27.5).

A ruptured aneurysm presents with abdominal pain or collapse. The aneurysm develops on a large artery, feeding a large spleen, and thus rupture causes a rapidly progressive, usually fatal, hemoperitoneum. Diagnosis is with CT angiography, but immediate resuscitation and emergency surgery is required.

Surgical relevance

1 Detection of these aneurysms should be part of the long-term follow-up of adolescent patients with chronic portal hypertension using abdominal ultrasound as a screening method.
2 Left-sided upper abdominal pain in patients with chronic portal hypertension and large splenomegaly should trigger CT angiography, with a very early arterial contrast phase, to identify and define anatomically a possible distal splenic artery aneurysm.
3 Surgery is difficult because the aneurysms are often located distally on the artery, and there is a risk of necrosis of the spleen. The surgeon's role includes standing by at a radiological intervention, treating the complications (including a splenectomy) for massive necrosis, or even primary surgery, when appropriate.

Portal biliopathy

Portal biliopathy has been poorly understood and inadequately managed for decades, but is now better understood [17–19]. The recent success in managing chronic portal hypertension in patients with an extrahepatic cavernoma means that many patients survive into adult life – when portal biliopathy becomes clinically relevant and is associated with complications.

High portal pressure drives the development of a collateral circulation through the veins that connect the splanchnic venous area with other systemic areas. These veins do not develop between areas of similar pressure and will increase in size with increased flow. It is thus uncommon to observe a cavernomatous transformation of the liver hilum in patients with cirrhotic livers. The liver is a high-pressure area, and flow will not develop in the liver. On the contrary, in patients with a normal liver and with extrahepatic portal hypertension, the liver is a low-pressure area and multiple small veins in the hilum, and in particular the pericholedochal veins, grow and flow towards the liver [17]. Anatomically speaking, these veins belong to the epi- and/or paracholedochal venous system and are located within the wall of the choledochus. They drain directly into the liver venous system. While they are increasing in size, these choledochal veins can expand either "externally" to the choledocal wall, or into the lumen. They then present as varices in the lumen of the biliary tree – possibly filling the lumen by confluence, and cause a mechanical, not structural, obstruction of bile flow (a portal pressure that is higher than that of the bile flow secretion).

Figure 27.5 Aneurysm of the splenic artery (arrow) in an adolescent patient with chronic portal hypertension (thrombosis of the portal vein secondary to neonatal umbilical catheter use). (A) Arterial CT phase showing the distal position of the aneurysm on the splenic artery course (within the splenic hilum). (B) Three-dimensional reconstruction. (C) Arterial and venous phase showing how the aneurysm is sitting in an area where numerous large arteries and veins can make for a difficult positive diagnosis.

Structural lesions (biliary strictures) develop, perhaps as a result of ischemic injury of the bile duct wall. Both types of anomalies are associated with a moderate dilation of the intrahepatic biliary system (see Figure 27.4C). The cholangiogram (retrograde, prograde, or at MRI) may mimic a cholangiocarcinoma or sclerosing cholangitis (hence this particular aspect is called "pseudocholangiocarcinoma" or "pseudo-sclerosing cholangitis") [17]. Lastly, the bile stasis is often complicated by sludge or stone formation, usually in the gallbladder or in the liver [18].

Patients present with jaundice, cholangitis, and biliary obstruction.

Surgical relevance

1 Most children with extrahepatic portal hypertension have a minor or moderate degree of portal biliopathy, but complications are rare. Treatment is with ursodeoxycholic acid.
2 The degree of luminal obstruction depends on the portal pressure: lowering or normalizing the portal pressure resolves the problem but endoscopic stenting may increase the risk of sludge formation and cholangitis [17, 18].
3 Late complications, including secondary biliary cirrhosis, may be the result of long-term chronic portal biliopathy so consideration should be given to performing a portosystemic shunt to prevent progression of disease [19].
4 In children with a patent Rex recessus, a meso-Rex bypass is a cure and should be considered pre-emptively [20].
5 Complicated gallbladder lithiasis in a patient with extrahepatic portal hypertension and no active shunt is a complex condition because the cavernomatous transformation of the liver hilum encompasses the gallbladder. Hence, a cholecystectomy can be challenging and should be performed in an expert center [17, 18].

Hepatic tumors

Reactive, benign, regenerative-type lesions, or focal nodular hyperplasia or adenoma, may develop in patients with portal hypertension of any cause, even in the absence of cirrhosis [21]. In these patients, portosystemic shunting – congenital or acquired – seems to encourage the development of hepatic regenerative lesions that appear to be secondary to hemodynamic and architectural changes, as observed in animal models. It is possible that portal vein blood deprivation is a stimulus for uncontrolled liver growth and regeneration because these lesions are often found after the experimental creation of a portosystemic shunt, or observed spontaneously in patients with a congenital absence of the portal vein (see Figure 27.4F) [21].

Surgical relevance

1 Adenoma can grow rapidly and rupture with massive hemorrhage, or become malignant. In contrast, hepatocarcinoma may mimic benign liver regeneration nodes.
2 All patients with portal hypertension with or without cirrhosis, and those with portosystemic shunting (surgical or not), should be carefully screened for liver nodules by abdominal ultrasound.
3 The appearance of a new liver node should trigger detailed imaging with CT or MRI, a liver biopsy if feasible, and measurement of α-fetoprotein (AFP).
4 Benign reactive regeneration nodes can be simply followed up by ultrasound.
5 Patients with a rapidly growing adenoma or with high AFP levels, and those who are confirmed by biopsy as positive for B-catenin or hepatocarcinoma features, should be considered for resection or for a liver transplantation (see Chapter 28).
6 Hepatic regeneration nodules may disappear after a meso-Rex bypass suggesting that portal blood flow deprivation may be the cause.

Anatomical basis for portal hypertension surgery

The need for surgical shunts has been much diminished with the development of effective endoscopic management, radiological interventional procedures, and liver transplantation. There are now few remaining indications for shunt surgery, which is mostly used as a last resort in patients for whom no alternative exists.

In children, the use of these techniques has been limited in the past and few teams have developed an expertise with these operations in children on a large scale. Contributing factors for this were:

- Chronic liver disease and portal hypertension is less frequent in children compared with adults, so general pediatric surgeons do not have experience of portosystemic shunting.
- The high thrombosis rate in shunts performed in young children, related to the small caliber of the "major" veins of the splanchnic area, has been a major deterrent.
- Liver transplantation is now standard management in those with a chronic and irreversible liver disease, and precludes the realization of a (temporary) portosystemic shunt.

The most important and relevant anatomical aspects for the surgeon when dealing with portal hypertension in children are:

1 The anatomy of the major venous trunks (mesenteric, splenic, and portal veins).
2 The vicinity of relationships between the portal system and the caval system.
3 The intrahepatic portal system and the Rex recessus.

Extrahepatic portal system

The extrahepatic portal system includes all of the veins in the splanchnic area. It drains the venous blood from the gut (from the lower esophagus level up to the mid portion of the

rectum), the pancreas, and the spleen. This venous drainage system is anatomically organized into five separate areas, with each area draining into a major vein, and with the major veins subsequently joining to form a unique trunk that flows into the liver (the portal vein) (see Figure 27.1A,B): These five areas and veins are:

1 The splenic vein that drains the spleen, pancreas, and main part of the stomach.
2 The inferior mesenteric vein that drains the left transverse and descending colon, and the upper portion of the rectum.
3 The superior mesenteric vein that drains all of the small intestines and cecum.
4 The gastric vein that drains the lower esophagus and small curvature of the stomach.
5 The right colonic vein (or "Henle" venous trunk) that drains the ascending and right transverse colon; it lies between the duodenopancreas and mesocolon transverse.

Any of these five veins, and especially the first three in the list – which are larger in diameter – is of relevance for the surgeon because he or she will have to approach one or more of these, either for portal hypertension surgery or for a liver transplantation. These veins are the usual inflow sites for shunting or a graft reperfusion. It is useful to know a bit more about the other (smaller) veins:

- The gastric vein is a common route for the collateral flow developing from the splanchnic area to the thoracic azygos system through the gastric and esophageal venous network. This may become so distended that it may be used as an inflow site in selected cases.
- The Henle venous trunk is less relevant for a shunt operation, but deserves to be mentioned because the surgeon must identify it and appropriately divide this trunk during the various procedures, and, especially, when approaching the superior mesenteric vein between the pancreas head and the mesocolon.

Topographic anatomy

The topographic anatomy shows how close the portal system and the caval system are to each other (see Figure 27.1C). The caval system runs in the retroperitoneum along the posterior abdominal wall, from the pelvis to the diaphragm, and connects with an alternative (or compensatory) venous pathway that runs parallel towards the thorax (azygos system), or the liver in its retrohepatic portion. The caval system lies close to the major veins of the portal system, close enough to consider creating a shunt between both systems. These are:

- The portal vein and portion of the vena cava between the liver and the renal veins, respectively.
- The left renal vein and splenic vein, respectively.
- The left renal vein and inferior mesenteric vein, respectively.
- The inferior vena cava and mesenteric vein, respectively.

Although dependent on the patient's particular anatomy, many shunts can be created without interposing bypass material using direct anastomosis (with the transposition of a vein), end-to-side anastomosis, or side-to-side anastomosis. However, creating a shunt between the inferior vena cava and the mesenteric vein (mesocaval shunt) always necessitates the interposition of bypass material – except if the vena cava is sacrificed and rerouted to the mesenteric vein as in the Clatworthy technique.

Meso-Rex bypass procedure

The meso-Rex bypass procedure was developed for children with extrahepatic portal hypertension, and encouraged study of the anatomy of the intrahepatic portal system and precise imaging of it, particularly the recessus of Rex [1, 5, 16].

When it enters the liver, the portal vein trunk divides into two or three veins (one or two for the right liver and one trunk for the left liver. All three trunks subsequently divide within the liver into segmental branches, feeding the corresponding segments. The left portal vein trunk anatomy is particularly interesting in that it runs parallel to the liver surface and remains mostly extrahepatically all along the porta hepatis, toward its end in the umbilical vein remnant. From the main portal bifurcation, it runs for a 2–4 cm length toward the left, and then the main trunk changes its direction – from a frontal to a sagittal route – and follows the umbilical scissure plane. The latter portion of the vein (running sagittally in the umbilical scissure) is named after the German anatomist who described it (Hugo Rex). From this section of the vein arise the many venous branches for segments 2, 3, and 4, and for the caudate lobe. Although it is often necessary to divide the bridge of the parenchyma, formed by the fusion of the liver capsule at the edges of segments 3 and 4, the ventral aspect of the recessus of Rex is free of branches and is covered by only a thin layer of peritoneum without arteries or bile ducts.

As there are no valves, portal venous flow can run in a reverse mode along the left portal branch and feed the right liver, so the Rex recessus is an ideal entry site to reperfuse the liver in the case of a portal vein trunk thrombosis [3]. Furthermore, because the cavernomatous transformation that involves the porta hepatis area makes surgery at the porta hepatis challenging, approaching the Rex recessus is simpler for the surgeon. The surgical area is central in the abdomen and lateral to the liver hilum, outside the cavernoma.

However, if the intrahepatic portal system has been thrombosed as an extension of the thrombotic process, or as a primary site (typically by an umbilical catheter) [1] with thrombosis of the Rex recessus, this preclude the formation of a meso-Rex bypass. It is thus essential that careful imaging of the intrahepatic portal system is conducted before planning the intervention, and before identifying a patent Rex recessus. Although a Doppler ultrasound, CT angiography, or MRI can provide an adequate image in some cases, the current

recommendation is to perform transjugular retrograde portography, which allows a direct anatomical assessment.

Surgical techniques

Interventions for arterioportal fistula

All patients with an arterioportal fistula should be managed promptly, as the flow through the fistula can grow rapidly, and can cause acute portal hypertension and a subsequent transformation of the portal system. The timing and severity of these complications are not predictable, and all fistulae should be closed for this reason (see Figure 27.2).

Inversion of the portal flow within a major portal trunk (left, right, or – worse – the portal trunk) and/or the development of portal hypertension must prompt intervention, surgical if necessary, to interrupt the arterial flow into the portal system. Failure to intervene may cause major endothelial damage, in the liver and splanchnic veins, consisting of intrahepatic partial thrombosis and saccular deformation of the veins, markedly thickened by the portal venous system, and even extensive thrombosis [6].

A simple vascular fistula/malformation and even a solitary hemangioma is best managed by radiological intervention, which is a safer, simpler, more selective, and more efficient procedure for such lesions. Surgical devascularization interventions – consisting of the division of major hepatic arteries (right or left or even the hepatic trunk) to control either a congenital fistula or vascular malformation, in order to diffuse hepatic hemangiomatosis or a large, solitary, infantile hemangioma of the liver – have little or no role these days [4–6].

Very complex arterioportal malformations should be discussed by a multispecialty team because the risk of failure due to embolization is high. It is important to be clear about the best strategy, the type of procedure to be performed, and how much anatomical area to preserve, and to leave "space" for a possible rescue management by surgery if necessary. Radiological interventions and a repetitive embolization can create a vascular remodelling that makes any future liver resection surgery (including the option of a liver transplantation) difficult.

Surgery might be considered as a first option in some cases where the malformation could be easily controlled surgically. Surgery may be either direct arteriovenous disconnection/devascularization, or by partial resection of the liver. Exceptionally, surgery may be necessary for complications after radiological management, as for example in a thrombosis of the portal system due to migration in the portal system of coils or material used for the embolization of a large fistula [4]. Rarely, a total hepatectomy and liver transplantation may be a last resort intervention if all other therapy has failed and portal hypertension and liver dysfunction develops [6].

Portosystemic shunts

Prior to effective variceal endoscopic management and the development of radiological interventions – allowing a surgeon to create a shunt percutaneously (TIPSS), or to close the splanchnic collaterals in a retrograde manner (balloon-occluded retrograde transvenous obliteration procedures) – surgeons created a variety of procedures aimed at decreasing portal pressure by diverting the portal blood flow into the systemic circulation (Figure 27.6). The drop in portal pressure depends upon how much flow is diverted, and this varies according to the diameter of the anastomosis, the shunt type, and the location of the site chosen for the portal blood diversion. Shunts can be tailored to the need of the patient by adapting the caliber and length of the shunt, and by the use or not of prosthetic material.

However, with the development of interventional endoscopy and radiology, and the success of liver replacement as a radical cure, shunting procedures are nowadays rarely performed. The remaining indications in children are in those with chronic liver disease and a well-preserved liver function (pediatric end-stage liver disease (PELD) socire <10) (e.g., congenital hepatic fibrosis), who develop refractory complications to conservative management (i.e., ascites or variceal bleeding). Exceptionally, it may be a bridge to transplant, managing ascites and portal hypertension.

Non-surgical interventions such as TIPSS are preferred because the latter procedure has the advantage of: (i) preserving the integrity of the abdomen; (ii) allowing percutaneous management of the stent (pressure gradient checks, balloon dilation, etc.); and (iii) allowing for removal of the stent, and thus closure of the shunt, at the time of a liver replacement.

Definitions: central, distal, lateral, and H

Surgical shunts are designated by referring to the surgeon who introduced the technique (e.g., Clatworthy, Auvert, Warren, Sarfeh, and Mitra), or by successive epithets (central or distal, end-to-side, side-to-side, or H type) and anatomical references (veins of origin and the end of the shunt) (Figure 27.6).

Distal or central refers to that portion of the portal system that is drained (distal), and not the main portal system (central). It also refers to the "selectivity" of the drainage, with distal being selective and central being non-selective. A distal or selective shunt is supposed to avoid the risk of an excessive, or total, diversion of the portal flow into the systemic circulation, reducing the liver portal flow and with collateral effects (encephalopathy and portopulmonary syndromes). Unfortunately, with time, even distal shunts get to "centralize" (development of numerous collaterals connecting drained and undrained areas at the splanchnic level), and render the distal shunt less selective.

The epithets end-to-side, side-to-side, or H-type describe how the connection between the portal and the caval system

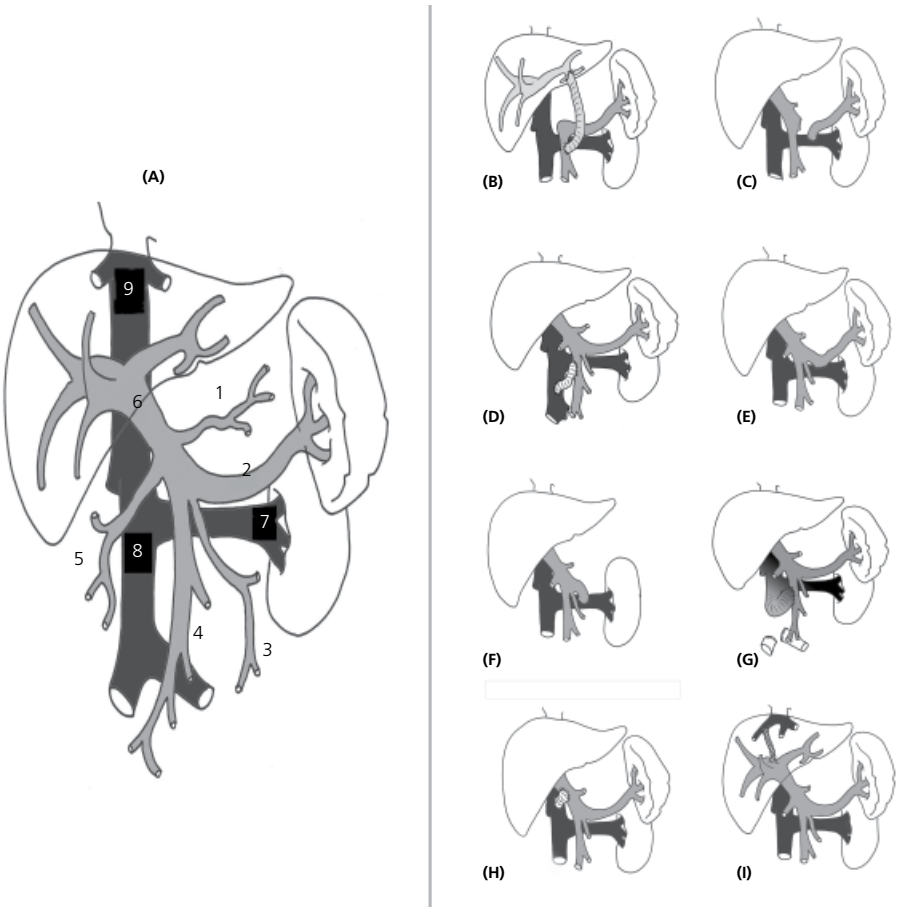

Figure 27.6 Intervention types for the management of portal hypertension. (A) Schematic view of the splanchnic veins (1, gastric; 2, splenic; 3, inferior mesenteric; 4, superior mesenteric; 5, Henle trunk), portal vein (6), and the inferior caval system (7, left renal vein; 8, infrahepatic inferior vena cava; 9, retrohepatic vena cava and hepatic veins). (B) Meso-Rex bypass. (C) Distal splenorenal shunt (Warren). (D) Mesocaval shunt (H type). (E) Side-to-side splenorenal shunt (Mitra). (F) Proximal (central) splenorenal shunt (with splenectomy). (G) Cavomesenteric shunt (Clatworthy). (H) Portocaval shunt (H type). (I) Intrahepatic portohepatic shunt (TIPSS).

is technically realized. End-to-side means that a splanchnic vein is divided and rerouted to end terminally in the caval system (portocaval, splenorenal, cavomesenteric).

A TIPSS is a central (non-selective) H-type portohepatic shunt that uses prosthetic material inserted under fluoroscopic guidance by an interventional radiologist.

Portosystemic shunts: surgical types

Selective or distal shunts

The best example is the distal end-to-side splenorenal shunt (Warren shunt) (Figure 27.7) [22]. The splenic vein is divided close to the mesenteric vein and brought to the left renal vein into which the splenic stump is anastomosed. In the conventional procedure, a devascularization of the short gastric curvature is performed by division of the gastric vein. By dividing the splenic vein at its junction with the mesenteric vein, mesenteric blood flow toward the liver is preserved, while the stomach, pancreas, and spleen are decompressed. It is useful for reducing spleen size, in treating hypersplenism, and in reducing the risk of variceal or gastric bleeding. It is also a good option for children with extrahepatic portal hypertension who are not candidates for a meso-Rex bypass, because of a thrombosed Rex recessus, as the procedure maintains flow towards the liver through the cavernoma.

Although it is a selective shunt, and is supposed to reduce the risk of postoperative encephalopathy, the progressive centralization of the shunting is associated with hyperammonemia [13–15]. Long-term patency rates of >90% have been reported, with a favorable long-term survival and with a low rebleeding rate [22].

Non-selective or central shunts

A direct, non-selective communication is opened between the portal system and the systemic circulation which, depending on the flow through the shunt, reduces portal pressure. These shunts are associated with a significant risk of hepatic encephalopathy and should be avoided whenever possible, or used late in life, as a possible preservation of the neurocognitive function is particularly important in children.

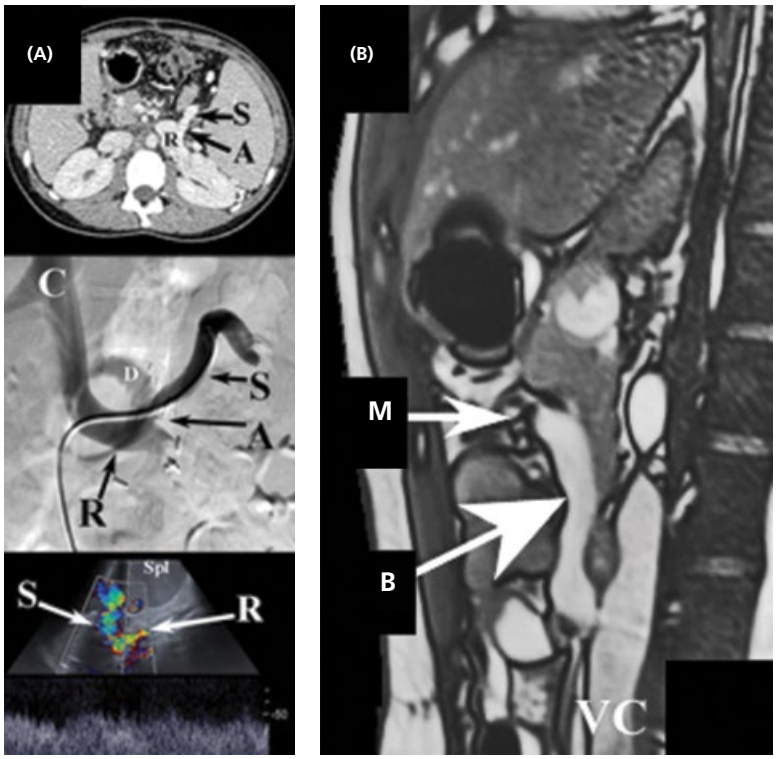

Figure 27.7 Portosystemic shunts. (A) Distal splenorenal shunt. (B) Mesocaval shunt. A, anastomosis; B, bypass conduit; D, duodenum; M, mesenteric vein; R, left renal vein; S, splenic vein; Spl, spleen; VC, vena cava.

These shunts correspond anatomically to Figure 27.6:

- Portocaval shunt (of any type: end-to-side, H, or side-to-side).
- Proximal splenorenal shunt: the spleen is removed and the splenic vein stump is brought into the left renal vein (end-to-side).
- Mesocaval shunt: this is usually an H-type shunt interposing with a conduit (prosthetic tube or autologous vein graft). In children, a mesocaval shunt with a jugular vein conduit has been used with satisfactory results.
- Cavomesenteric shunt: the Auvert and Clatworthy shunts are fashioned after the division of the iliac veins or vena cava, respectively. After mobilization of the inferior vena cava, the stump is anastomosed onto the superior mesenteric vein. Late venous complications are reported in relation to the interruption of both iliac veins.
- Side-to-side and H splenorenal shunt: this non-selective shunt has the advantage of preserving the spleen when compared with the central proximal splenorenal shunt. A Mitra or Denton Cooley shunt is a side-to-side splenorenal shunt. In this shunt, the spleen is preserved and the shunt is fashioned between the splenic vein and left renal vein. It may reduce the risk of encephalopathy compared with other non-selective shunts because it does not completely steal portal flow from the liver; good results have been reported.

Tailored central shunts

To limit the amount of blood shunted through the connection and to avoid collateral effects, surgeons have proposed a "calibrated" shunt to make it more selective. These shunts are less effective in reducing portal pressure and hypersplenism, with a higher risk of thrombosis or occlusion in the long term. They are not recommended for children.

A Sarfeh shunt is an H-type portocaval shunt using an 8 mm diameter GoreTex® graft. Similarly, a mesocaval H shunt can be fashioned using an 8 mm diameter GoreTex® graft.

Meso-Rex bypass

The meso-Rex bypass was first used for portal vein thrombosis, following transplantation, and was later used for children with an idiopathic cavernoma. The recessus of Rex is the portion of the portal system that was part of the umbilical vein path before birth. It provided inflow into the body and liver, before birth, and can easily be reconverted to a new inflow for decompressing the portal system. The umbilical fissure is a favorable place for accessing the portal vein system as it lies outside the porta hepatis, where the cavernoma develops. Lastly, the blood flows easily from left to right in the portal venous system and this allows for maximal flow and pressure decompression.

A meso-Rex shunt is indicated in children with a portal vein thrombosis and cavernomatous transformation who

have no liver disease and normal liver function. The anatomy of these patients allows for rerouting of the portal flow from the splanchnic area (high pressure) into the intrahepatic portal system (low pressure).

A liver biopsy may be required to confirm that the parenchyma is normal and that there is no evidence of hepatic fibrosis, cryptogenic cirrhosis, or obliterative portal venopathy. These diseases are rare in children and a liver biopsy should only be indicated in children with an abnormal finding on ultrasound or an abnormal portocaval gradient measured by a wedged catheterism during retrograde portography [1]. A diagnosis of obliterative venopathy or other parenchymal liver disease is a contraindication for considering a meso-Rex bypass [1, 5, 23].

When the liver parenchyma is normal, the liver has a low venous pressure and resistance to the portal flow is entirely extrahepatic. Portal vein thrombosis is usually limited to the trunk of the portal vein in most cases, although it may extend into the splenic and/or mesenteric veins in around a third of cases, with an occlusion of the confluence between these veins, and the development of huge collaterals around and through the duodenopancreatic area (see Figure 27.1D,E,F). Even with the latter complications, the superior mesenteric vein is patent and accessible for the surgeon, just below the pancreas in most patients. Although the veins may be hypoplastic and small in diameter (3–4 mm), if the intrahepatic system is patent it may be revascularized with success. These veins may not be visible on conventional imaging (e.g., Doppler ultrasound, CT angiography, angio-MRI, or even with conventional angiography) and can only be adequately imaged by retrograde portography [1, 5, 23].

The technique for this procedure has been described in detail elsewhere [2]. The inflow site for the meso-Rex bypass is preferred from the superior mesenteric vein, and is immediately below the edge of the pancreas, but alternative sites can be used (Figure 27.8), for instance the coronary vein, splenic vein, inferior mesenteric vein, or even the large gastroduodenal vein. The conduit itself is preferably an autologous vein of an adequate diameter. The best results are obtained using the patient's own internal jugular vein. Other alternatives are more complex (e.g., a spiraled saphenous vein) and/or are associated with less good outcomes (e.g.,

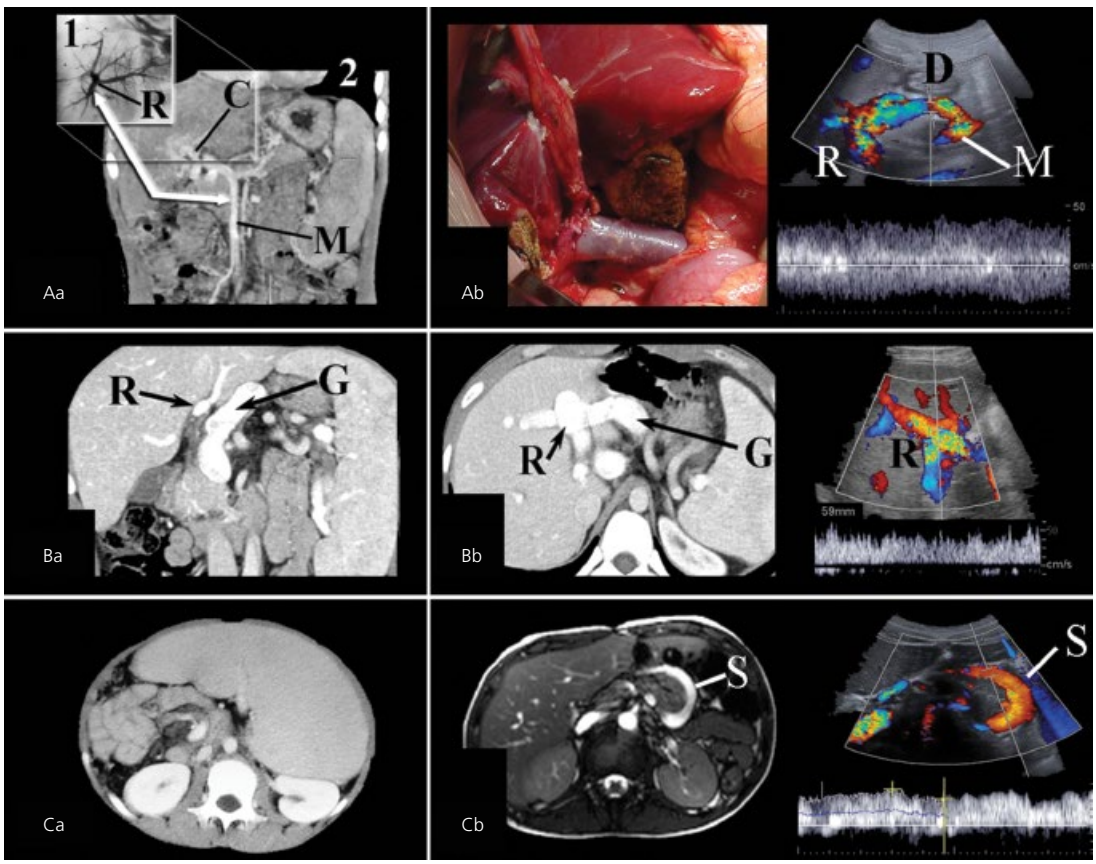

Figure 27.8 Management of extrahepatic portal hypertension by bypasses into the Rex recessus (R). (A) Bypass from the superior mesenteric vein (M) with a standard technique: 1, retrograde portography showing that the intrahepatic portal system and the Rex recessus are patent; 2, CT reconstruction. (B) Bypass from the gastric vein (G) in a patient with a favorable anatomy consisting of a large gastric vein sitting close to the Rex recessus. (C) Bypass using the splenic vein (S) in a teenager complaining of a huge splenomegaly (Ca) and who underwent a splenectomy with spleno-Rex bypass (Cb). (Aa, Ba, and Ca are preoperative views; Ab, Bb, and Cb are operative and postoperative views.) C, cavernoma.

recanalized umbilical vein, cryopreserved allogeneic vein, or prosthetic GoreTex®).

Alternative surgery

Alternative surgery has been used in the past and consists of:

- Splenectomy.
- Splenopexy/transposition.
- Spleen embolization.
- Gastric/esophageal devascularization.

These procedures are palliative as there is usually residual portal hypertension, and are best avoided in children except for selected patients who have no other alternative because of complex anatomical conditions (e.g., extensive splanchnic thrombosis).

Splenectomy

Although an increase in the size of the spleen is the rule in patients with portal hypertension, the associated hypersplenism is rarely a major issue in children. Even with low figures, such as <50,000 platelets or <3500 white cell count, patients do not have significant complications (see Chapter 21). Isolated splenectomy is a palliative operation that has a temporary effect on portal hypertension and may cause thrombosis of the splenic vein, portal vein, or superior mesenteric vein and is best avoided [5].

Vaccinations against meningococcus, *Haemophilus influenzae*, and pneumococcus, should be given a month in advance, and long-term postoperative antibiotic prophylaxis is recommended.

Indications include:

- Patients with thrombosis of the splenic vein and with major splenomegaly/hypersplenism; the spleen can be removed on the condition that the splenectomy is performed very distally (i.e., very close to the splenic capsule), preserving all of the veins of the hilum and the perisplenic collaterals (natural portosystemic neocollaterals), in order to preserve drainage of the gastropancreatic area.
- An adolescent with a huge splenomegaly, who is not compliant with treatment, with a lifestyle that puts himself/herself at risk of fatal bleeding from a splenic rupture or from gastrointestinal bleeding, or is far from medical support or has significant abdominal discomfort or cosmetic concerns associated with a splenomegaly. Any splenectomy must be associated with the creation of a splenorenal central shunt.
- Patients with severe hypersplenism, with associated severe hypertensive gastropathy, or recurrent esophageal varices, in the absence of a possible cure for portal hypertension (i.e., a meso-Rex bypass). A Warren splenorenal shunt should be proposed instead of a splenectomy.

More recently, the use of a partial splenectomy has been proposed. This seems to be a reasonable alternative as it helps preserve splenic vein flow, and avoids the need for performing a portosystemic shunt. Long-term results and benefits are still unclear, although it seems to be a palliative measure that provides good results only in the short and medium term.

Splenopexy or transposition

This procedure was proposed decades ago and involved transposing the spleen above the diaphragm and scarring its capsule. It aimed to create collaterals between the splanchnic area and the thoracic veins, and thus allow portal blood to pass through the spleen to drain into a low-pressure area. This is a palliative procedure that should be abandoned.

Splenic embolization

Total embolization of the spleen is contraindicated as it causes necrosis and complications, but partial embolization is possible. It is a palliative procedure, with a limited effect on splenic flow and hypersplenism, and may make further regional surgery more demanding. Effective embolization may also cause a hyposplenic state, exposing the patient to postsplenectomy fulminant sepsis. It cannot be recommended in children unless no other option is possible. Vaccinations against meningococcus, *Haemophilus influenzae*, and pneumococcus are recommended, as for splenectomy.

Surgical ligation of varices and esophageal transection

Endoscopic variceal eradication has replaced these historical operations and has greater success, efficiency, and safety. A direct ligation of the varices was performed through a thoracic or abdominal approach; this was later replaced by a more standard transection of the upper stomach, reducing the risk of fistula (Sugiura procedure and others). The operation works by direct interruption of the intramural vein and variceal network, but portal hypertension is not affected and the effect on variceal bleeding is limited. A number of modifications of the Sugiura operation exist for children and it is still performed in developing countries with limited surgical and medical management options. The procedure is palliative and has a high incidence of recurrence and creates numerous adhesions that make further abdominal surgery more difficult. Other procedures should be preferred whenever possible.

Liver transplantation

Transplantation of the liver restores normal physiological flow through the hepatic filter, and is the "ultimate" cure for portal hypertension and portopulmonary syndromes (hypoxia and hepatopulmonary syndrome; see Chapter 31). As any child with chronic portal hypertension may become a candidate for a liver transplantation in his/her life, surgeons should take this into account when proposing abdominal interventions, as abdominal scarring, adhesions, or previous shunt surgery make a future transplant more challenging [19].

Strategies in the 21st century

The choice of intervention based on the literature and accumulated clinical experience allows an estimate of the completeness of therapy (residual portal hypertension, likelihood of recurrence), the need for continuing therapy (anti-platelet therapy, anticoagulation, antacid, immunosuppression), the risk of collateral effects (encephalopathy, portopulmonary syndromes, hepatic nodes), the risk of aggravation of the portal system condition (an extension of thrombosis), and the added risk of complications from the procedure (hepatic decompensation, adhesions and scarring of the abdomen, necrosis or abscess of the spleen, esophageal secondary problems). Based on these, the management types can be listed by order of decreasing preference as follows:

1. A meso-Rex bypass in patients with a cavernoma and a favorable anatomy.
2. Endoscopic and medical long-term management in the absence of refractory or untreatable complications.
3. A liver transplantation in patients with cirrhosis and a liver dysfunction.
4. A liver transplantation in patients with a normal liver and severe encephalopathy or a portopulmonary syndrome.
5. An attempt at a meso-Rex bypass in patients with a cavernoma and an unfavorable anatomy.
6. Selective (distal) shunting.
7. Non-selective shunting or mesocaval or splenorenal side-to-side or H-type shunts.
8. TIPSS when anatomically feasible.
9. Other non-selective shunts.
10. Splenectomy with a non-selective proximal shunt.
11. Splenectomy alone in patients with a cavernoma of the splenic vein.
12. Other alternative surgical procedures in patients with normal hepatic function.
13. A liver transplantation in patients with other complicated conditions.
14. A multivisceral transplantation in patients with an extensive thrombosis and major complications that are not amenable to other management.

Algorithm for extrahepatic portal hypertension

1. There is now general consensus that a meso-Rex bypass is the optimal procedure for children with portal vein thrombosis, normal liver function, and a patent Rex recessus [1, 14, 20]. It should be performed early in the course of the disease to halt progression toward major splenomegaly and hypersplenism, secondary coagulopathy, or biliopathy. In all patients with extrahepatic portal hypertension, the first approach should be to assess the feasibility of a meso-Rex bypass (Figure 27.8) [1, 2, 16, 20, 24]. Surgery is recommended even in the absence of complications, and even if the child could be managed conservatively, unless the patient has primary genetic severe thrombophilia. A meso-Rex bypass can be performed in a pre-emptive and prophylactic manner with a probable success rate of >95% [1, 2, 16, 20, 24]. An adequate preoperative assessment is necessary to select candidates for a pre-emptive, or prophylactic, meso-Rex bypass to avoid submitting some children to a rescue portosystemic shunt much earlier than otherwise recommended.

   The ideal preoperative assessment consists of the following investigations:
   - Ultrasonography of the liver and the portal system.
   - Exclusion of liver parenchymal anomalies, heterogeneity, or associated liver diseases by liver biopsy.
   - Transjugular retrograde hepatic portography to image the Rex recessus and check its patency and that of the intrahepatic portal system [1]. During this examination, the free and wedged pressures should be recorded, and the gradient should be found to be <8 mmHg. If the gradient is found to be >8 mmHg a liver biopsy is recommended in order to exclude a parenchymal disease such as an obliterative portal venopathy, hepatic fibrosis, or preclinical cryptogenic cirrhosis.
   - Doppler ultrasound, CT angiography, or angio-MRI to demonstrate the splanchnic venous anatomy and patency of the superior mesenteric vein.
   - Doppler ultrasound of the neck vessels to check patency. A similar caliber of both the right and left internal jugular veins is ideal. If their diameter is different by >25%, a head magnetic resonance angiograph with an assessment of the intracranial venous system for a R-L anastomosis is recommended. The resection of the internal jugular vein on one side is not recommended in the case of a variant anatomy with an insufficient R-L anastomosis.
   - Exclusion of a primary genetic deficiency causing thrombophilia.

   Postoperative antithrombotic prophylaxis is recommended (a low dose of heparin during the first week, followed by antiplatelet agents (aspirin and dipyridamole) for 3–6 months).
2. In patients with a less favorable anatomy [1], a conservative approach and endoscopic management is justified in the absence of complications such as hepatic regenerative nodules, portopulmonary syndrome, or (sub)clinical encephalopathy [24]. In these patients, the meso-Rex procedure should be attempted first (surgical exploration), followed by a Warren or mesocaval shunt intraoperatively if a meso-Rex bypass cannot be performed [22].
3. In patients with a thrombosis of the splenic vein and a cavernomatous transformation, an isolated splenectomy can be considered in the case of recurrent or severe bleeding from the stomach and/or a major hypersplenism. The operation must be done with preservation of the perisplenic veins and collaterals.
4. In patients with a complete thrombosis of the portal and splanchnic venous system, time can be gained by using a

splenectomy alone, but at some point a multivisceral transplant may be the ultimate solution.

5 Patients with collateral complications such as portopulmonary syndrome or encephalopathy, a meso-Rex bypass might be a possibility although liver transplantation may be preferable [13, 14].

6 Portal biliopathy is usually asymptomatic in children and requires no treatment [17, 18]. If there is symptomatic bile duct obstruction, endoscopic retrograde cholangiography should be performed in order to assess the presence of anatomical strictures or a cholelithiasis. A biliary stent insertion may be useful. Sphincterotomy and balloon maneuvers are not recommended as large venous collaterals may have developed locally causing bleeding. If the latter therapeutic approach fails, shunt surgery with an adequate (central, non-selective) shunt may be effective. In the case of an anatomical fixed biliary stricture, biliary surgery (stone removal or stricturoplasty) may be considered afterwards [17, 18].

7 The role of radiological interventional procedures and a portal stent insertion for prehepatic portal hypertension has been considered by several authors, but these are less likely to be successful in children [25]. Successful attempts were limited to those cases with a large intrahepatic portal system with sufficient transhepatic access. Interventional radiological attempts should be reserved for the few patients with a large intrahepatic portal system and with a very short segment of portal vein thrombosis. Interventional radiology with stenting is the treatment of choice for the Budd–Chiari syndrome.

Algorithm for portal hypertension from other causes

Portosystemic shunt surgery was developed decades ago in order to manage patients in whom the control of variceal bleeding failed, either acutely or in the longer term. This approach was successful in adults with well-compensated cirrhosis but not in adults with a Child–Pugh score of C and even B. In contrast, it is rarely successful in children and infants, possibly because of their small-caliber vessels.

The successful introduction of liver transplantation and good endoscopic management has reduced the need for shunt surgery for chronic portal hypertension in children, with only a few exceptions, particularly in countries where liver transplantation is not an option. The use of microsurgical techniques, anesthesia, and intensive care means that surgeons can now offer excellent technical outcomes of shunt surgery, even in smaller children, when necessary. Selective shunts have a relative advantage in children because of a long life expectancy, and they should be preferred to protect patients from encephalopathy, although they become less selective and more "centralized" with time. Alternatively, non-selective H-type shunts also have good clinical results. It is important that all options for treatment are considered by the multidisciplinary team to individualize the treatment.

Role of surgical management in emergencies

Surgical management no longer has a place in the emergency management of variceal bleeding (see Chapter 21) with the current medical management of somatostatin or octreotide, interventional endoscopy, and radiology [7, 24, 26, 27]. If a portosystemic shunt is indicated, a TIPSS is preferable [28]. In the cirrhotic patient, acute hepatic decompensation is an indication for transplantation. Emergency surgery is essential for a ruptured splenic aneurysm.

Organ transplantation: role and timing

Liver transplantation is a cure for portal hypertension and should be considered the definitive treatment for portal hypertension. The timing depends on the severity of hepatic decompensation, and whether the complications of portal hypertension can be controlled (see Chapter 31). If a portosystemic shunt has been created it must be closed at the time of liver replacement in order to ensure a satisfactory portal flow towards the liver. This makes an indication for a surgical shunt in a cirrhotic patient a critical step for the future of the child, as it may render a transplant operation more difficult.

Patients with extrahepatic portal hypertension are not candidates for liver replacement as their liver is healthy. In those with portopulmonary syndromes, multiple liver adenomas that are increasing in size and are B-catenin positive, and in those with secondary biliary cirrhosis caused by portal biliopathy, liver transplantation may be indicated.

Results and complications of portal hypertension surgery

Portosystemic shunt surgery

As a result of draining the portal blood directly into the caval system, portosystemic shunts are associated with a satisfactory drop of pressure in the portal system and a correction of most of the related complications. In skilled hands, it is an efficient and safe procedure. Long-term patency rates (89–97%) are reported in the best series [22]. Successful shunt surgery allows for the prevention of a recurrence of a gastrointestinal hemorrhage, decreasing splenomegaly and hypersplenism, and improving portal biliopathy. A catch up and acceleration of growth are reported and there is a significant improvement in the quality of life. An unfavorable anatomy, thrombophilia, and diffuse thrombosis of the portal venous system are associated with a low success rate.

Shunt thrombosis is a most serious problem. A Doppler ultrasound assessment during the first week should detect early problems, and early surgical revision may rectify any technical problems (kinking, compression, etc.). Postoperative antithrombotic prophylaxis is recommended (a low dose of heparin during the first week, followed by antiplatelet agents (aspirin and dipyridamole) for 3–6 months). In the medium and long term, an assessment of the

patency and the absence of a gradient can be conducted by radiological intervention, with the option of a balloon angioplasty in the case of a stricture.

Shunt patency and rerouting most of the splanchnic blood into the caval system may cause serious side effects:

- Encephalopathy is the most common problem; it can be clinically evident in patients with cirrhosis, and it can develop subclinically in those with no parenchymal disease (extrahepatic portal hypertension) [10, 12, 13].
- Portopulmonary syndrome (hepatopulmonary syndrome and pulmonary hypertension) is rare in children. A 1% rate of pulmonary arterial hypertension after portosystemic shunts for prehepatic portal hypertension has been reported. Because these complications may develop slowly, and quite late after shunt surgery, long-term follow-up is recommended, including a cardiopulmonary assessment (e.g., echocardiography, resting, and stressed O_2 saturation measurements).
- Liver nodules (liver cell adenomas, focal nodular hyperplasia) may develop with time, and mostly in those patients with a prolonged survival, who have not required transplantation. Ultrasonography-based screening over the long term is suggested.

Meso-Rex bypass

A meso-Rex bypass has a high success rate of >90% in expert hands [2, 16, 29, 30], even in children with a very low weight (Figure 28.9). The outcome following other technical variants, such as variation of the inflow for the bypass (on the splanchnic side, i.e., splenic, gastric, and inferior mesenteric veins, and other sites) is not clear. The success is also dependent on the type of material used for the bypass. The best results have been obtained with the internal jugular of a patient's own vein [2, 16, 29, 30]. Other bypass types have been proposed (e.g., spiraled saphenous vein, recanalized umbilical vein, cryopreserved allogeneic vein, or prosthetic GoreTex®), but they are associated with less successful outcomes. The bypass is directly accessible for radiological maneuvers (portography and gradient measurement, angioplasty, etc.) if required [31].

The meso-Rex bypass restores the physiological hepatic flow [3, 16] and allows the portal pressure to revert back to normal values, if the bypass is of an adequate diameter. With time, there is resolution of all the complications related to portal hypertension, in a physiological manner which is superior to that of other shunts [14, 16]. Portosystemic collaterals tend to close spontaneously and varices regress. Coagulation abnormalities, neurocognitive ability, and subclinical encephalopathy improve [15], and growth and body mass index increase in those who had growth retardation. Isolated reports suggest that a meso-Rex bypass reverses the hepatopulmonary syndrome, nodular regenerative, or adenomatous transformation of the liver. The procedure has little morbidity or mortality, even in low-weight infants (Figure 27.9).

A meso-Rex bypass also restores the portal flow towards the liver in patients with portal vein thrombosis after a liver transplantation and is usually technically feasible. If there is ongoing graft damage, severe fibrosis secondary to chronic rejection, or biliary problems, this alters the parenchyma, decreasing its conductance, and is a contraindication for the procedure [29].

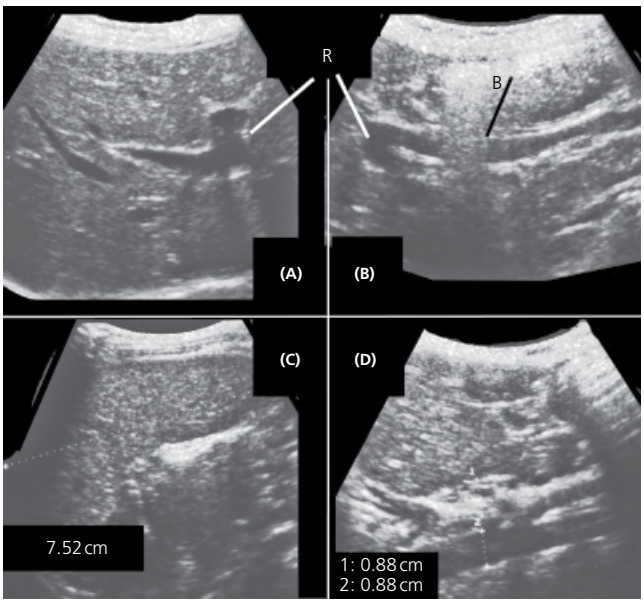

Figure 27.9 Outcome after a meso-Rex bypass in a 5 kg infant operated on at 5 months of age for recurrent variceal bleeding, refractory to endoscopic management. (A,B) Two years after the operation, the meso-Rex (R) bypass (B) is patent and well functioning. The spleen maximal span (C) is 7.52 cm (within the range for the age) and the small omentum thickness is equal to the anteroposterior diameter of the abdominal aorta (D).

Conclusion

Modern medical and surgical management has transformed the outcome of surgical portal hypertension and is dependent on effective multidisciplinary team working.

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CHAPTER 28

Primary Hepatic Tumors

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Key points

- Liver tumors are rare in childhood.
- A multidisciplinary team approach is required for a successful outcome.
- Accurate staging and risk-adapted treatment delivers the best outcome in hepatoblastomas.
- Cisplatin is the most effective therapeutic agent in the management of hepatoblastomas.
- Surgical resection of malignant tumors is essential to effect cure.
- Liver transplantation is required for inoperable tumors with an excellent outcome even in high-risk cases.
- Management of hepatocellular carcinoma remains challenging and is the focus for therapeutic advances with targeted chemotherapy agents.

Epidemiology

Table 28.1 indicates the nature and frequency of hepatic tumors seen in children [1]. The incidence of hepatic tumors in childhood is consistently quoted from many series as being in the region of 0.5–2.5 per million population. Data collected from the West Midlands Regional Children's Tumor Registry in the UK has reported the incidence to be 1.2 per million person-years [2]. The incidence of hepatoblastoma (the commonest malignant tumor) was 0.77, and for hepatocellular carcinoma (HCC) it was 0.09 – somewhat lower than other published series.

Thus, on average in the UK, approximately 10–15 children with hepatoblastoma and one or two children with HCC are diagnosed each year. There is a male preponderance of 1.8 : 1 for all malignant tumors, consistent with other series. Hepatoblastoma presents in a younger age group, being an uncommon diagnosis over the age of 4 years. Hepatocellular carcinoma has its peak onset in early adolescence, although the range is wide. The older age at onset for HCC may well reflect its close association with other underlying disease processes.

These data were confirmed in an overview conducted by the Automated Childhood Cancer Information system [3]. At a population level, there has been a dramatic increase in survival in countries where a modern health system has been implemented, although the increased survival is lower for HCC in comparison with hepatoblastomas.

Etiology

Many etiological factors have been linked with the development of malignant hepatic tumors in childhood (Table 28.2). Broadly speaking, genetic influences are particularly important in the development of hepatoblastoma, whereas environmental factors and coexisting liver disease are strongly associated with HCC.

The link between hepatoblastoma and congenital overgrowth abnormalities – including Beckwith–Wiedemann syndrome [6], Simpson–Golabi–Behmel syndrome, and hemihypertrophy – has been well described. Molecular interest has focussed on the short arm of chromosome 11 and common genetic links with other embryonal tumors such as Wilms tumor and rhabdomyosarcoma. The WAGR (Wilms tumor, aniridia, genital anomalies, and mental retardation) locus at 11p13, and a second Wilms tumor locus at 11p15.5, have been the stimuli for much of the research efforts. Loss of heterozygosity for a recessive allele at 11p15.5 in two patients with hepatoblastoma, mapping to an area in the region of insulin-like growth factor-2 (IGF-2), has been demonstrated. The loss of heterozygosity for 11p15.5 in a patient with

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Table 28.1 The incidence of hepatic tumors in childhood. (Adapted from Weinberg and Finegold 1986 [1].)

Type of tumor	N	%
Malignant		
Hepatoblastoma	532	43
Hepatocellular carcinoma	284	23
Sarcoma	79	6
Benign		
Hemangioma/hemangioendothelioma	166	13
Mesenchymal hamartoma	75	6
Adenoma	22	2
Focal nodular hyperplasia	22	2
Other	57	5

Table 28.2 Conditions associated with hepatoblastoma and hepatocellular carcinoma.

Hepatoblastoma	Hepatocellular carcinoma
Beckwith–Wiedemann syndrome	Hepatitis B
Hemihypertrophy	Hepatitis C
Familial adenomatous polyposis	Tyrosinemia type 1
Gardner syndrome	Progressive familial intrahepatic cholestasis 2
Glycogen storage disease type I	α1-Antitrypsin deficiency
Trisomy 18	Cirrhosis secondary to any liver disease
Fetal alcohol syndrome	Glycogen storage disease type I and IV
Prematurity and low birthweight	Neurofibromatosis
Maternal exposure to:	Familial adenomatous polyposis
Oral contraceptives	Drug/toxin exposure to:
Gonadotropins	Androgens
Metals	Oral contraceptives
Petroleum products	Methotrexate
Paints and pigments	Aflatoxins
Paternal exposure to:	Fanconi anemia
Metals	
Meckel diverticulum	

Beckwith–Wiedemann syndrome and hepatoblastoma has also been reported. The same locus has been linked with the development of rhabdomyosarcoma. The likely mechanism for tumorigenesis is the loss of a tumor-suppressor gene from this region. Other investigators have been able to demonstrate that in addition to loss of heterozygosity, loss of genomic imprinting can result in hepatoblastoma. There appears therefore to be compelling evidence to suggest a common genetic link between the pathogenesis of hepatoblastoma, rhabdomyosarcoma, and some cases of Wilms tumor, 11p15.5, and the Beckwith–Wiedemann syndrome.

At a molecular level the main focus has been on the Wnt/β-catenin pathway, frequently noted to be mutated in cases of hepatoblastoma. The mutation results in loss of degradation of β-catenin [4]. The resultant accumulation of β-catenin within the nucleus results in uncontrolled cell proliferation. Two further molecular pathways influence tumorigenesis via the β-catenin pathway. Hepatocyte growth factor is a ligand for c-met and activation of this pathway also results in β-catenin accumulation. Adenomatous polyposis coli (APC) protein forms a complex with β-catenin and axins 1 and 2, leading to its degradation. Mutations of APC as seen in Gardner syndrome, mapped to chromosome 5q, result in loss of function of *APC* gene function and β-catenin protein accumulation. Mutations of phosphorylation sites on exon 3 of the *APC* gene have been associated with other types of cancer, and in recent years a high incidence (up to 80%) of such mutations have been observed in hepatoblastomas. It has been suggested that as many as 5–10% of cases of hepatoblastoma may be associated with familial adenomatous polyposis (FAP). There are important health implications for survivors of childhood hepatoblastoma with a family history of FAP. These children may have up to a 50% incidence of adenomatous polyps in later life, and it is important that they receive appropriate screening. This raises the question of screening for hepatoblastoma in infants of parents with a known history of FAP, and colorectal screening for FAP for family members of children who develop hepatoblastoma.

With the availability of *APC* germline mutation analysis, many now advocate mutation screening in all patients diagnosed with hepatoblastoma. Other β-catenin-independent mechanisms of hepatoblastoma tumorigenesis include activations of the Notch and Hedgehog signaling pathways seen in a number of other pediatric malignancies in addition to liver tumors.

There is a strong link between HCC and hepatitis B virus (HBV) infection. The incidence of HCC in chronic HBV carriers is approximately 100 times greater than in the HBV-negative population [5], and HCC is more common in areas that have high rates of endemic HBV infection. Although integration of the HBV genome into the HCC genome can be demonstrated at a molecular level, this event in itself is not necessarily oncogenic, and a second, as yet unidentified, promoter is probably necessary for the development of tumor. It has been postulated that environmental influences may be the reason why the incidence of HCC varies geographically and that it may not simply be a reflection of the prevalence of endemic HBV infection. Promoters could also be genetic variations specific to a population, modifying cellular growth characteristics that in turn would encourage neoplasm formation independently of a direct genetic HCC-type aberration. The fall of HBV due to neonatal vaccination has led to a reduction of cases in childhood.

Although hepatitis C is a known risk factor for HCC in adults, it is rare in children, and there is only a single case report of this occurring that required transplantation. Nevertheless, evidence from the hemophiliac population suggests that patients infected with hepatitis C virus also have an increased cumulative risk of developing liver cancer and that this risk may be significantly increased when there is coinfection with human immunodeficiency virus-1 (HIV-1).

The relationship between the development of HCC and cirrhosis is unclear. Cirrhosis of any origin and dysplastic regenerating nodules have long been considered to be the likely precursors of HCC because of their frequent association with HCC occurrence, but other genetic mechanisms may be involved. From the cytogenetics, it appears that some HCC genetic alterations may be specific to etiological factors (particularly HBV infection). It has recently been suggested that progressive familial intrahepatic cholestasis 2 (PFIC 2), associated with the mutation ABCB11 – causing deficiency of the bile salt export pump (BSEP), a membrane canalicular bile acid transporter – represents a specific and previously unrecognized risk for HCC in young children.

α1-Antitrypsin deficiency has a slightly different mechanism for carcinogenesis, in which liver injury would result not from the classic concept of a "gain of toxic function" mechanism, but from abnormal and chronic regenerative signaling from the sick cells to younger, less sick hepatocytes. Chronic regeneration in the presence of tissue injury leads to adenomas and ultimately to carcinomas. It is suggested that the latter mechanism may explain hepatocarcinogenesis in other chronic liver diseases, e.g., genetic disorders, viral hepatitis, or non-alcoholic steatohepatitis. This hypothesis could be evaluated using multicenter analysis for rare disease, such as glycogen storage disease type III, in which a specific risk for HCC has recently been reported.

Tyrosinemia 1 (fumarylacetoacetate hydrolase deficiency) is an autosomal recessive inborn error of tyrosine metabolism that leads to liver failure and cirrhosis. Prior to therapy, there was a high risk of HCC in childhood or early adolescence. The development of therapy with nitisone (2-[2-nitro-4-(trifluoromethyl)benzoyl] cyclohexane-1,3-dione), which prevents the production of cytotoxic tyrosine metabolites in combination with a tyrosine- and phenylalanine-restricted diet, has altered the natural history of tyrosinemia, and has reduced, but not eliminated the risk of HCC.

HCC is also associated with glycogen storage disease types I and IV.

Although the histological features of HCC in children are similar to those seen in adults, there is underlying cirrhosis in only about one-third of pediatric cases. This is in contrast to adult HCC, in which 70–90% of tumors are associated with cirrhosis. In cases associated with tyrosinemia type 1, cirrhosis is an invariable finding. In cases associated with biliary atresia, the development of HCC is not universally associated with cirrhosis. Thus, whilst the development of cirrhosis clearly has a part to play in oncogenesis, the exact relationship remains unclear.

From the genetic point of view, dedifferentiation leading to HCC is characterized by increasing chromosomal instability and insertional mutagenesis, leading to an accumulation of structural chromosomal aberrations with losses and gains of defined chromosomal regions. Gains of regions 1q and 8q and losses of 8p are very frequently found using high-resolution microarray comparative genomic hybridization. At a molecular level, mutations and/or altered expression of Wnt, SHH, TGFb, RB1, p53, PTEN, MAP/Ras, and IGF are observed. In adult HCC it may be possible to utilize such genetic signals to define a molecular risk stratification. In pediatric HCC we still need to evaluate in larger prospective series whether this entity is biologically distinct from adult HCC. Recently, a molecular marker for fibrolamellar HCC has been described involving a deletion in chromosome 19 resulting in an active fusion protein PRKACA.

Malignant tumors

Clinical features

Hepatoblastoma

Hepatoblastoma is most commonly seen in children under the age of 18 months and is rare after the age of 3 years. There is a male predominance of 3 : 2. The commonest presenting feature is of a palpable abdominal mass with abdominal distension, but other features, including anorexia, weight loss, pain, vomiting, and jaundice have also been reported (Table 28.3).

One of the more unusual presenting features of hepatoblastoma is its association with sexual precocity due to the release of human chorionic gonadotropic hormone (β-HCG) by the tumor. Osteoporosis is said to occur in up to 20% of cases and when severe can lead to bone fractures and vertebral compression. The tumor may rupture spontaneously, producing an acute abdomen and hemoperitoneum.

Physical examination should also focus on possible syndromes that may be associated with hepatoblastoma (Beckwith–Wiedemann, hemihypertrophy, etc.; see Table 28.2). During history taking, inquiries should be made about familial associations such as hereditary polyposis coli, drug and toxin exposure, etc.

Hepatocellular carcinoma

The clinical features are very similar to those seen with hepatoblastoma (Table 28.3). HCC tends to present at a slightly older age than hepatoblastoma and is very rare in infancy. Jaundice, whilst uncommon, occurs slightly more often in HCC than in hepatoblastoma. Sexual precocity and osteoporosis are not features of HCC. A search for signs of underlying liver cirrhosis (splenomegaly due to portal

Table 28.3 Signs and symptoms of liver tumors in children.

	Hepatoblastoma (%)	Hepatocellular carcinoma (%)
Abdominal mass	71	58
Weight loss	24	21
Anorexia	22	22
Pain	18	16
Vomiting	13	10
Jaundice	7	10

hypertension, spider nevi, etc.) should be sought as a possible clue to underlying etiological factors.

The rare *fibrolamellar type of HCC* is seen in an older age group (median age 26.4 years) and occurs in non-cirrhotic livers. Abdominal mass is the commonest presentation and systemic symptoms are unusual. There remains some controversy in the pediatric age group whether these patients have a better prognosis than standard HCC patients and whether the response to neoadjuvant chemotherapy is the same [7].

Other malignant tumors

Malignant mesenchymal tumors of the liver represent approximately 10% of the primary malignant tumors of childhood. The commonest of these is undifferentiated (embryonal) sarcoma. Malignant mesenchymal tumors tend to present in a similar fashion, with abdominal pain, mass, and fever. In some cases, the presentation may be relatively acute after the tumor bleeds within the liver, with anemia and abdominal pain caused by the sudden volume increase and liver capsule distension. This mode of presentation is relatively typical of embryonal sarcoma. The latter tumor is often cyst-like, with the tumor mass being very poorly structured and myxoid, mimicking liquid on computed tomography (CT) or ultrasonography. The dislocation of the tumor by the hematoma may make the formal diagnosis difficult, and this typical mode of presentation in small children should help in diagnosing these rare cases. α-Fetoprotein (AFP) is normal, there are no abnormalities of the full blood count and liver enzymes, and serum bilirubin is rarely elevated.

Epithelioid hemangioendothelioma is a rare and unique neoplasm, now considered to be a separate entity from hemangioma and angiosarcoma, and has a low-to-intermediate grade of malignancy. It is mostly seen in young adults, but can be observed in teenagers and children, in whom it appears to have more aggressive behavior, with rapid growth and a tendency toward extrahepatic spread. Coincidental diagnosis is possible, but most cases are diagnosed late with symptoms related to tumor growth – abdominal pain, Budd–Chiari phenomena, portal hypertension, or liver failure. Since this tumor tends to be multifocal and widespread in the liver at diagnosis, only a limited number of patients can be considered for surgery, and the role of transplantation in children is not clear. New chemotherapy approaches appear to be useful and may help in selecting candidates for transplantation on the basis of the response to chemotherapy, as has already been done successfully for hepatoblastoma cases.

Diagnostic investigations

Laboratory tests

Full blood count

Anemia (usually normocytic, normochromic) is seen in at least 50% of children with hepatoblastoma. The platelet count is also often abnormal, with up to one-third of patients demonstrating thrombocytosis (often in excess of 1000×10^9/L), and fewer patients having thrombocytopenia. Thrombocytosis is thought to be related to increased levels of circulating thrombopoietin. Thrombocytosis is less common in HCC patients, but polycythemia is sometimes seen.

Liver function tests

These are commonly normal in hepatoblastoma, but are more frequently abnormal in HCC, presumably due to the greater incidence of underlying cirrhosis or hepatitis.

α-*Fetoprotein*

α-Fetoprotein is a useful diagnostic and prognostic marker and can be helpful in monitoring disease progress and the response to therapy. From 28 days' gestation onward, the developing fetus produces AFP, reaching maximum levels around 14 weeks. There is a steady decline to term, and the fall continues over the first year of life, when normal adult levels are usually reached. AFP levels may be higher in premature infants. The vast majority of hepatoblastoma patients and over two-thirds of HCC patients have elevated levels of AFP at presentation. Levels tend to be higher in patients with more bulky disease and metastases and therefore have prognostic significance. A return of AFP levels to normal following treatment indicates remission, while persistently abnormal results should alert the clinician to the possibility of residual tumor or relapse.

In addition, the presence of low AFP at diagnosis (<100 ng/mL) confers an especially poor prognosis. Tumors associated with low AFP tend to be bulky and less responsive to chemotherapy, with a predominance of unfavourable, small-cell, undifferentiated histological subtype. Two-year event-free survival has been reported to be as low as 24% in these cases and appears to be the most important determinant of poor outcome in hepatoblastoma [8]. Malignant yolk sac tumors may rarely present as a primary hepatic tumor. These tumors also secrete AFP, and the presence of elevated serum AFP is therefore not specific for hepatoblastoma/HCC. If β-HCG is elevated at diagnosis, it can be used like AFP to monitor the response of hepatic tumors to therapy.

Other markers

The fibrolamellar variant of HCC is usually associated with normal AFP values, but elevation of vitamin B_{12}-binding proteins, especially transcobalamin I100, makes them useful markers that can also be used to monitor disease response and progression. Other markers, including elevated levels of urinary cystathionine, serum cholesterol, IGF-2, ferritin, carcinoembryonic antigen, and γ-prothrombin, have all been reported in malignant liver tumors, but are non-specific and of limited benefit in diagnosis and follow-up.

Imaging

The aim in imaging assessment of any childhood liver tumor is to determine the site and characteristics of the lesion and to establish whether any metastases are present, whilst also

giving some indication of the suitability of the lesion for surgical resection.

Abdominal radiography

A plain radiograph of the abdomen will often show a mass effect from the hepatic lesion itself, and in addition the presence of any calcification. This investigation provides very little diagnostic information alone, and since the majority of children will probably have an ultrasound or CT scan as the first-line imaging method, it is not mandatory in all patients.

Ultrasonography

This will often confirm that the lesion originates in the liver and can help distinguish between other common pediatric abdominal tumors that present with a palpable mass (e.g., Wilms tumor and neuroblastoma). The size of the tumor can be defined and measured and information can be obtained on the presence of cystic features, which may be suggestive of a benign lesion. Vascular structures can be identified (hepatic arteries, hepatic veins, portal veins, and inferior vena cava) and Doppler studies performed to confirm flow rates and patency. Ultrasonography may be particularly helpful in differentiating benign hepatic hemangiomas or hemangioendotheliomas, by defining the vascular features of these tumors. However, magnetic resonance imaging (MRI) is now the preferred imaging technique for vascular tumors. Although some authors have reported a reasonable predictive accuracy for the extent and resectability of liver tumors using ultrasound, the additional information gleaned from CT and/or MRI makes these mandatory additional explorations in the investigation of liver tumors.

Computed tomography

Computed tomography scanning provides detailed information about the anatomical limits of liver tumors. Intravenous contrast medium should always be used in order to define vascular landmarks and assess the patency of vessels (Figures 28.1 and 28.2). Typically, malignant tumors are of low attenuation and demonstrate patchy enhancement with contrast. Vascular lesions may be hard to define without contrast, but show dense peripheral enhancement with gradual filling of the central areas following intravenous contrast. CT scanning will also define the presence of para-aortic lymph node metastases, although these tend to be uncommon.

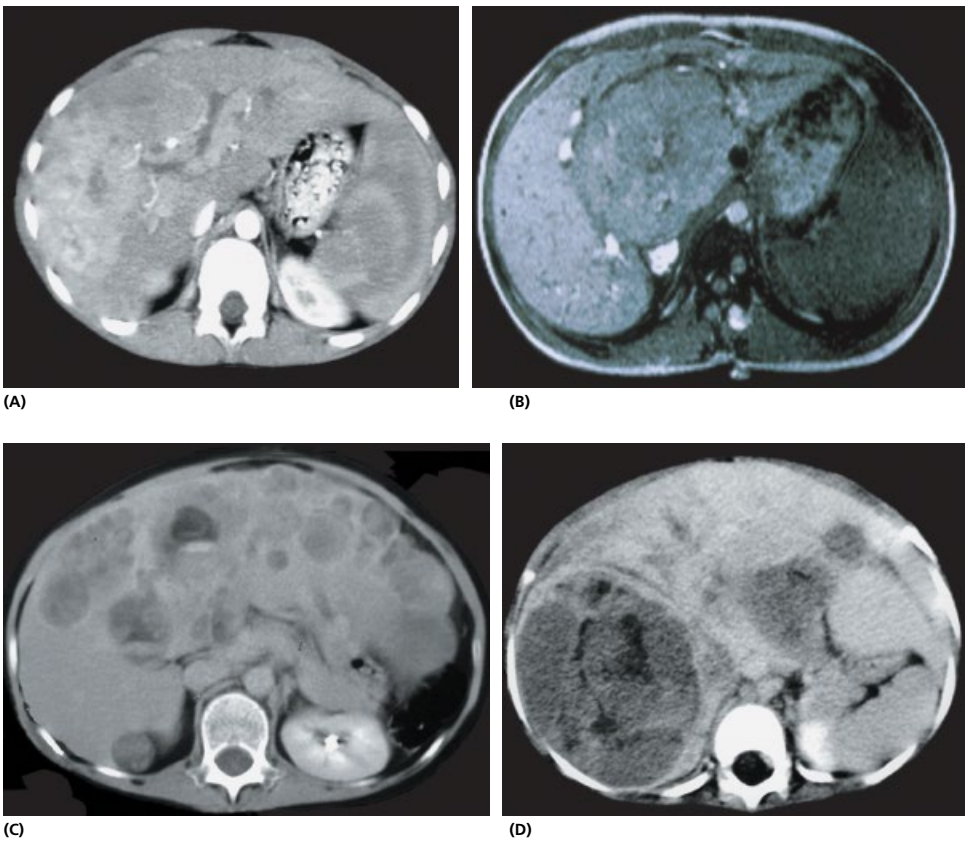


Figure 28.1 Computed tomography appearances of hepatocellular carcinoma. (A,B) Fibrolamellar hepatocellular carcinomas. (C,D) Classic hepatocellular carcinomas, originating in normal liver. Hepatocellular carcinomas are usually multifocal and invasive, commonly involving both lobes at diagnosis. Areas of hemorrhage and necrosis are common in the usual type. Vascular invasion and thrombosis (portal or hepatic veins) are usually associated with rapid progression and a poor short-term prognosis. Fibrolamellar hepatocellular carcinomas are fibrous, solid tumors, often better delineated, and have remarkably slow growth.

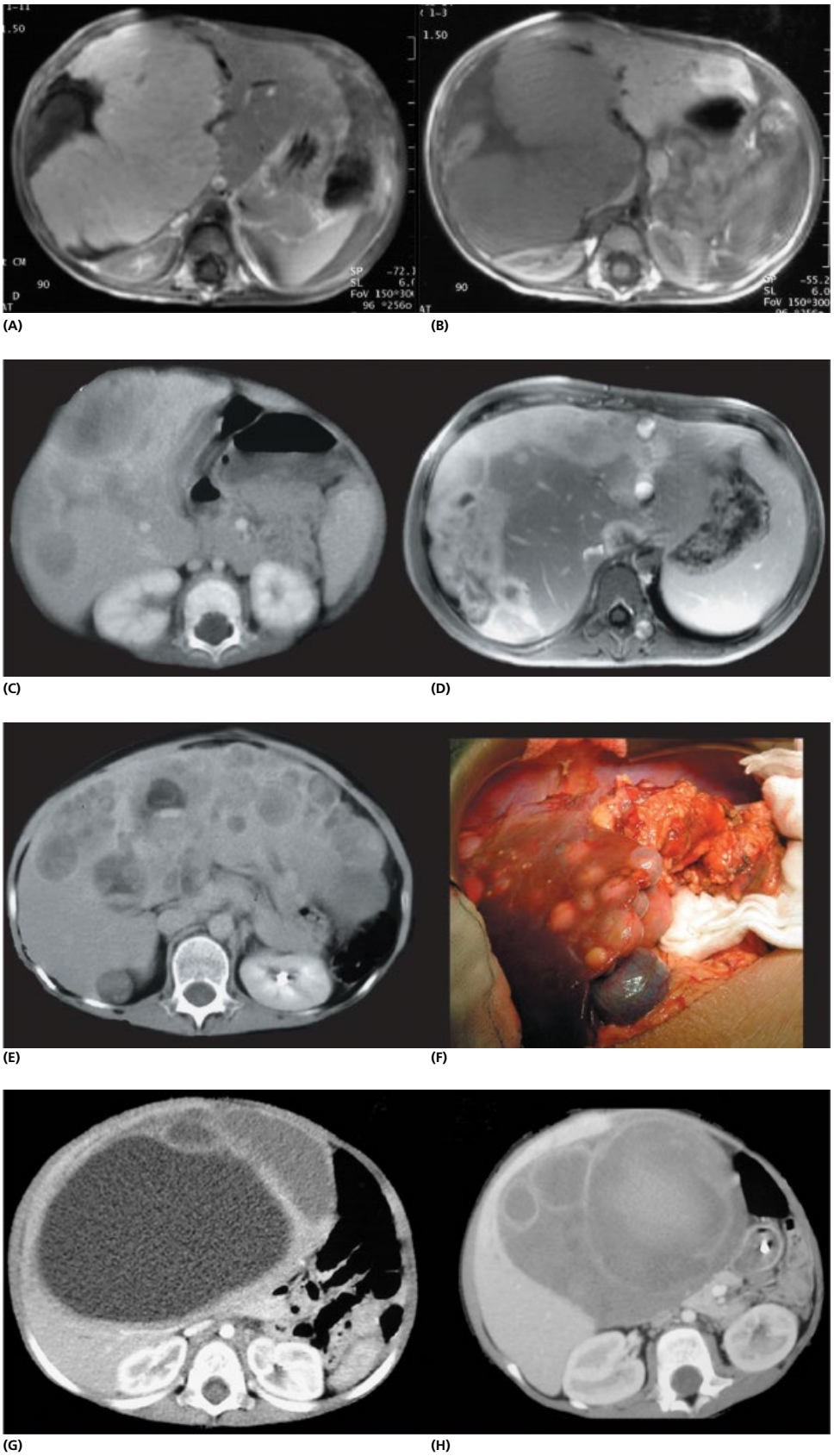


Figure 28.2 Hepatic tumors imaged by CT scanning, which demonstrates a large hepatoblastoma before (A) and after (B) intravenous contrast. It may also help to differentiate solid tumors such as hepatoblastoma (C), epitheloid hemangioendothelioma (D), and hepatocarcinoma (E) from cystic tumors such as mesenchymal hamartoma (G) and sarcoma (H). The cut surface of a hepatocarcinoma is shown in (F).

Magnetic resonance imaging

Magnetic resonance scanning provides good definition of a tumor and surrounding infiltration, allowing accurate assessment of segmental involvement. This has important implications for clinical staging and any proposed surgical interventions. The vascular anatomy can also be demonstrated, and this may avoid the need for hepatic angiography (Figure 28.3).

Hepatic angiography

This may be required before surgery if the vascular anatomy has not been clearly demonstrated on MRI.

Chest radiography

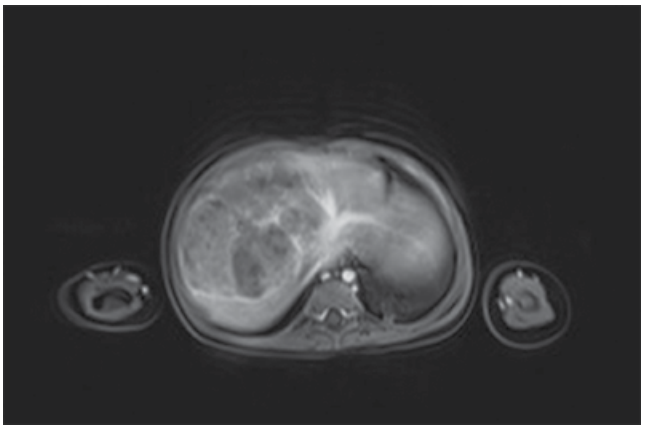
A chest radiograph should be performed to exclude pulmonary metastases. If normal, a CT scan of the chest should be undertaken before the start of therapy, since up to 50% of metastases may not be visible on chest radiography alone.

Positron-emission tomography

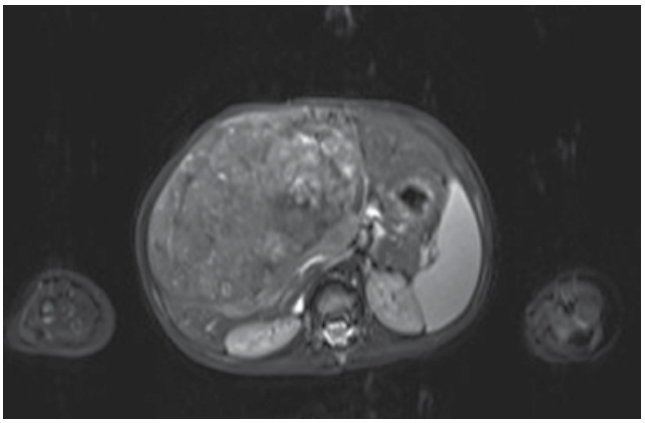
This technique has not been validated in the management of hepatic tumors of childhood, and interpretation of results should be used with caution.

Biopsy

Although clinical and laboratory clues can lead to a presumptive diagnosis in the majority of children with liver tumors, caution has to be exercised at all times. For example, although a 2-year-old child with grossly elevated AFP and a liver mass is most likely to have a hepatoblastoma, it is important to exclude other diagnoses, such as malignant germ cell tumor or HCC. This is particularly relevant when chemotherapy is being contemplated prior to definitive surgery. In the majority of cases, therefore, it is mandatory to take an initial biopsy. Due to the multifocal nature of many liver tumors, a "blind" biopsy may not yield samples that contain tumor. It is therefore best to perform a percutaneous biopsy of the liver mass using a Tru-Cut or Menghini needle under CT guidance. In addition, the use of imaging may alert the operator to bleeding from the biopsy site. Fine-needle aspiration may be unsatisfactory, as only minimal amounts of tissue may be obtained, which can be notoriously difficult to interpret with any degree of certainty. Caution must be exercised in attempting biopsies in lesions that are highly vascular, which may be difficult to distinguish clinically from a benign vascular anomaly or a highly vascular malignant tumor. In these circumstances, interventional radiologist services becomes paramount as the only other alternative approach is to undertake an open biopsy through a mini-laparotomy in order to be able to visualize the liver and treat any bleeding at the time of surgery. There is a risk of seeding malignant cells along the biopsy track, although the risk is greatest in hepatocellular tumors, which are less responsive to subsequent chemotherapy than hepatoblastomas.



(A)



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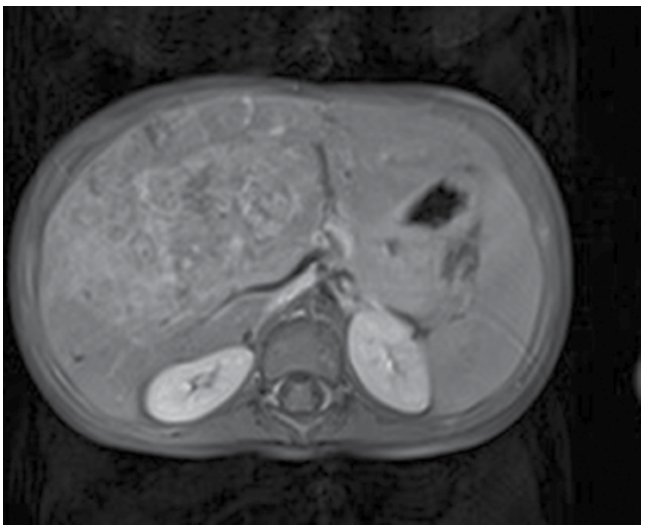

(C)

Figure 28.3 MRI scan of a PRETEXT III hepatoblastoma. (A) T1-weighted image: the tumor extends to the middle hepatic vein and confluence of all three hepatic veins. (B) T2 STIR image: the tumor extends to the bifurcation of the portal veins with the right portal vein closely applied to the medial border of the tumor. (C) Post chemotherapy image showing very little response and continuing tumor proximity to the portal veins.

Table 28.4 Hepatoblastoma pathological classification. (From Lopez-Terrada *et al*. 2014 [9]. Reproduced with permission of Nature Publishing Group.)

Classification	Description
Epithelial	
Fetal	"Well-differentiated": uniform (10–20 μm in diameter), round nuclei, cords with minimal mitotic activity (<2 per x 10/400 microscopic fields), EMH "Crowded" or mitotically active (>2 per x 10/400 microscopic fields); conspicuous nucleoli, less glycogen "Pleomorphic, or poorly differentiated" moderate anisonucleosis, high N/C, nucleoli "Anaplastic" marked nuclear enlargement and pleomorphism, hyperchromasia, abnormal mitoses
Embryonal	10–15 μm in diameter, high N/C, angulated nuclei, primitive tubules, EMH
Macrotrabecular	Epithelial hepatoblastoma (fetal or embryonal) growing in trabeculae of >5 cells thick (between sinusoids)
Small-cell undifferentiated	5–10 μm in diameter, no architectural pattern, minimal pale amphophilic cytoplasm, round to oval nuclei with fine chromatin and inconspicuous nucleoli, ± mitoses; ± INI1[a]
Cholangioblastic	Bile ducts, usually at periphery of epithelial islands, can predominate
Mixed	
Stromal derivatives	Spindle cells ("blastema"), osteoid, skeletal muscle, cartilage
Teratoid	Mixed, plus primitive endoderm; neural derivatives, melanin, squamous and glandular elements

EMH, extramedullary hematopoiesis; N/C, nuclear/cytoplasmic ratio.
[a] Pure small-cell undifferentiated needs to be differentiated from malignant rhabdoid tumors (discohesive, eccentric irregular nuclei, prominent nucleoli, abundant cytoplasmic filaments including cytokeratin and vimentin, negative nuclear INI).

Pathology

The histopathological classification of hepatoblastoma has recently been revised in an international consensus workshop (Table 28.4).

The well-differentiated fetal subtype of tumors has a particularly favorable outcome. The diagnosis can only be made in untreated resected tumors, but current evidence suggests that assuming resection is complete, these tumors require no adjuvant chemotherapy.

Conversely it appears that pure, small-cell, undifferentiated tumors confer a poor prognosis. Before the routine application on INI1 immunostaining it is likely that many of these tumors would have been incorrectly labeled hepatoblastoma. We now know a proportion of these tumors lack nuclear INI1 expression and should be regarded, and treated, as malignant rhabdoid tumors. Whilst it is not uncommon to see isolated islands of small undifferentiated cells within a hepatoblastoma this is not believed to necessarily confer poor prognosis.

The histological features of HCC, especially in patients with underlying cirrhosis, share morphological features similar to HCC in adults. However in non-cirrhotic liver disease, the diagnosis of HCC can be challenging and there is definite overlap with some of the histological features seen in hepatoblastoma. The microscopic features distinguishing HCC from hepatoblastoma are the presence of tumor cells larger than normal hepatocytes, broad cellular trabeculae, considerable nuclear pleomorphism, nucleolar predominance, frequent tumor giant cells, and an absence of hemopoiesis.

The fibrolamellar variant of HCC is a discreet subtype characterized by large eosinophilic hepatocytes with prominent nucleoli. It appears these tumors are less sensitive to chemotherapy than conventional HCC, but survival is probably similar, perhaps related to the absence of underlying liver disease in fibrolamellar tumors.

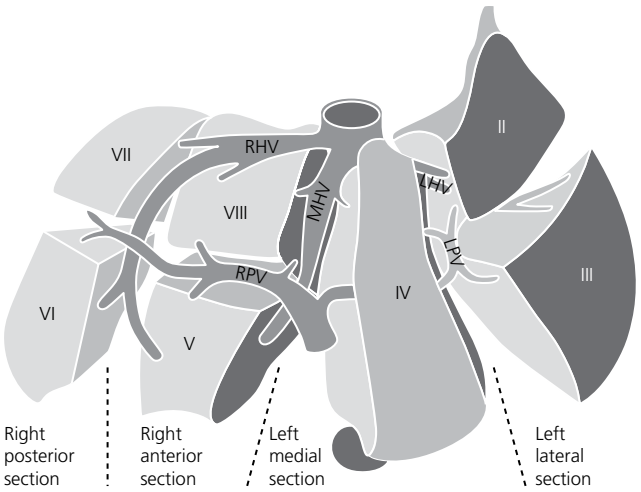

Figure 28.4 Exploded frontal view of the segmental anatomy of the liver. The liver segments are grouped into four sections: segments II and III (left lateral section); segments IVa and IVb (left medial section); segments V and VIII (right anterior section); and segments VI and VII (right posterior section). LHV, MHV, RHV, left, mid, and right hepatic veins; LPV, RPV, left and right pulmonary veins. (From Roebuck *et al*. 2007 [10]. Reproduced with permission of Springer.)

Clinical staging

The International Society of Pediatric Oncology (Société Internationale d'Oncologie Pédiatrique, SIOP) has introduced a staging system based on preoperative assessment and the location of the tumor. The original *pret*reatment *ext*ent of disease (PRETEXT) staging system divides patients into four groups, depending on the section or sections (groups of segments) involved (Figure 28.4 and Table 28.5) and also takes into account vascular involvement of the portal veins and/or inferior vena cava (IVC), extrahepatic extension, and distant metastases. In a revision of the PRETEXT system, greater clarity has been provided about the liver sections themselves, including the caudate lobe, which was previously ignored. Much greater

detail is also provided for documenting extrahepatic disease (Table 28.6) [10]. The PRETEXT system has now been evaluated in a large number of children with hepatoblastoma. It demonstrates a slight tendency to overstage patients, but has reliable interobserver reproducibility and, perhaps most importantly, has a superior positive predictive value for survival in comparison with other staging systems.

Risk stratification

The SIOPEL group was able to demonstrate the importance of both PRETEXT and the presence of metastatic disease on outcome following its first clinical trial SIOPEL-1. This formed the basis for the subsequent SIOPEL trials based on standard risk (localized PRETEXT I–III) and high risk (PRETEXT IV and/or metastatic disease). With an increasing portfolio of studies and patient numbers, further clinical risk factors have been demonstrated to effect outcome, the most potent of which appears to be the presence of low levels of AFP resulting in poor survival.

In a recent analysis low-, intermediate-, and high-risk groups have been identified based on PRETEXT, metastatic disease, AFP, and age (Figure 28.5) [11]. Further refinement of these risk factors is ongoing with an international collaborative effort, CHIC (Childhood Hepatic Tumor International Consortium). Moving forward, attempts at identifying molecular signals to aid clinical risk stratification are being developed, an example of this being a 16-gene microarray demonstrating an aggressive phenotype in β-catenin-activated tumors with resultant upregulation of myc signaling [12]. Plans are now in place to evaluate some of these molecular profiles in prospective clinical trials.

Table 28.5 Definitions of the PRETEXT numbers. (From Roebuck *et al*. 2007 [10]. Reproduced with permission of Springer.)

PRETEXT no.	Definition
I	One section is involved and three adjoining sections are free
II	One or two sections are involved, but two adjoining sections are free
III	Two or three sections are involved, and no two adjoining sections are free
IV	All four sections are involved

Treatment

Modern management of malignant hepatic tumors consists of a combination of chemotherapy and surgical resection, with the highest cure rates being associated with complete surgical resection.

Chemotherapy

Hepatoblastoma

The 3-year overall survival rates of children with hepatoblastoma have improved from 25% to around 80% over the past 20 years as a result of progress in the evaluation of chemotherapy

Table 28.6 PRETEXT staging system: additional 2005 criteria. (From Roebuck *et al*. 2007 [10]. Reproduced with permission of Springer.)

Caudate lobe involvement	C	C1	Tumor involving the caudate lobe	All C1 patients are at least PRETEXT II
		C0	All other patients	
Extrahepatic abdominal disease	E	E0	No evidence of tumor spread in the abdomen (except M or N)	Add suffix "a" if ascites is present, e.g., E0a
		E1	Direct extension of tumor into adjacent organs or diaphragm	
		E2	Peritoneal nodules	
Tumor focality	F	F0	Patient with solitary tumor	
		F1	Patient with two or more discrete tumors	
Tumor rupture or intraperitoneal hemorrhage	H	H1	Imaging and clinical findings of intraperitoneal hemorrhage	
		H0	All other patients	
Distant metastases	M	M0	No metastases	Add suffix to indicate location
		M1	Any metastases (except E and N)	
Lymph-node metastases	N	N0	No nodal metastases	
		N1	Abdominal lymph node metastases only	
		N2	Extra-abdominal lymph node metastases (with or without abdominal lymph node metastases)	
Portal vein involvement	P	P0	No involvement of the portal vein or its left or right branches	Add suffix "a" if intravascular tumor is present, e.g., P1a
		P1	Involvement of either the left or right main branch of the portal vein	
		P2	Involvement of the main portal vein	
Involvement of the IVC and/or hepatic veins	V	V0	No involvement of the hepatic veins or IVC	Add suffix "a" if intravascular tumor is present, e.g., V3a
		V1	Involvement of one hepatic, vein, but not the IVC	
		V2	Involvement of two hepatic veins, but not the IVC	
		V3	Involvement of all three hepatic veins and/or the IVC	

IVC, inferior vena cava.

	Prognostic factors							
Prognostic strata	PRETEXT I/II/III	PRETEXT IV	Age > 5 years	AFP > 1.2×10^6	Multi-focal	M+	AFP < 100	SCU
1: Good	+	–	–	–	–	–	–	–
2: Intermediate	+/–	At least one				–	–	–
3: Poor	+/–	+/–	+/–	+/–	+/–	At least one		

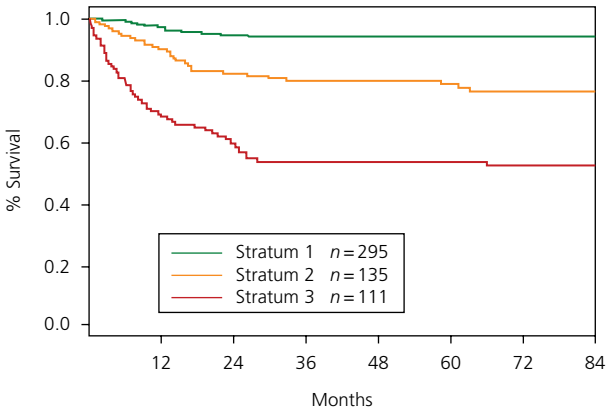

Figure 28.5 Hepatoblastoma prognostic factors and prognostic strata: + factor must be present; – factor must be absent; +/– factor can be present or absent. Overall survival estimates are based on the three risk strata. AFP, α-fetoprotein; M+, metastatic disease; SCU, small cell undifferentiated histology subtype. (From Miabach *et al.* 2012 [11]. Reproduced with permission of Elsevier.)

regimens. Although surgical resection is essential for long-term cure, the emphasis in each therapeutic modality has changed over time. Evans *et al.* [13] noted that the use of postoperative adjuvant chemotherapy conferred a survival advantage on children with completely resected hepatoblastoma and HCC, whereas the preoperative use of similar chemotherapy regimens in unresectable tumors was disappointing. Combinations of cyclophosphamide and vincristine (VCR) with other agents including doxorubicin, actinomycin-D, and 5-fluorouracil (5-FU) demonstrated that despite an initial response, only 12% of children were disease-free at 2 years. The introduction of cisplatin in the early 1980s led to a subsequent improvement in the response rate in patients treated either with cisplatin alone or in combination with vincristine/5-FU and in those who had proved resistant to previous chemotherapy. As doxorubicin had also been shown to be effective as a single agent, chemotherapy regimens containing both cisplatin and doxorubicin (PLADO) were a logical step forward. In the first International Society of Pediatric Oncology Liver Trial (SIOPEL-1), PLADO chemotherapy was tested in a multinational setting for the preoperative management of malignant liver tumors. The 5-year event-free and overall survival figures were 66% and 75%, respectively [14].

In a large randomized study by the Children's Cancer Group and Pediatric Oncology Group, PLADO chemotherapy was compared with cisplatin, 5-FU, and VCR. No significant differences in the overall or event-free survival (EFS) were seen between the two chemotherapy schedules, although PLADO consistently outperformed cisplatin/5-FU/VCR (5-year EFS 69% vs. 57%; $P = 0.09$). More toxicity was observed in the PLADO arm, including two cardiac deaths from cumulative doses of 640 mg/m^2 of doxorubicin – dose levels we today would never consider appropriate in these young children.

Building on the good results achieved with a combination of cisplatin and doxorubicin, and an ability to stratify patients into those with good risk and poorer risk, the SIOPEL group planned a pilot study (SIOPEL-2) of cisplatin monotherapy in standard risk patients (defined as PRETEXT I–III tumors, without vascular invasion, extrahepatic disease, or metastases) and an intensified chemotherapy approach in high-risk tumors with the addition of carboplatin to PLADO or "SuperPLADO." For the standard risk group, the 3-year overall survival with cisplatin monotherapy was 91%. For high-risk patients, the SIOPEL-2 study achieved a 3-year EFS of 53%.

SIOPEL-3 built on the results of the SIOPEL-2 pilot study and standard risk patients were randomized between PLADO chemotherapy and cisplatin monotherapy delivered at a dose of 80 mg/m^2 every 2 weeks. High-risk patients continued to receive the SuperPLADO regimen (Figure 28.6). In the standard risk population no difference was observed in event free and overall survival between the 2 randomised arms.[14] The 3-year overall

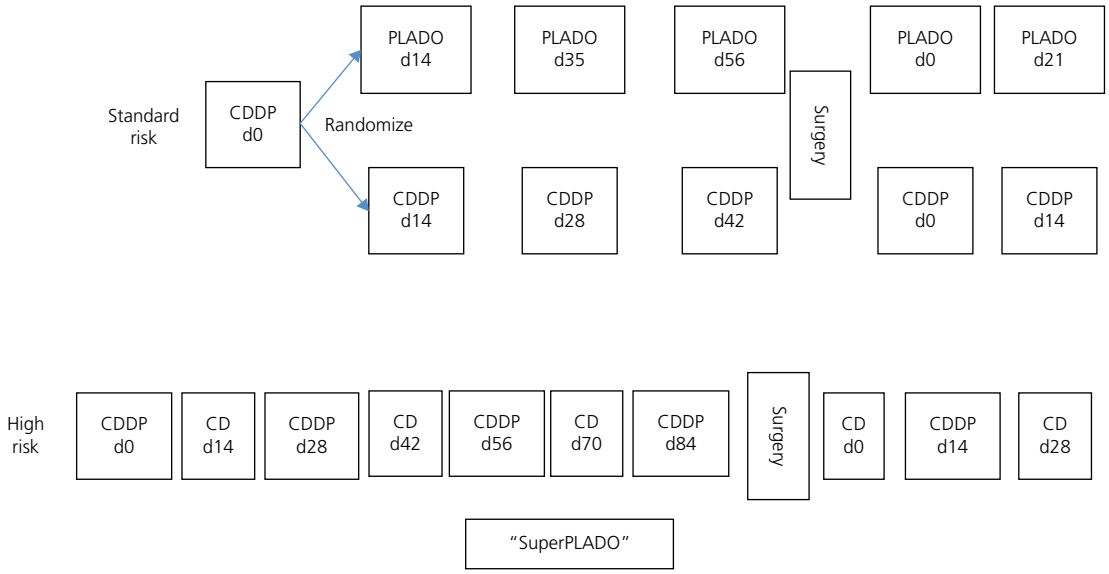

Figure 28.6 Treatment plan for the SIOPEL-3 study chemotherapy regimen: CDDP, cisplatin 80 mg/m² every 14 days (four cycles preoperatively, two cycles postoperatively); PLADO, cisplatin 80 mg/m², doxorubicin 60 mg/m² every 21 days (three cycles + 1 CDDP alone preoperatively, two cycles postoperatively); SuperPLADO, day 1 cisplatin 80 mg/m², day 15 carboplatin 500 mg/m² and doxorubicin 60 mg/m² (every 28 days), four courses CDDP, three courses CD (carboplatin/doxorubicin) preoperatively, two courses CD, one course CDDP postoperatively.

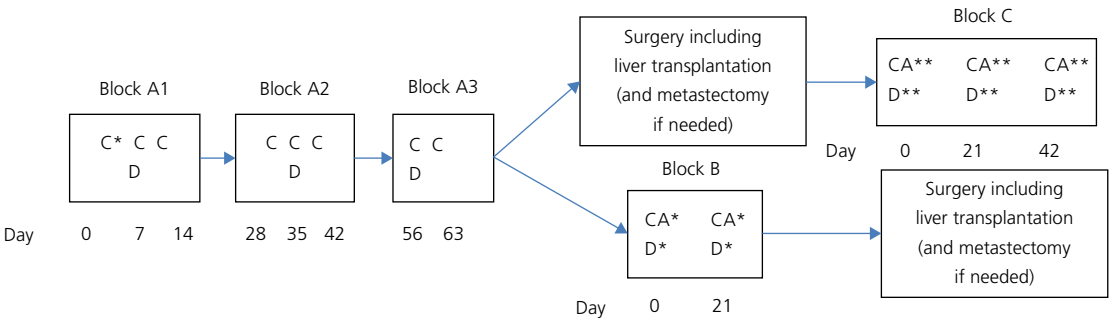

Figure 28.7 Treatment plan for the SIOPEL-4 study chemotherapy regimen: C*, cisplatin 80 mg/m² day 0; C, cisplatin 70 mg/m² days 7, 14, 28, 35, 42, 56, 63; D, doxorubicin 30 mg/m² days 7, 8, 35, 36, 56, 57; CA, carboplatin area under the curve (AUC) 10.6 mg/mL/min/day days 0 and 21; D*, doxorubicin 25 mg/m² days 0, 1, 2, 21, 22, 23; CA**, carboplatin AUC 6.6 mg/mL/min/day days 0, 21, 42; D**, doxorubicin 20 mg/m² days 0, 1, 21, 22, 42, 43.

survival rate of 95% for the cisplatin monotherapy arm is a remarkable achievement and is being adopted as the standard of care for many international groups around the world.

For high-risk patients treated with SuperPLADO, improvements over historical controls were maintained with a 3-year EFS of 65% and 3-year overall survival of 69%, respectively [16]. When analyzed separately patients with PRETEXT IV tumors had an EFS of 68% and overall survival of 69% whilst patients with lung metastases fared slightly worse with an EFS of 56% and overall survival of 62%. Despite a high response rate to chemotherapy, 24% of patient did not achieve a complete resection of their liver tumor, highlighting the importance of surgery in the cure for these patients. This was despite the increased use of liver transplantation, 20.6% in this study population. In an effort to improve the surgical resection rate for these high-risk patients and to improve the metastatic response rate, thereby rendering more patients eligible for liver transplantation, the SIOPEL-4 study was conceived. Based on the observation that cisplatin appeared to be the most effective agent in the treatment of hepatoblastoma and in the absence of dose-limiting myelotoxicity investigators developed a dose-dense cisplatin induction regimen. The schedule involved weekly administration of cisplatin in three blocks (A1–3) with the incorporation of one cycle of doxorubicin in each block (Figure 28.7). Consolidation therapy, usually delivered after tumor resection, was provided by a combination of carboplatin and doxorubicin. Results appeared superior to SuperPLADO in this small pilot study with a 3-year EFS of 76% and overall survival of 83% [17]. Despite the increased dose intensity of therapy, toxicity appeared quite acceptable. Of particular interest was the observation that the response rate of lung metastases was very high and 19 of

20 patients who cleared their metastatic disease with chemotherapy remained in continuing complete remission.

SIOPEL currently recommends cisplatin monontherapy for standard-risk tumors, SuperPLADO for non-metastatic high-risk tumors, and SIOPEL4 therapy for metastatic tumors. Future therapeutic strategies in the treatment of hepatoblastoma aim to build on the following:

- Improvements in risk stratification of patients based on clinical parameters and molecular genetic signatures will allow treatment planning to be refined.
- For a proportion of low-risk localized tumors, primary resection should be considered if a safe margin can be ensured against adjacent vascular structures. For such patients, limited adjuvant chemotherapy is probably sufficient. In the case of well-differentiated fetal histology, no adjuvant chemotherapy is necessary.
- For standard-risk patients, cisplatin monotherapy provides very high levels of cure. Strategies to reduce long-term toxicities, especially ototoxicity, by reducing cumulative exposure or the addition of otoprotectants are under investigation.
- High-risk patients have complex primary tumors and/or metastatic disease which require skillful multidisciplinary planning and early consideration for liver transplantation. Good metastatic response to chemotherapy may convey excellent opportunity for cure.

Hepatocellular carcinoma

Although traditionally HCC has been managed in a similar fashion to hepatoblastoma, the impact of chemotherapy is less well defined. There is no doubt that responses to PLADO chemotherapy can be achieved, with response rates in the order of 50% [18]. Subsequent complete surgical resection, however, is only possible with a smaller percentage of HCC tumors than with hepatoblastoma.

In the SIOPEL experience, response rates of 49% and resection rates of 36% were achieved, but 51% of patients never became operable. Overall survival rates at 5 years were 28%. Experience in the USA is very similar. Patients with localized disease, although this is a rare occurrence (17% in the series), had an encouraging outcome, with 5-year EFS estimates of 88%, but for all patients the 5-year survival was only 19%. This probably reflects the surgical nature of the disease, rather than the impact of chemotherapy. In the American study, no difference was observed in randomization between vincristine, 5-FU, and cisplatin versus cisplatin and doxorubicin chemotherapy.

Recent reviews of the adult literature highlight the problems with HCC, including a lack of translational research, difficulty in conducting multicenter clinical trials, lack of availability of new agents, inadequacy of tools for measuring meaningful clinical benefits, etc. In the SIOPEL group, an attempt to conduct a large international trial of HCC in children and young adults using thalidomide in combination with PLADO as an anti-angiogenic drug floundered, mainly as a result of the complexities of conducting such studies in rare tumors across many sites under the impact of the European Clinical Trial Directive's bureaucracy. Future studies are planned to capitalize on the many molecular-targeted therapeutics available. Sorafenib, the multitargeted tyrosine kinase inhibitor with anti-angiogenic properties has shown evidence of response and prolongation of event-free survival in combination with doxorubicin in adult HCC and will be investigated in the upcoming international pediatric liver tumor trial PHITT. Other targets, including inhibition of the mTOR pathway, may prove attractive given the use of agents such as everolimus in the management of post-transplantation immunosuppression.

The fibrolamellar variant of HCC is usually a slow-growing tumor, which metastasizes late and can be treated surgically without the need for adjuvant chemotherapy. It was at one time thought that this variant of HCC had a more favorable outcome, but a recent study in the USA suggests that the proportion of patients with advanced disease, the response to chemotherapy, and the outcome do not differ from those in conventional HCC.

Other tumors

Other malignant tumors of the liver are relatively rare and historically have a poor prognosis. Treatment should be dictated by the histological diagnosis.

Sarcomatous lesions tend to be managed, like hepatoblastomas, with combined surgical and multiagent chemotherapy regimens, as well as adjuvant radiotherapy treatment.

Undifferentiated embryonal sarcoma of the liver has been considered a very aggressive neoplasm with an unfavorable prognosis, but recent reports suggest modern chemotherapy regimens lead to significant tumor mass reduction, thereby allowing radical surgery in an increasing number of cases.

In the pediatric population, *epithelioid hemangioendothelioma* is uncommon and typically presents as a non-resectable tumor; it appears to be a more aggressive tumor in comparison with those in adults, and transplantation may not be a suitable management option as in adults. The role of chemotherapy as the first-line or only treatment is not clear. Ifosfamide-based chemotherapy is not effective, but a regimen combining carboplatin, cisplatin, and doxorubicin stabilized the disease in one patient, and carboplatin and etoposide brought about a partial response followed by stabilized disease in another. A complete response after carboplatin and etoposide in an aggressive form of pleural epithelioid hemangioendothelioma has also been reported. Overall, using carboplatin and etoposide and further studying their effect on epithelioid hemangioendotheliomas appears worthwhile. Alternatively, utilizing TACE, also reported in the adult literature, with a view to rendering tumors surgically resectable should be considered.

Response to therapy

The response to therapy can be monitored by serial assessment of:

- Imaging findings. Repeat CT scans or ultrasonography will document shrinkage of the hepatic tumor, while chest radiography or chest CT scans will monitor the progress of pulmonary metastases.
- AFP levels in tumors that secrete AFP. Patients with a good response to chemotherapy have a rapid fall in serum AFP levels, whereas a failure to return to normal limits in the absence of radiographically evident disease is highly suspicious for minimal residual disease. An increase in AFP after the initiation of chemotherapy is a sensitive marker of relapse or treatment failure.
- Transcobalamin levels in fibrolamellar HCC may be a guide to response.
- Eighty-six percent of hepatoblastomas respond to PLADO chemotherapy, while the response rate in HCCs is only 43%.

Surgery

Selection for surgery

It is clear that complete cure depends on effective resection of the tumor after chemotherapy. Careful assessment of post-chemotherapy imaging with CT scans, MRI, or hepatic angiography is essential to establish that the a branch/main portal vein, branch/main hepatic artery, hepatic vein to drain the remaining liver, and branch/main/bile duct is patent and that there will be sufficient liver remaining after resection. In the majority of cases, the tumor will be localized to one half of the liver and compensatory hypertrophy of the contralateral side occurs, so that liver insufficiency after surgery is unusual. Tumors that remain multifocal, central in location, or involve the portal vein after chemotherapy are not resectable, and these patients should be considered for liver replacement unless there is extrahepatic disease that has not responded to chemotherapy.

Surgical resection techniques

Conventional resection techniques are based on the segmental anatomy of the liver. Precise knowledge of the anatomical landmarks and previous surgical experience are the most important factors for obtaining a high rate of complete resection, and concentrating these cases in expert surgical units in which these operations are carried out regularly and with a minimum of risk should be recommended. In the majority of cases, resection of the hemiliver (left or right) or extended resection of one side plus additional segments from the opposite side is required (Figure 28.8). Major liver resections can be performed with minimal perioperative morbidity and mortality. For well-selected patients with conventionally "unresectable" tumors, liver transplantation is emerging as an essential approach. In children, the use of special techniques such as ex vivo surgery followed by autotransplantation of the liver remnant, or prolonged vascular exclusion with cold perfusion of the liver, are extremely rarely used as the indications are limited and because of the increased technical risks associated with the small size of the vessels.

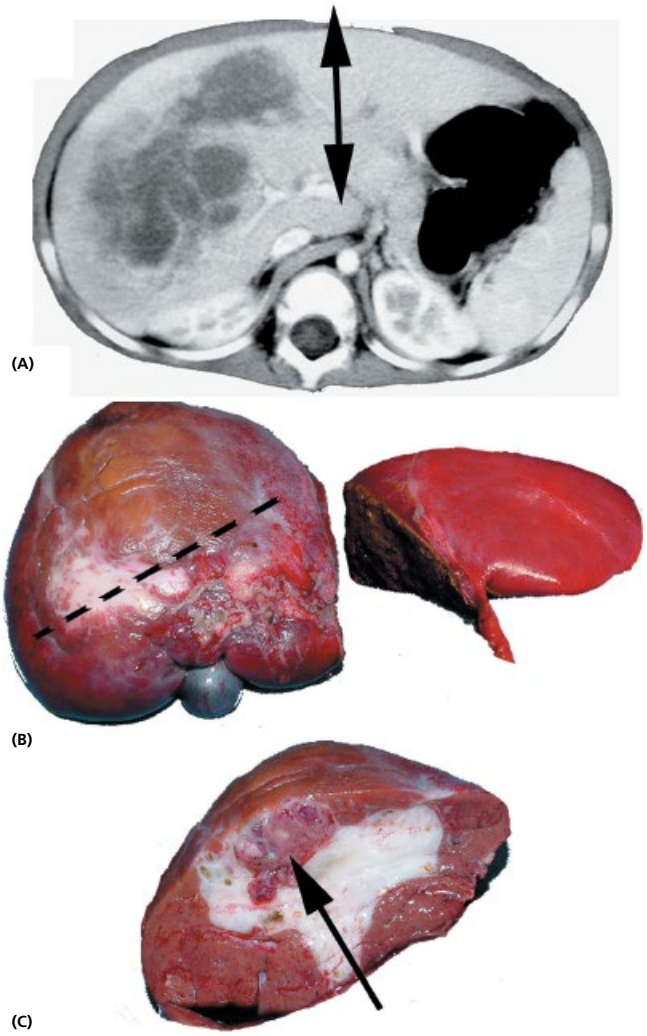

Figure 28.8 Extended right hepatectomy for large hepatoblastoma. (A) Preoperative CT scan showing the tumor location and extension from the right liver toward the left. The arrow indicates the liver resection line. (B) Intraoperative view showing the resected liver (left side) and the remaining healthy parenchyma (right side). (C) Macroscopic appearance of the tumor at section (resection along the dotted line shown in B). The large white areas represent fibrotic and necrotic transformation of the tumor, but a viable node is still visible (arrow).

Surgery is carried out through a wide upper transverse or J-shaped incision. Standard techniques include isolation and suture ligation of the hepatic artery and portal vein branches to the portion of the liver being resected, followed by division of the liver parenchyma along the line of demarcation that has been produced. Most liver surgeons employ

special instruments such as an ultrasonic scalpel or a water jet for dividing the liver. These techniques divide the liver parenchyma but leave the vascular and biliary structures intact for subsequent coagulation with diathermy for small structures or suture ligation. Latest technologies utilize simultaneous sealing and cutting devices (using combined ultrasonic and advanced bipolar energy or pure ultrasonic vibration for dividing the liver). With these devices the smaller vessels are sealed/coagulated and divided along with the parenchyma. The hepatic veins draining the area can be suture ligated early on in the procedure, but if access is difficult this step can be delayed until after complete division of the liver. Clamping of the vascular supply (portal vein and hepatic artery) or vascular exclusion (vascular inflow and vena cava) has been suggested in order to reduce blood loss during difficult hepatectomies. While this accelerates the procedure and eventually limits the blood loss, it also causes liver ischemia, thus increasing the risk of postoperative liver dysfunction (especially when the residual liver is small).

Most liver surgeons now prefer a meticulous division of the liver, steadily achieving hemostasis and avoiding vascular clamping. While the operating time may be prolonged, this approach is associated with more rapid hepatic recovery and patient discharge. Hemostasis at the cut surface is aided by the use of bipolar diathermy for dividing the liver and of argon diathermy for final hemostasis of the cut surface. Application of a layer of fibrin glue, or the application of various hemostatic patches, reduces the postoperative risk of leakage of blood or bile.

Currently, surgery for HCC developing in a cirrhotic liver are based on experience gained in adult patients, known as the Milan criteria: no more than three tumors, each no more than 3 cm in size; or a single tumor no more than 5 cm in diameter. In adult patients, survival in the range of 70–80% can be expected in highly selected patients. Recent studies suggest that in an otherwise normal liver, it may be possible to increase the current size cut-off points to 6.5 or 7 cm. In HCC, vascular invasion and lymph node involvement are contraindications for transplantation.

Epithelioid hemangioendothelioma typically presents as a non-resectable tumor, both in adults and in children; in the pediatric group, the tumor behavior is more aggressive than in adults, with rapid growth and a risk of recurrence. Transplantation may not be a suitable management option, and it is possible that only patients who are responsive to chemotherapy should be considered for transplantation.

In selected patients with benign liver tumors, transplantation may be considered when the tumor is associated with local complications (compression, ascites, Budd–Chiari phenomenon), or even life-threatening problems, such as cardiac failure in multifocal neonatal hemangiomas.

Hepatoblastoma

Hepatoblastoma is the most frequently resected tumor. Although some patients present with resectable disease, most patients do not, and all such patients should undergo chemotherapy before surgery. As chemotherapy produces a shrunken and sclerotic tumor mass, the resection line may pass close to this without fear of incomplete resection, as long as the margin itself is clear of tumor. This is different from the situation with other malignant tumors, in which a clear margin of at least 1 cm is recommended. Histological examination of hepatoblastomas resected after chemotherapy reveals that much of the residual tumor mass is in fact sterilized disease, with smaller nests of persistent tumor within the hepatoblastoma.

The other option for surgical management of hepatoblastoma is consideration for liver transplantation (Figure 28.9), where careful patient selection is crucial. In suitably selected candidates this is a viable option for local therapy. The most significant predictor of a good outcome after liver transplantation appears to be the demonstration of chemosensitivity, and Birmingham Children's Hospital, UK observed a 100% survival rate in this group in comparison with 60% survival

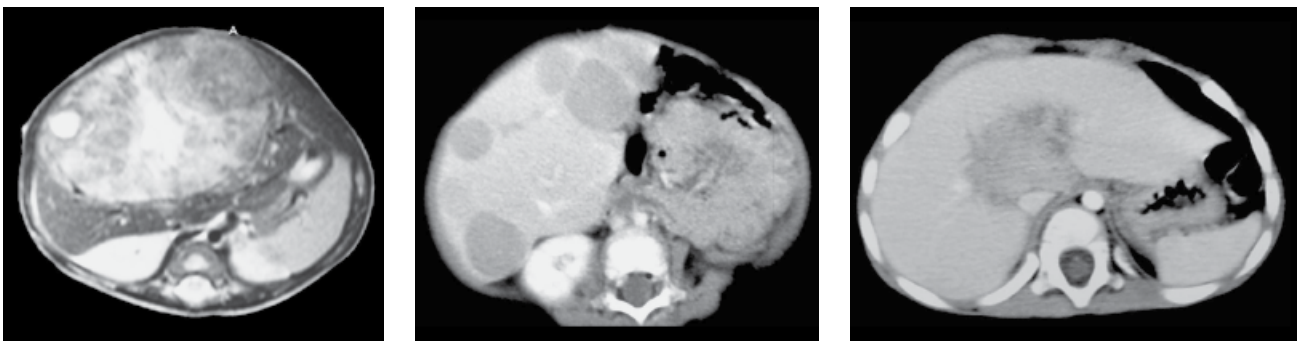

Figure 28.9 Non-resectable hepatoblastomas are an indication for transplantation. Typically, PRETEXT IV hepatoblastomas (B) are unresectable and must be considered for transplantation unless there is extrahepatic spread. Selected cases with PRETEXT III (A) or PRETEXT II (C) tumors may be considered for primary transplantation due to their anatomical position, the difficulty in achieving a radical resection, and/or the risk of secondary liver failure related to insufficient residual liver mass or vascular complications. In the latter cases, heroic attempt at difficult resection should not be encouraged.

in poor responders to chemotherapy, as has been suggested in other series [19]. This is in line with the further observation that the presence of lung metastases at diagnosis is not a contraindication for transplantation if the metastases clear during chemotherapy.

Liver transplantation

Liver transplantation has a definitive role to play and must be considered for the treatment of patients with unresectable liver tumors. Indications include patients with hepatoblastoma who have one of the following:

- Multifocal tumor (PRETEXT IV at diagnosis).
- Large, solitary tumor (PRETEXT IV, involving all four sectors of the liver).
- Unifocal, centrally located PRETEXT II and III tumors involving the main hilar structures or all three main hepatic veins.

Plus:

- No extrahepatic metastases.

In the context of organ shortage, case selection and correct timing are essential not only for the appropriate use of this surgical option, but also for a successful outcome. Transplantation may avoid unnecessary attempts to intensify chemotherapy to achieve surgical resectability in difficult cases, along with the related morbidity that involves. A good response of the main tumor site following chemotherapy is not a prerequisite for a good outcome after transplantation, but indicates that the biological behavior of the tumor is favorable.

Macroscopic venous invasion (portal vein, hepatic veins, vena cava) and previous lung metastases in hepatoblastoma patients are not a contraindication for transplantation if these sites are completely resected during the transplant operation or are cleared after chemotherapy.

Liver transplantation is a poor option for patients with recurrent disease or in whom incomplete excision was achieved previously. In a review of the worldwide experience of 147 liver transplantations for hepatoblastoma by Otte *et al.* [20], the 6-year post-transplant survival was reported to be 82% for patients receiving "primary" transplants and only 30% for patients receiving "rescue" transplants (Figure 28.10).

Liver transplantation is associated with special challenges for the administration of chemotherapy. This is why early identification of patients is essential, so that they can be listed and donor searches initiated. In many cases in the past, donors were not found before planned preoperative chemotherapy had been completed. This then leads to a need to "tread water" whilst a donor is found, with the risk of additional cumulative doses of doxorubicin and the danger of cardiotoxicity. Currently, an active split liver transplant program allows the allocation of a liver graft around the time of conventional surgery and avoids additional chemotherapy. Another excellent alternative is to procure a left liver graft from a living related donor, allowing planning of this elective operation at the right time in accordance with the chemotherapy schedule. In addition, the added problems of cumulative cisplatin exposure can add to concerns about cross-reactivity with critical nephrotoxic drugs administered after transplantation (e.g., ciclosporin). However, in patients in whom early transplantation can be undertaken, the administration of postoperative chemotherapy should be considered. With close scrutiny of serum levels of immunosuppressive drugs during therapy, we have not encountered serious postoperative chemotherapy-related problems.

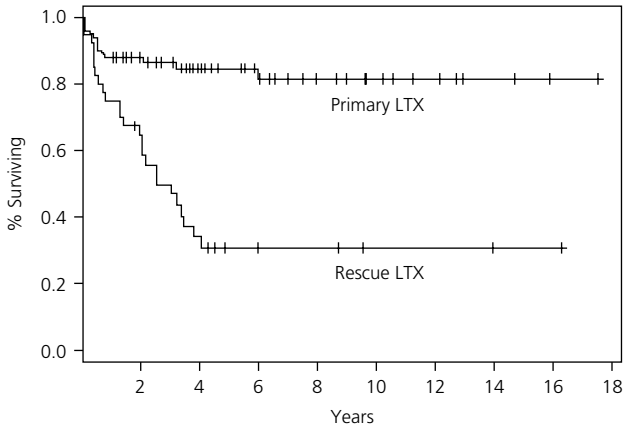

Figure 28.10 Overall survival outcomes after primary and rescue transplantation (LTX). (Reproduced with permission from Otte *et al.* 2004 [20]. Reproduced with permission of Elsevier.)

Hepatocellular carcinoma and other malignant tumors

Resection of HCC in children with cirrhotic livers secondary to an underlying metabolic disease is unlikely to achieve a cure, as these tumors are multifocal. Liver transplantation may thus be considered for unresectable HCC, but only for patients who have HCC nodes less than 5 cm in diameter and an absence of vascular invasion (absence of thrombosis). For those who need transplantation, the waiting time to obtain a liver graft may be a limiting factor, allowing the tumor to grow and spread; transplantation should be performed within 3–4 months of diagnosis. Using the option of living-donor transplantation may be a solution for those who are likely to have a longer wait on the list.

Patients with HCC and sarcomas in a non-cirrhotic liver should be considered for resection on the basis of the imaging findings, as described earlier. Techniques as outlined above are used, but it should be emphasized that a clear resection margin of at least 1 cm should be sought in order to maximize cure rates.

Epithelioid hemangioendothelioma is also often diffuse or multifocal in the liver at diagnosis and thus unresectable in most cases. Whereas in adults it is reported to be a low-grade malignancy with very slow growth (thus justifying liver

transplantation for quality of life reasons), its behavior appears to be much more aggressive in children and the role of transplantation may be questioned. Transplantation may be reasonable only for those with slowly growing tumors.

Patients with unresectable HCC and other rarer malignant tumors have received varying treatments, including embolization and hepatic artery ligation, but without any real evidence of benefit. Current research includes chemoembolization using agents injected into the hepatic artery at angiography, and the results with this are awaited.

Radiotherapy

It is difficult to define the role of radiotherapy in the management of hepatoblastoma, since patients have traditionally been managed with combinations of surgery and chemotherapy and have not received radiotherapy in isolation. Current studies do not recommend the use of radiotherapy for either the primary tumor or lung metastases. The use of radiotherapy is presently restricted to palliation of recurrent disease or treatment of residual disease following surgery.

Hepatic artery chemoembolization

Because of the overwhelming importance of rendering tumors resectable, other methods of reducing the bulk of the primary tumor have also been attempted. One such approach is hepatic artery chemoembolization (HACE), also known as transarterial chemoembolization (TACE), which may have a role to play in some cases, although it is not in widespread use. In this technique, intra-arterial chemotherapy is administered in combination with an embolizing compound. The theory behind HACE is that a higher concentration of anticancer drug can be applied directly to the tumor, whilst protecting the normal liver (supplied mainly by portal blood). The embolic effects of the treatment are aimed at prolonging the "dwell time" of the drug within the tumor, reducing the first-pass metabolic effects, and causing direct ischemic damage to the tumor. The use of HACE in adults with HCC has been well described, and some studies have been able to demonstrate improved survival. Experience with HACE in children with hepatoblastoma or HCC is limited, but a number of publications have suggested the procedure is safe, even in very small children, and that the majority of tumors shrink with treatment, often becoming resectable, so that the method can serve as a "bridge" whilst transplantation is awaited. The exact place of such therapy is unclear, but it needs to be considered as part of any combined therapeutic strategy in managing residual bulk tumor. As HACE may be associated with thrombosis of some of the hepatic artery branches, or even of the main (right, left, or both) arterial branches, it may interfere with subsequent surgical resection. HACE should thus be considered after a multidisciplinary discussion and with the surgical options at the end of the treatment being taken into account.

Benign tumors

Hemangiomas

The vascular lesion hepatic hemangioma is the most common benign endothelial cell neoplasm of the liver affecting fetuses and infants. The term hepatic hemangioma, however, has been used varyingly for almost any vascular liver lesion, including infantile hemangiomas, arteriovenous malformations of the liver, adult hemangioma liver lesions (benign venous malformations), and hemangioendotheliomas (vascular tumors that have the potential to metastasize). This confusion in terminology has resulted in inexact radiological findings and the development of various treatment algorithms. The treatment strategy of a child with suspected hepatic hemangioma ranges from simple observation to a series of complicated interventions requiring input from a multidisciplinary team composed of medical, radiological, and surgical subspecialists, using a variety of pharmacological agents and surgical approaches.

The Boston Children's Hospital have developed a data base and proposed a classification system for infantile hepatic hemangiomas [21]. In their classification, infantile hepatic hemangiomas can be focal, multiple, and diffuse. This subclassification has helped the development of protocols, treatments, and follow-up of the lesions, but confusion of nomenclature still exists as it has also been hypothesized that focal hepatic hemangioma are the hepatic equivalent of the cutaneous, rapidly involuting, congenital hemangioma, whereas multifocal and diffuse hepatic hemangioma are true infantile hemangiomas.

Hemangioma remains the most common benign tumor of infancy affecting 4–5% of white infants. Recent developments in the understanding of these lesions has resulted in the identification of two clinically and biologically distinct forms of hemangioma. Both groups have salient differences pertaining to natural history, clinical characteristics, histopathology, and radiographic imaging. The two groups are termed congenital hemangiomas, which are present at birth (some diagnosed antenatally), and infantile hemangiomas, which evolve after birth. There is no reason to believe that hepatic lesions share the same pattern of growth and involution as their more common cutaneous counterparts.

Congenital hemangiomas

These hemangiomas evolve during fetal life, are fully developed at birth, and do not undergo more than proportional postnatal growth. The lesions are rare and have an incidence

of about 3% of all hemangiomas. Histologically, they are different from infantile hemangiomas, being negative for glucose transporter-1 protein (GLUT-1) on histological examination.

Within the congenital hemangioma category, there are two subtypes:

1 Rapidly involuting congenital hemangioma (RICH) [22].
2 Non-involuting congenital hemangioma (NICH).

Focal hepatic hemangiomas

Focal hepatic hemangiomas have an equal sex distribution and are less commonly associated with accompanying cutaneous infantile hemangioma. In addition, immunoexpression of GLUT-1 is not observed in focal hepatic hemangiomas. These features support the concept that focal hepatic hemangiomas are congenital hemangiomas and that they develop antenatally.

This subtype can be identified on MRI as a well-defined, solitary, spherical tumor that is hypointense relative to liver on T1-weighted sequences and hyperintense on T2-weighted sequences. The typical tumor demonstrates centripetal enhancement on gadolinium contrast-enhanced sequences. Areas of central necrosis, thrombosis, or intralesional hemorrhage are heterogeneously enhanced. The solid non-thrombosed (non-involuted) areas of the lesion exhibit intense homogeneous enhancement.

Focal hemangiomas variably demonstrate the presence of high-flow shunts (arteriovenous or portovenous). Some focal lesions are associated with mild anemia or thrombocytopenia.

Infantile hemangiomas

Infantile hemangiomas are not usually identifiable at birth, or are very small, and then grow progressively. It is possible that the majority remain undetected because they are clinically silent. Some lesions are incidentally diagnosed on routine postnatal imaging indicated for other causes.

The natural history of the common infantile hemangiomas includes a period of rapid enlargement, occurring during the first few months of life, followed by a plateau in the growth and subsequent involution beginning around 9–12 months of age and lasting 5–7 years, akin to cutaneous hemangioma. It is during the rapid growth stage of the tumor, before spontaneous regression begins, that the infant is at the greatest risk for significant systemic manifestations, including:

- Cardiac failure secondary to high-volume shunting.
- Hypothyroidism secondary to overproduction of type III iodothyronine deiodinase.
- Fulminant hepatic failure.
- Abdominal compartment syndrome.

Infantile hemangiomas are positive for GLUT-1, which may be a specific marker for infantile hemangiomas. The behavior of true infantile hemangiomas must be distinguished from commonly misnamed hemangiomas of adulthood that are venous malformations and from the epithelioid hemangioendothelioma, a malignant tumor with metastatic potential. Confusingly, some infantile hemangiomas have been mislabeled hemangioendotheliomas, particularly in the histopathological literature. Infantile hepatic hemangiomas have also been confused with hepatic arteriovenous malformations since both can exhibit rapid flow, shunting, and cardiac consequences.

Infantile hepatic hemangiomas can be subtyped into multifocal and diffuse.

Multifocal infantile hemangiomas

These infantile hemangiomas present as homogeneously enhancing spherical tumors by MRI, hypointense relative to liver on T1 sequencing and hyperintense on T2-weighted sequencing. On CT, they are hypodense lesions with uniform or centripetal enhancement. Flow voids can be present in, or adjacent, to the lesions. These flow voids, along with enlarged hepatic arteries or veins or aortic tapering (distal to the origin of the celiac trunk), may indicate the presence of arteriovenous shunts. Whereas many multifocal lesions are asymptomatic and are only detected upon postnatal screening (because of the presence of multiple cutaneous hemangiomas, which may accompany visceral lesions), some are associated with high-output cardiac failure secondary to arteriovenous or portovenous shunting. These lesions undergo the typical course of involution of cutaneous infantile hemangiomas and demonstrate GLUT-1 immunoreactivity.

Diffuse infantile hemangiomas

Some infants with hepatic hemangiomas present with extensive hepatic involvement and near-total replacement of the hepatic parenchyma with innumerable centripetally enhancing lesions. These children are very likely to have a more serious clinical course. Massive hepatomegaly causes compression of the inferior vena cava and thoracic cavity, resulting in respiratory compromise. This mass effect can be so marked as to cause abdominal compartment syndrome and multiorgan system failure. Another complication of diffuse infantile hemangiomas is severe hypothyroidism owing to the overproduction of type III iodothyronine deiodinase. Hypothyroidism of this degree can cause cardiac failure (poor contractility with low output) and profound mental retardation in the developing infant. Although some patients demonstrate high-flow lesions, dilation of the hepatic veins will be less than expected given the size of the lesion. Furthermore, despite the enormous tumor burden, patients with diffuse involvement rarely develop high-output cardiac failure in the absence of hypothyroidism.

Management of hepatic vascular lesions

Confusing terminology has perpetuated improper diagnosis and misguided therapy for infants with hepatic hemangiomas. In the literature several algorithms have been proposed. Many hepatic hemangiomas, both congenital and

infantile, do not require any therapy because either they are asymptomatic or they involute spontaneously. A very small proportion lead to the development of critical pathophysiology. An algorithm for the management of hemangiomas in children is shown in Figure 28.11.

The natural history of these lesions is one of gradual progression and enlargement during the first 6 months of life. There may be other cutaneous hemangiomatous lesions that suggest the diagnosis.

The medical management of hemangiomas as outlined in the algorithm may involve prednisolone (0.5–10 mg/kg) administered for a period of 3 days to 9 months and propranolol (1–2 mg/kg body weight in three divided doses). Treatment with propranolol is generally started at 1 mg/kg in three divided doses and the child's pulse and blood pressure are monitored. If tolerated, the dose is increased to 2 mg/kg/day in three divided doses. The response is monitored with repeat ultrasound scans, and therapy is generally continued

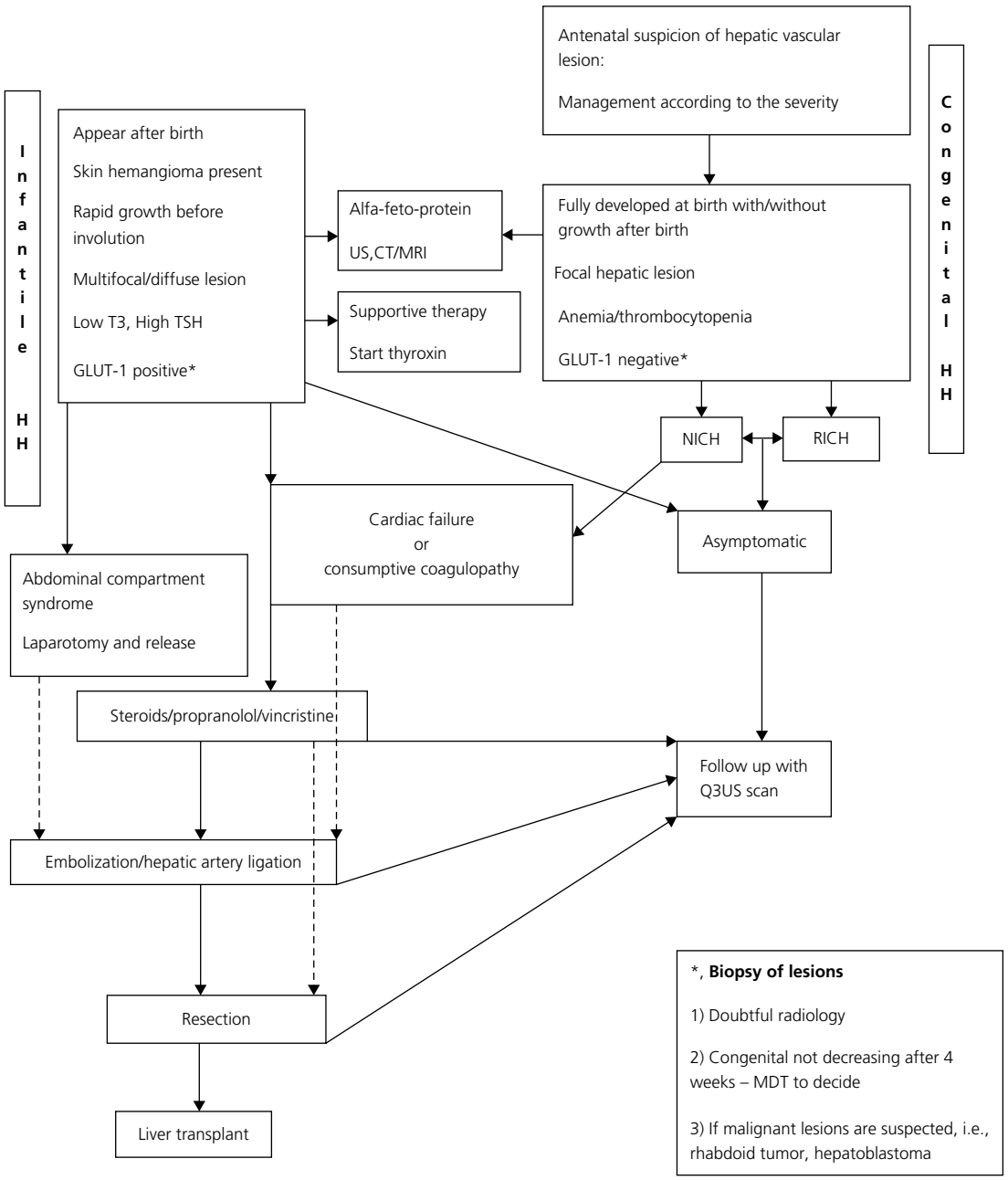

Figure 28.11 An algorithm for the management of vascular lesions of the liver in neonates, infants, and children. GLUT-1, glucose transporter-1 protein; HH, hepatic hemangioma; MDT, multidisciplinary team; NICH, non-involuting congenital hemangioma; Q3US, quarterly ultrasound scan; RICH, rapidly involuting congenital hemangioma; TSH, thyroid-stimulating hormone.

until the tumor has regressed considerably in size or became asymptomatic. Vincristine, at a dose of 1–2 mg/m^2 of body surface area, has been reported as an effective therapy for corticosteroid-resistant, life-threatening hemangiomas. Another recommended regimen is low-dose vincristine (9-week course, 0.05 mg/kg for 5 weeks, then 0.025 mg/kg for 4 weeks).

Both arterial and portal venous embolizations have been performed (Figure 28.12). In cases of multiple embolizations, the procedures are staged and performed a number of days apart to ensure that the embolization did not result in significant liver dysfunction as well as to assess response to the intervention. The choice of vascular system (arterial or portal venous) to embolize depends on the nature of lesion selection by the interventional radiologist based on the degree of shunting. A number of embolization agents including glue, PVA, and coils have been used.

Selective ligation of the hepatic artery has been reported in the literature to reduce the blood flow through the lesion. Extreme forms of therapy including resection/liver transplantation have also been reported in rare situations.

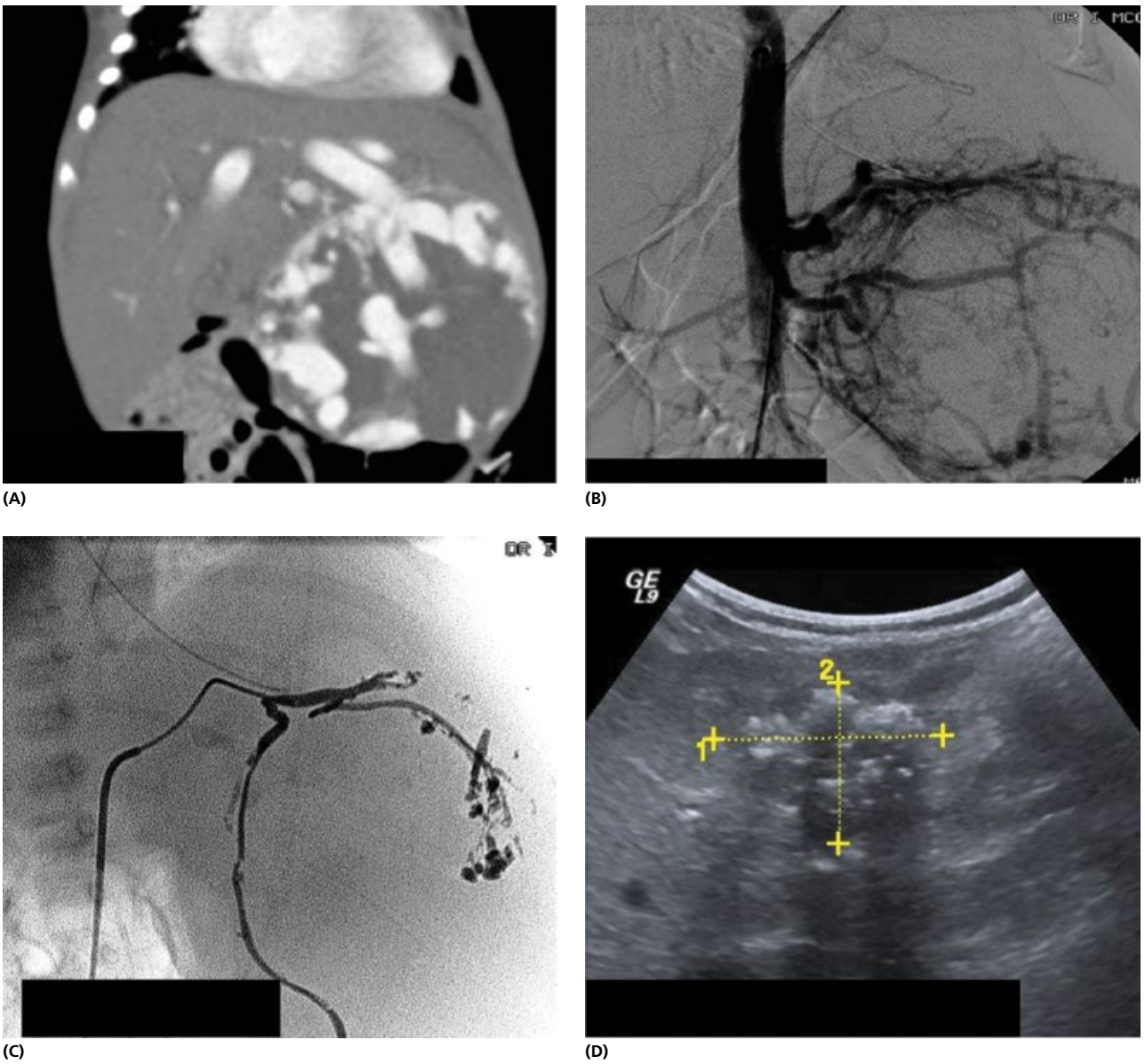

Figure 28.12 Congenital hemangiona. (A) CT scan showing persistent peripheral enhancement in the delayed phase with no "filling in" of the central aspect, with drainage via the very large left hepatic veins. (B) Flush aortogram showing marked vascularity of a hemangioma from a dominant single artery arising from the aorta, which split into three branches to supply the hemangioma. (C) Two branches embolized with glue with significant reduction in the flow. (D) Five-year follow-up ultrasound showing a small, mainly calcified, lesion.

Mesenchymal hamartomas

A mesenchymal hamartoma is a rare tumor, typically seen in children under 2 years of age. The majority of children present with abdominal distension, and the physical examination reveals a large, smooth, non-tender mass. The tumors are usually well demarcated, and resection by lobectomy (extended or not) is the treatment of choice.

Adenomas

Adenomas are extremely rare in the pediatric age group, but can occur at any age. In children, there is an association with glycogen storage disease type I and galactosemia, while in adults and adolescents there is a well-recognized link with oral contraceptive use – in these cases, cessation of the pill may allow resolution. The adenomas are usually diagnosed with ultrasound and confirmed by CT scan or radioisotope imaging using technetium-99 m sulfur colloid, as adenomas do not take up the isotope.

Persistent cases in adults require resection because of the risks of malignancy and rupture with intraperitoneal bleeding. Whilst adenomas are not thought to be premalignant in children, they can be difficult to distinguish from a well-differentiated HCC, and if there is any doubt resection should be undertaken.

Focal nodular hyperplasia

Focal nodular hyperplasia is also rare in the pediatric age group and occurs more commonly in girls than boys. The vast majority of cases are asymptomatic, although some patients present with an abdominal mass or pain. Histologically, the lesions have features of a well-localized area of liver cell hyperplasia around a fibrous central scar, which shows up clearly on CT or MRI scans. Colloid scans are usually positive, as there are sufficient reticuloendothelial cells within the mass to take up the isotope. Surgical resection is only required for symptomatic patients as there is no risk of malignancy.

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CHAPTER 29

Disorders of the Pancreas

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Key points

- Pancreatic disease in children is rare.
- Inherited or developmental factors play a major role in the etiopathogenesis of childhood inflammatory pancreatic disease.
- Most children can be managed conservatively without surgery.
- Imaging is based on judicious use of ultrasonograhy, computed tomography, magnetic resonance scanning, and endoscopic ultrasonography.
- Pancreatic tumors in childhood are very rare and different to those seen in adults.
- Outcomes after surgical resection are very good and these children are best managed at specialist centers.

Anatomy and physiology

Anatomy

The pancreas is an elongated organ placed transversely in the retroperitoneal space of the upper abdomen. It is divided into the head, uncinate process, neck, body, and tail. The head lies within the loop of the duodenum (Figure 29.1). The blood supply to the pancreas originates from the celiac trunk and the superior mesenteric artery. The pancreatic venous drainage is mainly into the portal, superior mesenteric, and splenic veins. The sympathetic and parasympathetic innervation will regulate pancreatic blood flow as well as exocrine and endocrine function. The endocrine component plays a central role in glucose homeostasis, while the exocrine cells are involved in the digestion of nutrients by the production of digestive enzymes.

Exocrine function

In adults, the pancreas synthesizes copious amounts of an enzyme- and electrolyte-rich juice per day. The secretion rate rises after stimulation from 0.2–0.3 mL/min to more than 10 times that amount, mainly by enhanced secretion of the ductal epithelial cells. The composition of the pancreatic juice depends on food supply; peptide hormones such as cholecystokinin (CCK) and parasympathetic efferent signals mediated by the vagus nerve increase the enzyme output of acinar cells, whereas the hormone secretin primarily promotes the bicarbonate secretion of ductal cells (Figure 29.2). The high content of bicarbonate neutralizes the acid gastric juice and thereby allows an optimal activity of the digestive enzymes in the intestinal lumen.

Approximately 90% of the pancreatic juice proteins are digestive enzymes, of which trypsin is the most important. All proteolytic enzymes are synthesized in the acinar cells as inactive precursors (zymogens). Only after entering the intestine, will trypsinogen be cleaved to active trypsin by the brush border membrane enzyme enteropeptidase (also designated as enterokinase). As consequence, the trypsin-mediated activation of further proenzymes such as chymotrypsin and carboxypeptidases starts. If a significant activation of pancreatic enzymes takes place within the pancreatic gland, autodigestion of the organ occurs, which presents clinically as pancreatitis.

The exocrine secretion is regulated by the nervous system and hormonal factors, and can be divided into a cephalic, a gastric, and an intestinal phase. The cephalic phase is mediated by the parasympathetic system (vagus nerve) and acetylcholine, which promotes enzyme secretion via muscarinic receptors, but does not influence bicarbonate output. The main driver of the gastric phase is distension of the stomach by food and a consecutive secretion of an enzyme-rich juice.

Diseases of the Liver and Biliary System in Children, Fourth Edition. Edited by Deirdre A. Kelly.

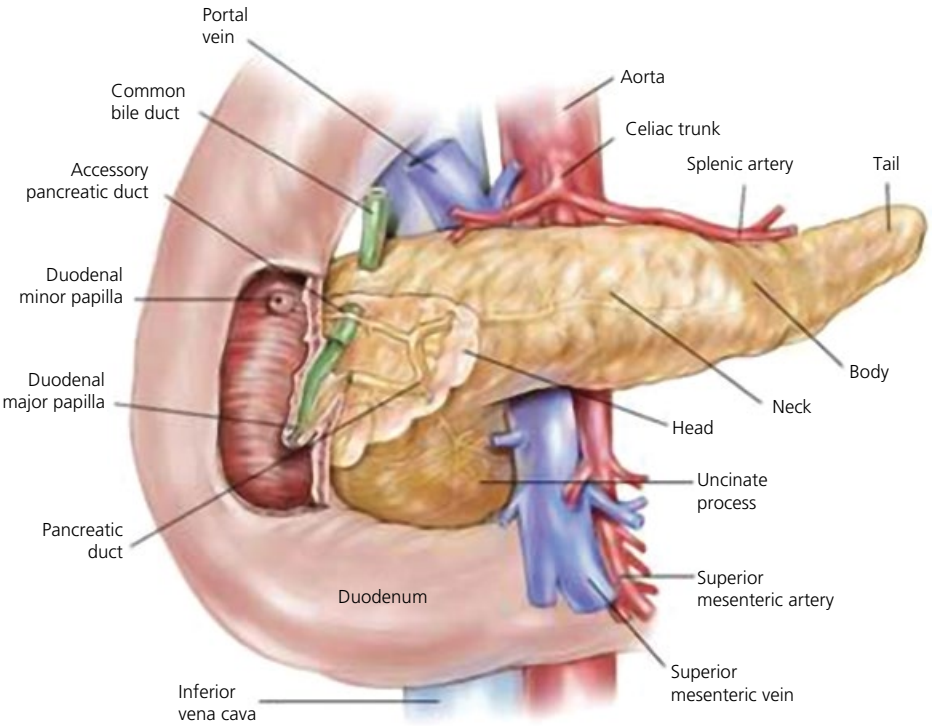

Figure 29.1 Anatomy of the pancreas.

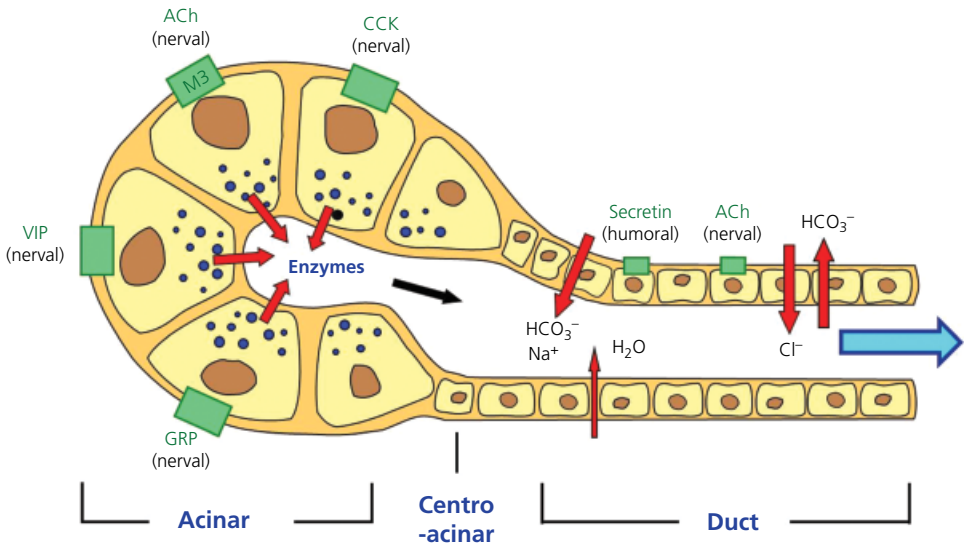

Figure 29.2 Schematic presentation of the exocrine pancreas. ACh, acetylcholine; CCK, cholecystokinin; GRP, gastrin-releasing peptide; VIP, vasoactive intestinal peptide.

During the intestinal phase, acidification of the duodenal juice by the gastric contents leads to the release of secretin and CCK. Secretin stimulates bicarbonate secretion, whereas CCK promotes enzyme secretion and contraction of the gallbladder.

Endocrine function

The endocrine function of the pancreas is mediated by hormone-producing islet cells that are essential for regulation of carbohydrate metabolism. Different cell types can be distinguished of which the α-cells produce glucagon, the β-cells

insulin, the δ-cells somatostatin, and the F cells pancreatic polypeptide. The main functions of these hormones are the storage of absorbed food as glycogen and lipids (insulin), the release of these energy reserves during fasting periods (glucagon), and the regulation of blood glucose levels and growth. The islet hormones also act on the exocrine pancreas by influencing the secretion of bicarbonate and digestive enzymes.

Embryology

The pancreas develops during the fourth gestational week from two separate buds of the foregut, the ventral and dorsal pancreatic buds (Figure 29.3). The ventral bud is located in the ventral mesentery immediately adjacent to the hepatic diverticulum, whereas the dorsal bud arises on the opposite side. In the following 2 weeks, the ventral part turns together with the orifice of the common bile duct around the back side of the foregut behind and below the dorsal bud (rotation). At the sixth week, the parenchyma and ducts of both buds merge and the dorsal part becomes the anterior part of the head, body, and tail of the pancreas, while the ventral bud forms the posterior head and uncinate process (fusion). The main pancreatic duct (duct of Wirsung) develops by the fusion of the duct of the ventral bud with the distal ductal part of the dorsal bud. The proximal part of the dorsal bud can degenerate completely, but persists in 60% of the population as an accessory duct (duct of Santorini).

In parallel with the organ development, the epithelial cells of the gland differentiate to acinar, ductal, and endocrine cells. Endocrine cells (islet cells) can be detected from the 12th gestational week and acinar cells from the 14th to 16th weeks. The formation of zymogen granules in the acinar cells as well as insulin secretion starts about the fifth gestational month. Exocrine pancreatic function in the mature newborn is incompletely developed and is subject to a postnatal maturation process which is completed in the second year of life. In particular, the secretion of amylase and lipase at birth is less than 1% and 10% of the adult values, respectively. Rapid growth is observed in the first year of life and the gland is relatively large in children [1].

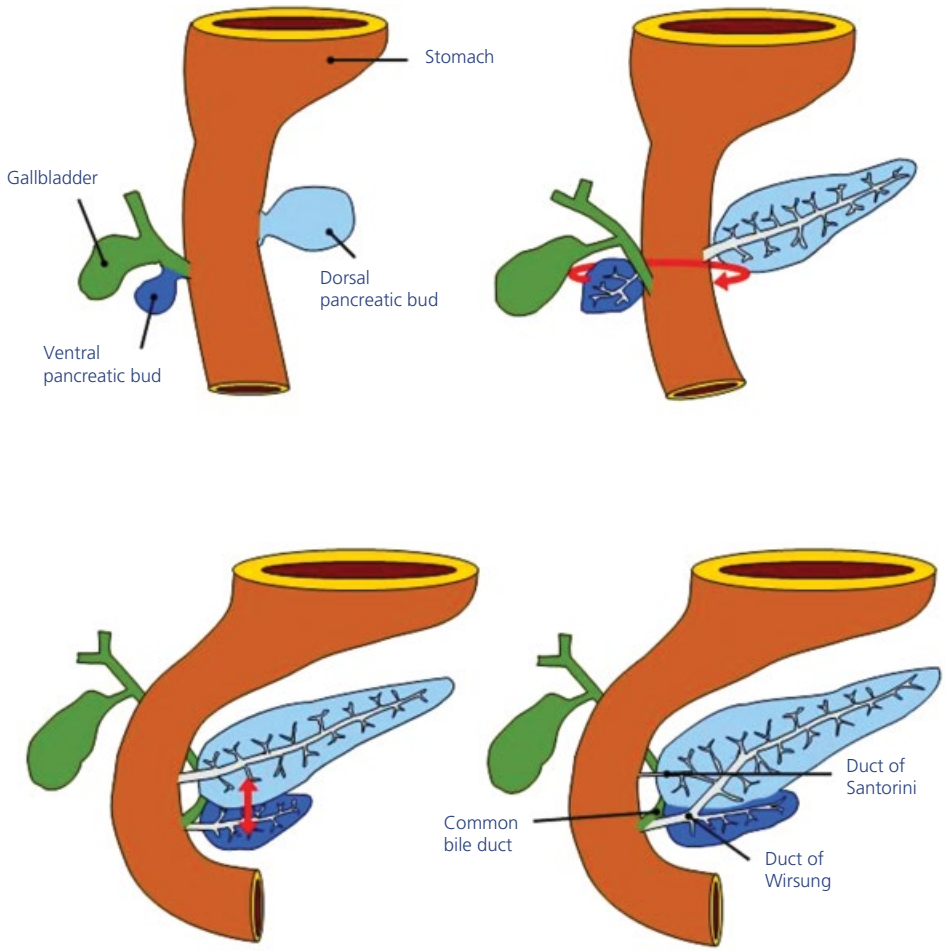

Figure 29.3 Embryological development of the pancreas.

Laboratory investigations of the pancreas

Routine blood tests to investigate a clinical suspicion of pancreatic disorder includes a full blood count, serum electrolytes, liver enzymes, and serum lipase and amylase levels. Diabetes due to endocrine pancreatic insufficiency is initially evaluated by serum fasting glucose or HbA1c measurement. Impaired levels are further investigated by a glucose tolerance test. Special investigations to assess exocrine function are discussed under the following subheading.

Exocrine insufficiency

Exocrine pancreatic insufficiency is defined as the functional restriction of pancreatic secretion with a subsequent reduction of secretion volume as well as a reduction of concentrations of bicarbonate and digestive enzymes. As a consequence, patients develop malabsorption and steatorrhea. If uncorrected, this leads to acute and chronic malnutrition with weight loss, linear growth failure, and fat-soluble vitamin deficiencies, which are more severe in infancy. Due to the large reserve capacity of the exocrine part of the gland, clinical symptoms develop only when function is reduced by more than 90%.

Exocrine function can be assessed by direct tests that directly measure pancreatic secretion (enzymes and bicarbonate) and by indirect tests.

Direct tests

Direct tests measure pancreatic enzymes (amylase, lipase, and trypsin) and bicarbonate after stimulation with secretin and CCK or its analog cerulein (secretin–cerulein or secretin–CCK test), or by the ingestion of a standard test meal (Lundh test). Test samples are collected by a tube placed in the duodenum over 1–2 h. The secretin–cerulein test has a high sensitivity and is assumed in literature to be the "gold standard" for the evaluation of exocrine pancreatic function. However, the procedure is time-consuming, unpleasant for the patient, non-standardized, and expensive and there are no reference values for the pediatric age group. These tests are no longer used because severe exocrine dysfunction can be assessed by fecal elastase measurement.

Indirect tests

Indirect exocrine function tests include fecal elastase-1, fecal chymotrypsin, the ^{13}C-mixed triglycerides breath test, the pancreolauryl test, and fecal fat quantification. None of these tests detect mild or moderate pancreatic dysfunction.

Pancreatic elastase-1 is only slightly degraded during intestinal passage and has a higher sensitivity and specificity compared with measurement of fecal chymotrypsin, which no longer needs to be determined. The normal reference range for fecal elastase-1 is >200 µg/g feces (lower in the first months of life). Normal values do not exclude exocrine insufficiency [2]. It is possible to exclude pancreatic insufficiency in 80% of children using the fecal elastase-1 test, because of the high specificity and negative predictive value of the test [3]. Patients do not have to stop enzyme replacement therapy because the test records the human enzyme only.

^{13}C-mixed triglycerides (^{13}C-MTG) breath test measures the degradation of triglycerides by measuring exhaled levels of $^{13}CO_2$ after ingestion of a fatty meal. Since ^{13}C is a stable isotope, no radiation exposure occurs. Sensitivity is lower compared with fecal elastase determination.

For *fecal fat quantification*, feces are collected over 3 days and fat is determined by titrimetry or near-infrared reflectance analysis (NIRA). A pathological fat secretion (>5 g/day) will be found in severe pancreatic insufficiency. Diarrhea may cause false positive results, whereas collection errors and a low-fat diet lead to false negative results. The test is the only valid method to prove steatorrhea, but is rarely used in clinical practice.

Imaging of the pancreas

Radiological diagnostic modalities are frequently used in the management of pancreatic disorders. The common tools are ultrasonography, computed tomography (CT), and endoscopic retrograde cholangiopancreatography (ERCP). With the advancement in imaging and endoscopic technologies there is an increased utilization of magnetic resonance imaging (MRI), magnetic resonance cholangiopancreatography (MRCP), and endoscopic ultrasound scan (EUS) in the diagnosis of pediatric pancreatic pathology. As a general rule, the head of the pancreas is relatively bulky in children and must be cautiously interpreted as a pathological process on imaging. The diameter of the pancreatic duct varies with age and a possible pathological enlargement should be correlated with the relevant clinical and laboratory data.

Ultrasound

This is the first investigation to use. Better quality images are achieved in children because of their small body habitus and fat-free abdominal contents. However, it is often difficult to visualize the entire gland and it may not be reliable in differentiating normal from abnormal echogenicity, especially in pancreatitis.

Computed tomography

Computed tomography is useful in differentiating between a variety of inflammatory and malignant pancreatic disorders and trauma. Multislice helical CT with its multidetector technology permits imaging of the pancreas in the clearly defined arterial phase, pancreatic (parenchymal) phase, and portal venous (hepatic) phase with a delay of 20, 40, and 70 s

following intravenous injection of iodinated contrast medium. Pancreatic and portal phase imaging is most useful for the detection and staging of neoplastic lesions, the diagnosis of pancreatic necrosis, and the characterization of cystic lesions. The arterial phase improves the diagnostic accuracy of vascular malformations and the assessment of pancreatic blood supply. Dynamic CT is the imaging modality of choice in the diagnosis and management of pancreatic trauma. Despite its benefits, CT should be used only for appropriate indications to limit the radiation-induced side effects.

Magnetic resonance imaging

Magnetic resonance imaging is an excellent imaging modality to characterize pancreatic mass lesions, inflammatory or infectious conditions, and vascular abnormalities. MRCP is the most effective, safe, non-invasive technique to evaluate the pancreaticobiliary ductal system and has replaced ERCP in the diagnosis of ductal anomalies. Recent advances in imaging techniques include diffusion-weighted imaging, magnetic resonance spectroscopy, and the use of secretin to stimulate the pancreas for a functional assessment. In younger children MRI may require sedation or a general anesthetic.

Endoscopic ultrasound scan

The role of EUS in the evaluation of pancreatic cyst and mass lesions is well established in adults, but although limited in the pediatric population, several published case series support its value in the diagnosis and treatment of pancreatic disorders. EUS and EUS-guided fine needle aspiration (FNA) is safe and may be useful in diagnosis [4].

Endoscopic retrograde cholangiopancreatography

The main role of ERCP is now in therapeutic applications such as sphincterotomy, stent placement, stricture dilation, and stone extraction. The low prevalence of pancreatic disorders as well as technical and equipment limitations resulted in a paucity of data on experience with ERCP in children. The incidence of post-ERCP complications, especially pancreatitis, is similar to that in adults. The risks and benefits should be carefully reviewed for a diagnostic ERCP, which should be avoided where possible.

Etiopathology of pancreatic disorders

Pancreatic disorders are rare in the pediatric population. The disease spectrum includes complex congenital anomalies as well as acquired pathology. Unlike adults in whom alcohol is a major risk factor, many pediatric disorders are genetic. Some diseases are seen only in young children, while others are similar to adult disease. In addition, the disease spectrum is changing with an increasing incidence of gallstone pancreatitis in children. With improved life expectancy of patients with cystic fibrosis and the detection of mild or atypical forms, pancreatic pathology is now presenting in adolescent and adult populations. An overview of childhood pancreatic disorders is presented in Table 29.1.

Table 29.1 Pancreatic disorders in children and adolescents.

Anatomical anomalies	Pancreatic agenesis
	Ectopic pancreas
	Annular pancreas
	Pancreas divisum
	Long common channel syndrome
	True congenital cyst
Pancreatic inherited diseases	Cystic fibrosis
	Hereditary pancreatitis
	Shwachman–Diamond syndrome
	Johanson–Blizzard syndrome
	Pearson syndrome
	Congenital enzyme deficiencies
Other inherited diseases affecting pancreas	von Hippel–Lindau disease
	Polycystic kidney disease
	Beckwith–Wiedemann syndrome
Inflammatory diseases	Acute pancreatitis
	Chronic pancreatitis
Pancreatic trauma	Accidental and non-accidental
Neoplasms	Pancreatoblastoma
	Solid-pseudopapillary tumor
	Endocrine (insulinoma, gastrinoma, etc.)
	Other (sarcoma, lymphoma, adenocarcinoma, etc.)

Anatomical anomalies

The pancreas has its origin from two endodermal structures of the primitive duodenum. During the embryonic development, the ventral part turns to dorsal (rotation) and subsequently joins with the dorsal part (fusion). Most congenital pancreatic anomalies are attributable to disturbances of the three critical developmental steps: tissue differentiation, rotation and fusion. Defects of differentiation and rotation are rare (with exception of ectopic pancreas) whereas defective fusion is frequent, but mainly asymptomatic.

Abnormal differentiation

Pancreatic tissue differentiates from the primitive foregut. A disturbed differentiation can cause absence (aplasia) or reduced formation (hypoplasia) of the gland or the origination of ectopic pancreatic tissue.

Aplasia and hypoplasia

Complete (aplasia) or incomplete (hypoplasia) agenesis of the pancreas are rare events and are confined to a handful of published case reports. Lumb and Beautyman classified

agenesis of the pancreas into: (1) complete; and (2) partial – (a) involving both endocrine and exocrine tissue; (b) involving endocrine tissue only; or (c) involving exocrine tissue only [5]. Partial agenesis is more common and it is referred to as congenital short pancreas. Agenesis of the dorsal pancreatic bud results in a short, rounded pancreatic head adjacent to the duodenum with absent neck, body, and tail.

Agenesis occurs either as an isolated agenesis or in combination with other defects such as polysplenia syndrome, congenital heart defects, or cerebellar agenesis. Mutations in several transcription factors (*PDX1*, *PTF1A*, *GATA6*) have been associated with pancreatic agenesis. The cause of hypoplasia is abnormal formation of one or both pancreatic buds, in which errors of the dorsal bud with missing formation of the pancreatic body and tail preponderate.

The clinical presentation is dependent on the degree of agenesis. Aplasia manifests clinically with neonatal diabetes mellitus and severe intrauterine growth retardation. Complete agenesis is incompatible with life, whereas the clinical presentation of hypoplasia varies from an asymptomatic course to endocrine and exocrine pancreatic insufficiency; pancreatitis may be an associated presentation. Sonography, abdominal MRI/MRCP, or CT are diagnostic. Therapy consists of substitution of insulin and pancreatic enzymes, respectively.

Ectopic pancreas

Ectopic pancreas, also designated as heterotopic, aberrant, or accessory pancreas, is defined as pancreatic tissue without an anatomical link to the pancreatic gland. The etiology is unclear but may be due to abnormal differentiation of pluripotent endodermal stem cells, or because pancreatic tissue became separated during embryonic rotation or it may be due to a variant vitellointestinal tract remnant [6]. It is more common than complete agenesis with a frequency of 0.5–15% in autopsy studies. In most cases, the ectopic tissue is localized in the submucosa of the upper gastrointestinal tract (stomach, duodenum, and jejunum).

Ectopic pancreas often represents an incidental finding in the course of a gastroscopy, where it appears as 0.3–3 cm big node. Clinical symptoms are rare during childhood, but include abdominal pain, intestinal obstruction, gastrointestinal bleeding, and intussusception. Acute pancreatitis, cystic degeneration, or malignant transformation of the exocrine or endocrine component have been reported [7]. The size of the lesion and extent of mucosal involvement may influence the symptoms. The diagnosis is difficult. A barium contrast study may demonstrate a mucosal filling defect with central umbilication. The treatment is surgery; excision is recommended due to the potential malignant transformation.

Abnormal rotation

Annular pancreas

In annular pancreas the duodenum is completely, or partly, surrounded by a ring of pancreatic tissue. The cause is a fixation of the ventral pancreas during embryonal development before the ventral part starts to rotate. As a consequence, the ventral pancreas persists. An annular pancreas may be asymptomatic or may present at any age. The incidence based on the increased use of radiological imaging is approximately 1 in 1000. It is frequently associated with other anomalies such as duodenal stenosis or atresia (40%), trisomy 21 (16%), tracheoesophageal fistula (9%), congenital heart defects (7%), intestinal malrotation, and anal atresia [1]. The majority of symptomatic cases present in the first week of life as duodenal compression with bilious vomiting and feeding intolerance, and is an important differential diagnosis in duodenal obstruction.

The diagnosis is suggested in >50% of cases during prenatal ultrasonography. A plain abdominal radiograph may demonstrate two large air-filled spaces, the so-called "double bubble" sign, if the obstruction is complete. The combination of abdominal ultrasound and upper gastrointestinal contrast series confirms the diagnosis in most cases. CT and MRCP are useful adjuncts to further delineate the anatomy. Surgical therapy with duodenal bypass either in the form of a duodeno-duodenostomy or a duodeno-jejunostomy treats the obstruction. Simple division of the annular pancreas should be avoided due to the risk of injury to the pancreatic duct resulting in a fistula [8].

In older children, annular pancreas presents mostly as pancreatitis. An abdominal X-ray is not sufficiently diagnostic and contrast media examinations or ERCP are required. The prognosis of annular pancreas depends on the age of onset and has the highest mortality in the newborn period because of other organ malformations.

Abnormal fusion

The fusion of the ventral and dorsal pancreas also merges the efferent ducts of both parts of the gland. Numerous anatomical variants of the pancreatic duct system may occur as a result. In most patients, the main pancreatic duct (Wirsung) is connected with an accessory pancreatic duct (Santorini). The main efferent duct flows together with the bile duct into the major papilla (Vateri), which solely drains the pancreatic juice, whereas the duct of Santorini becomes blind (a minor papilla might exist) (Figure 29.4A). In approximately 30% of cases the duct of Santorini ends via the minor papilla in the duodenum so that both ducts drain pancreatic secretions (Figure 29.4B). In pancreas divisum, the physiological fusion of both parts of the pancreas has failed. This results in a separate outlet of the ventral part into the papilla major and of the dorsal part via the duct of Santorini into the papilla minor, which drains around 80% of the pancreatic juice (Figure 29.4C). Sometimes, both duct systems are connected

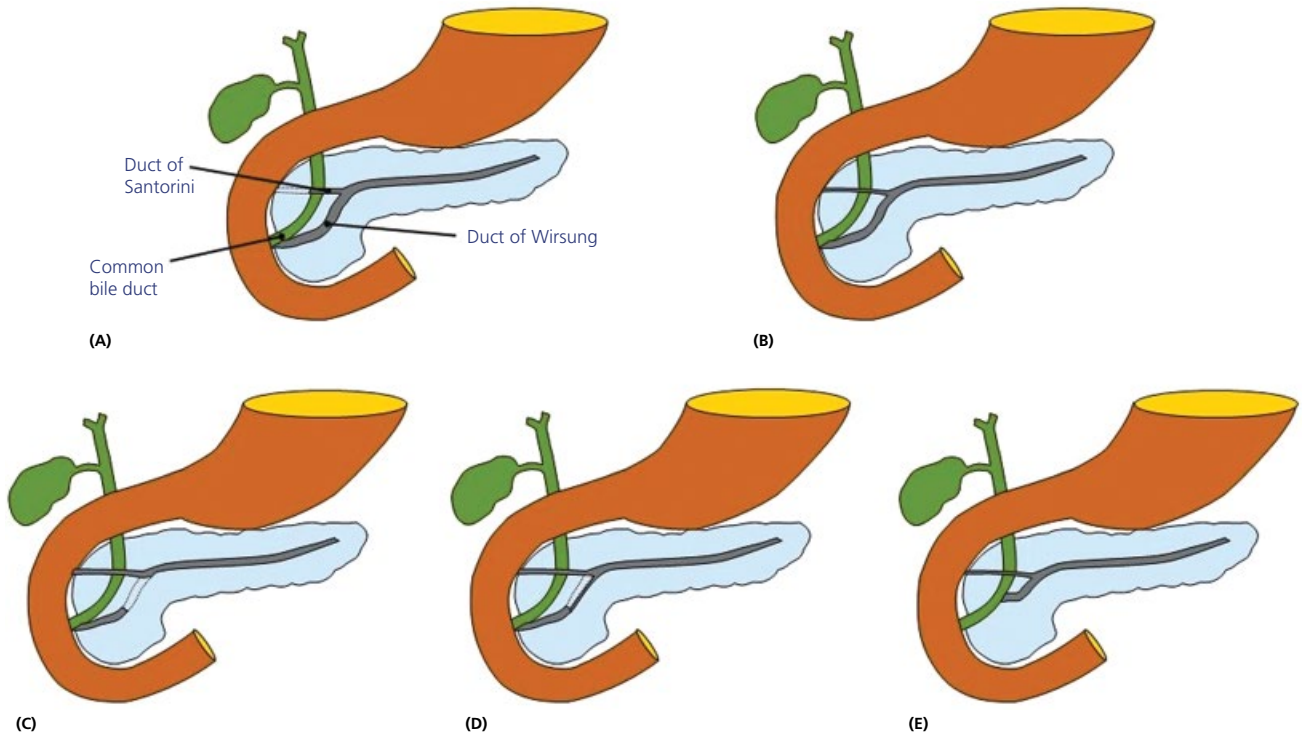

Figure 29.4 (A–E) Fusion anomalies of the pancreas, including the presence of a minor papilla and incomplete pancreas divisum as outlined in the text.

via a small side branch, which can be shown in MRCP as a subtle communicating duct (incomplete pancreas divisum) (Figure 29.4D).

The junction of the main pancreatic duct with the bile duct is also highly variable. In the majority, both ducts join to a short, 5 mm long common duct. However, separate orifices in the major papilla or an outlet in two distinct papillae are frequently found. Of clinical relevance are anomalies in which the pancreatic and bile duct join outside the duodenum and form a common segment longer than 1.5 cm (long common channel) (Figure 29.4E).

Pancreas divisum

Pancreas divisum is the most common fusion anomaly of the gland. The incidence is between 5% and 10% in autopsy studies and between 2% and 5% in ERCP studies. It may have no clinical significance as it may be a normal variant or it is possible that the narrow opening of the minor papilla insufficiently drains the secretions of the dorsal pancreas. This could cause a functional stenosis that predisposes to an obstructive pancreatitis. Based on the high frequency in the normal population, this anomaly is unlikely to cause pancreatitis alone, but may do so in combination with other environmental or genetic risk factors.

The diagnosis of pancreas divisum is based on MRCP imaging, which delineates the ductal anatomy (Figure 29.5). Secretin-enhanced MRCP (S-MRCP) is an alternative to conventional MRCP [9]. EUS and ERCP are invasive second-line investigations, although ERCP provides a simultaneous therapeutic intervention.

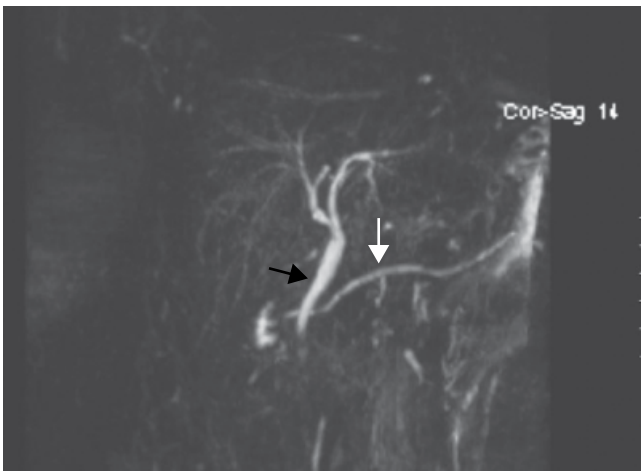

Figure 29.5 MRCP image showing a dorsal duct of Santorini draining separately into the minor papilla (white arrow). Black arrow, common bile duct.

Treatment includes endoscopic minor papilla sphincterotomy balloon dilation, or stenting to decompress the duct of Santorini. All have variable success rates. Surgical intervention in the form of transduodenal sphincterotomy is an alternative for patients who have failed endoscopic treatments.

Anomalies of the pancreaticobiliary junction

Most anomalies of the pancreaticobiliary junction are harmless variations within the normal range. Of clinical significance are pancreaticobiliary maljunctions, in which the biliary and pancreatic ducts unite outside the duodenum resulting in a long common segment (long common channel). It is seen in 1.5–3% of individuals, the common channel measures more than 15 mm proximal to the duodenal wall. Such anomalies are divided into three groups based on the type of fusion: (i) the common bile duct joins the major pancreatic duct; (ii) the major pancreatic duct joins the common bile duct; and (iii) the connection between the major pancreatic duct and common bile duct is too complex to be categorized as either (i) or (ii).

Anomalies of the pancreaticobiliary junction might promote a reflux of pancreatic juice into the common bile duct with consecutive inflammation and dilation of the duct, the formation of choledochal cysts, the development of pancreatitis and, in the long term, malignant transformation resulting in biliary carcinoma.

The incidence of pancreatitis is 3–31% in patients with these anomalies [10]. The most common clinical presentation is abdominal pain, jaundice, or pancreatitis. Inherited choledochal cysts, which are frequently associated with pancreaticobiliary maljunctions, may present as acute or recurrent pancreatitis.

Diagnosis is made by MRCP or ERCP. MRCP is the investigation of choice; contrast CT is useful in young children due to simplicity. S-MRCP is a dynamic test and has an added advantage of demonstrating associated reflux. Anomalies of the pancreaticobiliary junction in the presence of choledochal cysts should be treated with a surgical excision of the extrahepatic bile duct, cholecystectomy, and hepatico-jejunostomy due to the associated risk of malignant transformation (see Chapter 25).

Congenital pancreatic cysts

Most pancreatic cysts are acquired pseudocysts of inflammatory origin.

In contrast, congenital pancreatic cysts are lined with epithelium, may be single or multiple, and represent less than 1% of pancreatic cysts diagnosed in children. With improved radiological imaging, the diagnosis of incidental cystic lesions of the pancreas is on the rise. Most have little clinical significance; however a further characterization with EUS/MRCP is necessary in the majority to relieve anxiety (Figure 29.6).

Developmental anomalies in the pancreatic ductal system cause the formation of these cysts. They are classed into congenital developmental cysts, retention cysts, or duplication cysts [11]. They are frequently located in the tail or neck of the pancreas (62%).

Solitary cysts are generally asymptomatic, but can cause epigastric pain, vomiting, and jaundice by the compression of adjacent intestinal or biliary structures. The onset of symptoms is early and diagnosis is made usually in children aged less than 2 years. Multiple cysts are often combined with other disorders and are found in approximately 10% of patients with polycystic kidney disease and in 40–70% of patients with von Hippel–Lindau disease. In the latter disorder, the cysts may be the only abdominal manifestation and might precede other signs of the disease by several years.

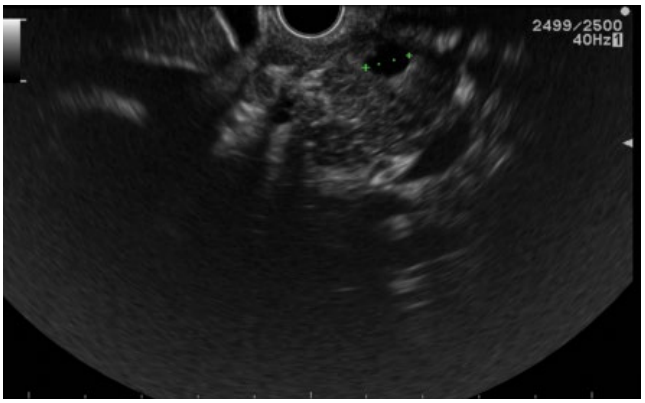

Figure 29.6 EUS linear probe demonstrating a cystic lesion in the tail of the pancreas (green marks).

Ultrasonography is a rapid and reliable initial investigation; however, for an accurate localization of the lesion MRI or CT imaging is necessary. The differential diagnosis includes pseudocysts, cystic pancreatic tumors, and gastrointestinal duplication anomalies.

Treatment strategies vary according to the location of the cyst. Symptomatic solitary cysts demand surgical resection as these could represent cystic malignancies. To avoid pancreatic resection, enucleation can be attempted if there is no communication with the ductal system. For lesions located in the tail or body, a spleen-preserving distal pancreatectomy is performed. EUS or laparoscopically guided internal drainage and biopsy of the cyst wall can be attempted in selected cases.

Pancreatitis

Definition and epidemiology

Pancreatitis is defined as an acute or chronic inflammatory disorder of the pancreas. The severity of acute pancreatitis varies from mild edematous pancreatitis to severe necrotizing inflammation. Whereas acute pancreatitis usually resolves spontaneously, chronic pancreatitis is a relapsing or continuing process, which is characterized by irreversible morphological changes and, in some patients, permanent impairment of exocrine function, endocrine function, or both. Morphologically, the pancreas in chronic pancreatitis demonstrates an irregular sclerosis with focal, segmental, or diffuse destruction of the parenchyma. Abnormalities of the pancreatic duct system such as dilations or strictures as well as intraductal plugs containing protein or calculi are frequent

findings. In children, chronic pancreatitis starts with relapsing attacks of acute pancreatitis with reversible morphological and biochemical alterations. Pancreatic insufficiency is a rare feature in this age group.

According to the Marseilles–Rome classification, which does not consider etiological factors, acute pancreatitis has been defined "not as a disease but as a spectrum of inflammatory lesions." Furthermore, it has been postulated that chronic pancreatitis is a cause and not a consequence of acute pancreatitis, and that the acute form only rarely progresses to chronic disease [12]. According to this concept, both presentations are two separate diseases that rarely merge. This concept, however, is incompatible with the presentation of pediatric hereditary pancreatitis, in which the predominant features are relapsing attacks starting in childhood with few signs of functional or morphological pancreatic damage. During the course of the disease, many patients develop all the morphological and functional signs of chronic pancreatitis. Consequently, acute and chronic pancreatitis should be considered as different stages of one dynamic disease process [13].

Over the last two decades, the incidence of pediatric pancreatitis is rising. It is estimated that 2–13 new cases are diagnosed annually per 100,000 children in industrialized countries, which may either represent a true incidence or be attributed to increased awareness [14]. The epidemiology and natural history of pediatric pancreatitis is poorly understood and is associated with significant morbidity in children. There are few published data and management strategies and outcome measures are extrapolated from the adult literature.

Pathogenesis

It is thought that pancreatitis results from pancreatic autodigestion. An inappropriate conversion of pancreatic zymogens to active enzymes within the pancreatic parenchyma may initiate the inflammatory process. A key role has been attributed to the activation of trypsinogen to trypsin, converting all proteolytic proenzymes to their active form. The identification of disease-causing mutations in a trypsinogen gene confirmed the significance of the pancreatic protease system in disease pathogenesis.

Several mechanisms protect the pancreas from autodigestion by activation of the pancreatic digestive cascade: intrapancreatic tryptic activity is prevented by the synthesis of digestive enzymes as inactive proenzymes (zymogens), by the localization of the activating enzyme enteropeptidase (enterokinase) outside the pancreas, and by a low glandular calcium concentration. In the normal pancreas, small amounts of trypsinogen are hydrolyzed to active trypsin in the parenchyma, but this tryptic activity is prevented by cosynthesized protease inhibitors such as SPINK1 and trypsin-degrading enzymes such as chymotrypsinogen C (CTRC). In inherited pancreatitis, the disorder may result from an imbalance of proteases and their inhibitors within the pancreatic parenchyma – and some evidence exists that this imbalance might be also important in other types of pancreatitis.

Etiology

Gallstone disease and alcohol are the most common etiologies for adult pancreatitis, whereas the most common etiologies in children are biliary and systemic disease, trauma, drugs, and metabolic, hereditary, and idiopathic causes (Table 29.2).

Mechanical/structural factors

Gallstone disease is noted in 10–30% of the pediatric population. Choledocholithiasis occurs in 2–7% of children with gallstone disease. Microlithiasis is more common and the incidence is almost 30%. Other obstructive causes for pancreatitis include structural defects such as choledochal cysts, annular pancreas, or pancreatic duct duplications, whereas it is not clear if pancreas divisum is a cause of pancreatitis.

Obstruction of pancreatic ducts by tumors or parasites (especially *Ascaris lumbricoides*) are occasional causes of acute pancreatitis.

Table 29.2 Causes of acute and/or chronic pancreatitis.

Mechanical/structural factors	Anatomical anomalies
Obstruction (gallstones, tumors, parasites)	
Trauma	
Metabolic factors	Hypertriglyceridemia
	Hypercalcemia
	Cystic fibrosis
	Dystrophy
	Malnutrition
	Renal insufficiency
	Diabetic ketoacidosis
Systemic disease factors	Shock
	Autoimmune pancreatitis
	Inflammatory bowel diseases
	Primary sclerosing cholangitis
	Systemic lupus erythematosus
	Rheumatoid arthritis
	Panarteritis nodosa
	Behçet disease
	Hemolytic uremic syndrome
	Sickle cell anemia
Drug/toxic factors	Drugs
	Toxins
Infectious factors	Viral
	Bacterial
	Parasitic
Hereditary/idiopathic factors	Cationic trypsinogen (*PRSS1*)
	Carboxypeptidase A1 (*CPA1*)
	Serine protease inhibitor, Kazal type 1 (*SPINK1*)
	Chymotrypsinogen C (*CTRC*)
	Carboxyl-ester lipase (*CEL*)
	Cystic fibrosis transmembrane conductance regulator (*CFTR*)

Trauma causes pancreatitis in 10–40% of cases. The common accidental injuries include bicycle handlebar trauma, motor vehicle accidents, sports injuries, and accidental falls. Other forms include blunt trauma in the setting of child abuse. Abdominal trauma usually causes self-limited inflammation, which might recur, if there is pseudocyst formation or rupture of the pancreatic duct. *Severe hemodynamic events* which lead to decreased oxygenation and/or reduced blood supply of the pancreas such as shock, can provoke pancreatitis.

Metabolic factors

Primary and secondary hyperlipoproteinemias with high serum triglyceride levels such as lipoprotein lipase or apolipoprotein C2 deficiency can provoke pancreatitis by unknown mechanisms.

Hypercalcemia in primary hyperparathyroidism, vitamin D intoxication, or excessive iatrogenic calcium supply can trigger pancreatitis, probably because calcium stimulates secretion of the exocrine pancreatitis. Pancreatitis has also been reported in up to 2% of patients with *diabetic ketoacidosis* and occasionally in patients with *renal insufficiency*.

Pancreatitis may be a consequence of *vasculitis* and may explain the link between pancreatitis and autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, polyarteritis nodosa, and Behçet disease. It is not clear whether immunological mechanisms, as in autoimmune pancreatitis, contribute to the pathogenesis of pancreatitis.

Systemic disease factors

The connection between *inflammatory bowel diseases* (IBD) and pancreatitis has been observed for a long time. It is possible that local inflammatory processes in the duodenum, a concomitant primary sclerosing cholangitis (PSC), or drug therapy may be related. More recent studies, however, suggest that pancreatitis develops independently and might precede clinical signs of IBD by years. An association with PSC (with or without IBD) is reported and may be caused by obstruction of the pancreaticobiliary duct system.

Patients with *hemolytic uremic syndrome* have transient elevations of pancreatic enzymes. Patients with *sickle cell anemia* are at a risk of pancreatitis; however, it is unknown if this is due to biliary stones or vaso-occlusive crises.

Although pancreatic insufficiency in *cystic fibrosis* (CF) is caused by inflammatory destruction of the gland, CF was not included into the Marseilles–Rome classification. One to two percent of CF patients suffer from recurrent pancreatitis and nearly all of these patients are pancreatic sufficient.

In non-industrialized countries, *protein energy malnutrition* is thought to be an important trigger factor. A deficiency of trace elements such as zinc or selenium and the consumption of toxic substances found in food may also play a role. More recent data, however, suggest that genetic factors such as *SPINK1*, *CTRC*, or *CPA1* mutations are major causes of so-called tropical calcific pancreatitis.

Drug/toxic factors

Numerous cases of *drug-induced pancreatitis* have been reported, but the causal relationship is substantiated for only a few drugs since in most cases patients have not been re-exposed after withdrawal of the suspected agent (Table 29.3). For instance, steroids have been incorrectly proposed as a trigger of pancreatitis for a long time. The time between drug exposure and pancreatitis varies. While azathioprine provokes pancreatitis mainly in the first months of treatment, sodium valproate might have a latent period of more than 10 years.

Infectious factors

Infectious causes include enteroviruses (coxsackie B and echoviruses), mumps, *Yersinia*, and *Ascaris* (Table 29.4).

Hereditary/idiopathic factors

Genetic factors play a major pathogenic role in children with both acute and chronic pancreatitis [15]. Recent genetic studies in children with pancreatitis have substantially changed the understanding of this disease. Hereditary pancreatitis was thought to be a rare disorder, but the recent findings of *PRSS1*, *SPINK1*, *CTRC*, *CEL*, and *CFTR* mutations in patients with so-called idiopathic chronic pancreatitis demonstrate that heredity is much more important than originally envisioned. These data challenge the differentiation between "hereditary" and "idiopathic" pancreatitis. Different mutations in different genes might lead to different phenotypic presentations and inheritance patterns, and even the same mutation in the same gene might have different consequences depending on the individual's genetic background and on environmental factors.

The classic form of hereditary pancreatitis follows an autosomal dominant trait. A gain-of-function mutation in the cationic trypsinogen (*PRSS1*) gene, p.R122H, is present in the majority of families. Additional *PRSS1* mutations

Table 29.3 Selected drugs that might induce pancreatitis.

Confirmed link	Probable/possible link
Asparaginase	Aminosalicylates
Azathioprine	Ciclosporin
Calcium	Clozapine
Didanosin	Foscarnet
Estrogens	Furosemide
6-Mercaptopurine	Interferon α
Statins	Metronidazole
Stibogluconate	Stavudine
Sulindac	Sulfonamides
Valproate	Tacrolimus (FK506)
Vincristine	Tetracyclines
	Thiazide diuretics

Table 29.4 Infectious disease associated with pancreatitis.

Viruses	Coxsackie B
	Echoviruses
	Hepatitis a, b, and e
	Herpesviruses (cytomegalovirus, Epstein–Barr virus, herpes simplex virus, varicella zoster virus)
	HIV (human immunodeficiency virus)
	Measles
	Mumps
	Rubella (German measles)
Bacteria	*Campylobacter*
	Escherichia coli[1]
	Legionella
	Leptospira
	Mycoplasms
	Salmonella
	Yersinia
Parasites	*Ascaris lumbricoides*
	Clonorchis sinensis
	Cryptosporidium parvum[2]
	Echinococcus granulosus
	Fasciola hepatica
	Toxoplasma gondii[2]

[1] Associated with hemolytic uremic syndrome.
[2] Predominantly found in immune-compromised patients (e.g., AIDS).

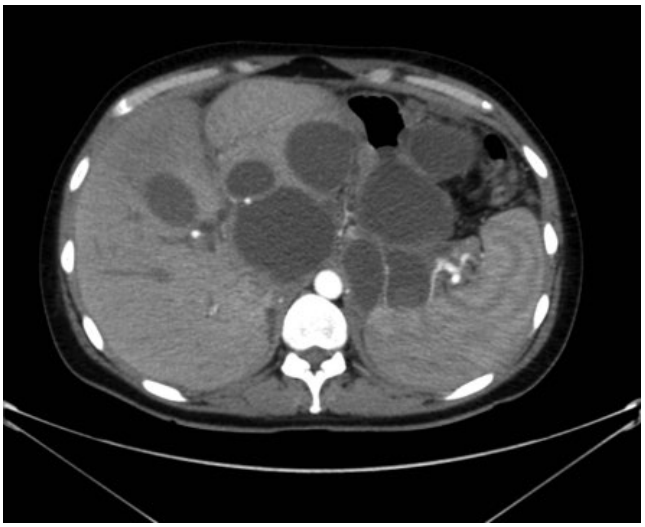

Figure 29.7 CT with pancreatic protocol showing multiple peripancreatic fluid collections following an episode of acute pancreatitis.

have been described in patients with or without a family history. According to the current concept, *PRSS1* alterations lead to an increased autoactivation and in part to degradation of the active enzyme.

Loss-of-function mutations in the serine protease inhibitor Kazal type 1 (*SPINK1*) are common in patients without a family history: 10–40% of patients with "idiopathic" chronic pancreatitis bear one or both alleles of the *SPINK1* p.N34S variant. Beside this alteration, numerous other *SPINK1* mutations have been identified. Genetic variations in the genes encoding chymotrypsinogen C (*CTRC*) and carboxylester lipase (*CEL*) are approximately four times more frequent in "idiopathic" chronic pancreatitis patients compared with controls. Cystic fibrosis (*CFTR*) gene mutations are also enriched in patients with chronic pancreatitis, but most of them are heterozygous carriers and few patients suffer from atypical cystic fibrosis with mutations on both alleles.

Recently, a strong association between mutations in the gene coding for carboxypeptidase A1 (*CPA1*) and pancreatitis have been described. It is proposed that these mutations provoke pancreatitis by induction of endoplasmic reticulum stress. *CPA1* variants are detected in patients with and without a family history, especially in very young patients. Ten percent of patients who develop pancreatitis before the age of 10 years bear a *CPA1* mutation.

Symptoms and complications

Abdominal pain accompanied by nausea, vomiting, and anorexia is the leading symptom of acute pancreatitis. The pain is typically sudden in onset and usually located in the epigastric area or diffusely in the upper abdomen. In childhood, pain rarely radiates to the back or lower quadrants. During an acute attack, abdominal tenderness with decreased bowel sounds are common physical findings. Additional signs include fever, tachycardia, and hypotension. In contrast to adults, chronic pancreatitis in children presents most commonly as recurrent attacks of acute pancreatitis and very rarely as chronic pain. Since recurrent abdominal pain is a frequent finding in children, the diagnosis of pancreatitis is often delayed.

Early complications include multiorgan dysfunction involving the lung and kidney due to overwhelming systemic inflammatory response. In severe cases, systemic reactions such as metabolic acidosis and decompensation (hyperglycemia, hyperkalemia, hypocalcemia), shock, organ failure, and disseminated intravascular coagulation (DIC) may occur.

Extrapancreatic complications such as pleural effusions, ascites, portal hypertension due to portal or splenic vein thrombosis, ulcer with gastrointestinal bleeding, or biliary obstruction may develop. Treatment includes the necessary organ support and control of sepsis in an intensive care unit.

Late-onset complications are mainly fluid collections, pancreatic necrosis, pancreatic ascites, and pseudocyst formation (Figure 29.7). Pancreatic pseudocyst is the most common cystic lesion and accounts for up to 75% of pancreatic cysts seen in children. Rarely, pseudocysts may rupture, become infected, or may cause local mechanical effects such as biliary or duodenal obstruction by compression of adjacent structures.

During the course of the disease some patients with chronic pancreatitis develop pancreatic insufficiency with bulky and greasy stools, weight loss, and insulin-dependent diabetes mellitus. However, steatorrhea develops only after a reduction of exocrine function to less than 10% of the normal level. Other complications are calcifications and necroses with the risk of subsequent pancreatic infection. The

development of pancreatic cancer is a late complication of chronic pancreatitis and the risk in hereditary pancreatitis is increased to 50–60 times compared with the general population.

Diagnosis

The diagnosis of acute pancreatitis is made through a combination of clinical symptoms, determination of serum lipase, and abdominal sonography or other imaging procedures. An extended work-up including genetic and further imaging diagnostics should be performed if there are recurrent attacks of acute pancreatitis or chronic pancreatitis

Laboratory parameters

- Serum lipase is a sensitive marker and pancreatitis is likely if there is greater than three-fold increase above the upper reference value. Serum lipase rises within a few hours after onset of the disease and shows a superior sensitivity and specificity compared with serum amylase, especially some days after onset.
- Serum amylase may be elevated in acute pancreatitis but is not always diagnostic, especially if measured late after the onset of symptoms. Urine amylase has a low informative value and should not be determined. The measurement of isoenzymes (pancreatic amylase) rarely adds additional information

There is poor correlation between the severity of the attack and the rise in pancreatic enzyme values. The serum concentrations of pancreatic enzymes are not influenced by meals. Increased serum enzyme values are also found after ERCP and abdominal processes affecting the pancreas such as ileus, cholecystitis, or cholelithiasis. Liver diseases, renal insufficiency, and viral or bacterial infections might also cause elevated enzyme values. Very low serum levels might be observed in cystic fibrosis or chronic pancreatitis; however, these findings are diagnostically irrelevant.

Serum markers such as pancreatic elastase, trypsinogen, trypsinogen activation peptide, or pancreatitis associated protein currently have no role in clinical practice.

- During an acute attack, serum lipase, blood cell count (hematocrit), C-reactive protein (CRP), blood gases, calcium, phosphate, glucose, urea, creatinine, and liver enzymes should be closely monitored in the first days.
- A high CRP value of >12 mg/dL and a high initial hematocrit suggest necrotizing pancreatitis and blood cultures should be performed.
- Sweat chloride measurement to exclude cystic fibrosis is mandatory.
- Ultrasound monitoring and, depending on the clinical course, MRI or CT monitoring should be performed.

Imaging

Abdominal ultrasound is the investigation of choice and may demonstrate an enlarged and echogenic organ. In addition, alterations of the pancreatic or biliary duct system, calcifications, pseudocysts, and low reflection formations at the rim of the organ suggesting fat necroses might be observed.

If the gland cannot be easily seen (e.g., due to overlying luminal air) or if necrotizing pancreatitis is suspected, MRI or contrast-enhanced CT should be performed. Because of the exposure to radiation, MRI is preferred, but CT is required to detect areas of non-perfusion of the pancreas and pancreatic abscesses. Ultrasound, MRI, or CT monitoring should be performed depending on the clinical course.

Investigation of underlying etiology

A careful medical history is crucial for further diagnosis. A family history of pancreatitis should be sought though an interview with both parents who should be asked about recurrent abdominal pain, pancreatic cancer, and diabetes mellitus. The history also includes medication, preceding abdominal trauma, and symptoms of other underlying diseases such as collagenoses or inflammatory bowel diseases.

Basic diagnostic tests are:

- Serum calcium and triglycerides to exclude metabolic causes.
- Pilocarpine iontophoresis to exclude cystic fibrosis.
- Viral, bacterial, and parasitic infections such as mumps or ascariasis.
- Genetic testing for mutations in the genes encoding *PRSS1*, *SPINK1*, *CTRC*, *CPA1*, and *CEL* should be performed in patients with a family history as well as in chronic pancreatitis patients without a family history (so-called idiopathic chronic pancreatitis) after exclusion of other underlying causes.
- In patients with idiopathic chronic pancreatitis, one might also consider *CFTR* analysis. However, atypical *CFTR* variants are often found in patients with chronic pancreatitis, which are only partially included in standard genetic cystic fibrosis tests.
- MRCP or ERCP should be performed to diagnose anatomical anomalies. If no simultaneous therapeutic intervention is intended, MRCP should be preferred.

Management

Treatment of acute pancreatitis is mainly symptomatic and directed toward pain and intensive care support in critically ill patients with necrotizing pancreatitis. Interventional procedures may be required in selected patients with complications of pancreatitis – pseudocyst, necrotizing pancreatitis, or pancreatic abscess.

Therapeutic strategies for chronic pancreatitis include pain relief, correction of exocrine and endocrine insufficiency, nutritional support, and rarely endoscopic or surgical intervention. Most children experience a mild attack and make an uneventful recovery.

Severity scoring systems may identify high-risk patients. The Ranson criteria, the Glasgow and modified Glasgow scores, or the Acute Physiology and Chronic Health Evaluation (APACHE) II score systems are validated, and widely applied in acute pancreatitis in adults. They are not validated in children and are not applicable for use. DeBanto *et al*. developed a pediatric acute pancreatitis severity (PAPS) score and reported a sensitivity of 70% and a negative predictive value of 91% in the pediatric population. However, further studies failed to reproduce these results and currently scoring systems have limited ability to predict disease severity in children and adolescents with acute pancreatitis.

Principles of treatment

- Adequate fluid resuscitation with correction of electrolyte abnormalities is important. Over or under fluid resuscitation are associated with worse outcomes and the clinical end points should be normalization of clinical (e.g., heart rate, urine output) and laboratory (e.g., urea, creatinine, hematocrit) markers.
- In the case of vomiting, short-time abstinence from food is advisable. Data about the optimal time point for oral refeeding is limited. A normalization of laboratory markers such as serum lipase is not necessary for reintroduction of oral food intake. Nutrition plays a crucial role in the recovery and early enteral nutrition reduces septic complications. Occasionally, jejunal tube feeding is effective. In severe and prolonged courses, parenteral nutrition and critical care monitoring might be required.
- Prophylactic administration of antibiotics for 2–4 weeks, is required, especially if pancreatic necrosis is present. Preferred drugs include imipenem or cefuroxime with metronidazole.
- Trials on secretion blockade (somatostatin, octreotide) or enzyme inhibitors (gabexate) failed to show any effect.
- Treatment of pain should be started with conventional analgesics such as paracetamol or metamizole. If pain relief is not achieved, additional prescription of opiates may be necessary.
- Other, but unproven, strategies for pain relief in chronic pancreatitis include inhibition of pancreatic enzyme secretion using pancreatic enzyme therapy and the use of antioxidants.
- Invasive approaches such as celiac plexus block, endoscopic procedures, and surgical drainage and resection have also been used as therapy for the pain of chronic pancreatitis, but none of these procedures has ever been the subject of controlled trials either in comparison with medical therapy or with no therapy.
- If a precipitating factor such as hypercalcemia, drugs, or obstruction can be identified, specific medical or surgical intervention is required.
- Other treatment modalities are minimally invasive or percutaneous drainage procedures and rarely open necrosectomy.

Nutrition

There is no such thing as a specific "pancreatic diet." In chronic pancreatitis, the intake of smaller but more frequent meals might be indicated. A restriction of fat intake is not necessary if the pancreatic exocrine insufficiency is compensated by enzyme replacement therapy. The restriction of dietary fat and the administration of medium-chain triglycerides (MCTs) is indicated only in cases of severe malabsorption refractory to treatment, since MCTs may worsen diarrhea in many patients. Deficiencies of fat-soluble vitamins are rarely found and should be supplemented in these specific cases.

If patients with chronic pancreatitis develop exocrine pancreatic insufficiency, enzyme replacement therapy should be initiated. In theory, pancreatic enzymes are indicated in patients with steatorrhea (fecal fat >5 g/day) and weight loss. In clinical practice, however, measurement of fecal fat is rarely performed so that the decision for enzyme replacement is based on an assessment of the patient's clinical state. The enzymes should be taken with meals in acid-protected (enteric-coated) formulations. The enzyme pellets should not be dissolved, chewed, or mixed with the food. Approximately 500–2500 IU lipase/kg body weight for main meals, and approximately half that for snacks, are recommended, but a higher dose or combination with a proton pump inhibitor may be required.

Diabetes in chronic pancreatitis is classified as type IIIc. However, the treatment is not different from that for patients with type I diabetes. Due to the coexisting deficiency of glucagon, patients with chronic pancreatitis have an increased risk of hypoglycemic events. This is a particular problem in patients with poor compliance. Acarbose and insulin sensitizers are ineffective.

Pancreatic pseudocysts

The management strategies for pancreatic pseudocysts in children are not well defined. There are two strategies – the Warshaw approach of intervention in pseudocysts >6 cm and the Vitas and Sarr approach of no intervention irrespective of size unless symptomatic – which are applied in the clinical management of adults. It is not known if these could be translated into pediatric clinical practice. The majority of pancreatic pseudocysts regress with a conservative approach, while drainage is indicated in the presence of symptoms or complications.

The experience with pediatric EUS is increasing and several studies supported the role of EUS as a primary diagnostic and intervention tool. The procedure is minimally invasive and should be offered as initial treatment for the drainage of pancreatic pseudocysts. Laparoscopic or open

surgical drainage is used for patients not suitable for EUS or failed endoscopic treatment. A percutaneous drainage should be avoided due the associated risk of pancreatic fistulae.

Interventional endoscopy

There is a longstanding controversy concerning the indication for interventional endoscopic therapy of complications such as duct stones, pancreatic or biliary tract stenosis, or pseudocysts even in adult patients. Due to the lack of randomized controlled studies, therapeutic decisions are made on the basis of technical skills available rather than scientific evidence. Many centers perform interventional therapy only in symptomatic patients with recurrent pain or acute attacks, associated with ductal dilation proximal to the stenosis or an obstructive stone. Interventional therapy is obligatory in patients with cyst-associated pain, gastric compression, or biliary obstruction when alternatives are absent, e.g., when surgery is refused by the patient or surgeon.

Surgery

Surgical therapy is reserved for patients who fail to respond to medical or endoscopic therapy, who have infected pancreatic necroses, common bile duct or duodenal obstruction, or a suspected pancreatic cancer. Like endoscopic therapy, surgical procedures for pain in chronic pancreatitis have never been subjected to randomized controlled trials comparing them with medical therapy or no therapy. In patients with pain and an inflammatory tumor of the head of the pancreas, a duodenum-preserving resection of the head (Frey, Beger, or Berne) is the method of choice in many centers. As an alternative procedure, a longitudinal pancreaticojejunostomy (Peustow's operation) may be considered if the main pancreatic duct is dilated to 7 mm or more.

Total pancreatectomy should, if at all, only be considered in combination with islet cell transplantation for patients with severe intractable pain or as a prophylactic procedure for pancreatic cancer in patients with hereditary pancreatitis or familial pancreatic cancer. Data for the pediatric age group are very limited.

Prevention

No medical relapse prevention exists and therapy with antioxidants (e.g., selenium) do not have sufficient scientific proof of their effectiveness.

Inherited pancreatic disorders of the exocrine pancreas

Cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive inherited, multisystemic disorder with variable phenotypical expression. It is characterized by chronic obstructive pulmonary disease with proximal bronchiectasis often resulting in lung failure, by exocrine pancreatic insufficiency with maldigestion and failure to thrive, and by elevated sweat chloride concentrations. Other clinical features include meconium ileus, liver fibrosis, gallstones, and male infertility due to obstructive azoospermia. In older age, patients show an increased incidence of Barrett esophagus and gastrointestinal tumors. For a detailed description of the disease see Chapter 16.

CF is caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene. With a frequency of approximately 1 : 2500 it is the most common cause of exocrine pancreatic insufficiency (EPI) in children, which is seen in 85–90% of CF patients. The pathological process starts in utero and continues into early childhood. Obstruction of the small ductules by mucoid secretions and inflammatory response results in loss of exocrine tissue and replacement by connective tissue. Pancreatic disease in CF can be divided into exocrine insufficiency, pancreatitis, and diabetes.

Fat malabsorption with steatorrhea are presenting symptoms of EPI. Exocrine function should be monitored in patients with pancreatic sufficiency every 1–2 years or in the case of weight problems. The treatment goal for pancreatic insufficiency is to correct the malnutrition with adequate enzyme replacement and thereby sustain normal growth and development. The dosage is based on body weight and fat intake, and patients are started on a low dose and up-titrated based on the clinical response. The recommended dose for children is 1000–2500 units lipase/g dietary fat with an upper limit of 10,000 units/kg body weight per day or 1000 units lipase/kg for each meal for patients under 4 years, and for those over 4 years 500 units lipase/kg per meal, respectively. Prophylactic supplementation of fat-soluble vitamins A, D, E, and K is recommended along with regular monitoring of levels.

Approximately 1–2% of all patients with CF and about 15% of CF patients without exocrine insufficiency suffer from recurrent attacks of pancreatitis. Ductal plugging due to inspissated secretions and/or increased intrapancreatic autoactivation of digestive enzymes are hypothesized as underlying mechanisms.

Diabetes in CF is due to insulin deficiency caused by islet destruction and usually develops between the 10th and 30th year of life. The incidence is rising due to improved therapy of CF, leading to increasing life expectancy. An oral glucose tolerance test should be performed from the 10th year of life on or in the case of clinical signs suggesting diabetes. An early and aggressive insulin replacement minimizes the systemic complications of diabetes and improves survival.

Shwachman–Diamond syndrome

Shwachman–Diamond syndrome (SDS) is an autosomal recessive inherited disorder characterized by a triad of exocrine pancreatic insufficiency with hypoplastic acinar

cells with fatty replacement (lipomatosis), bone marrow dysfunction, and skeletal abnormalities. SDS is caused by gene conversion mutations in the *SBDS* gene. There is no apparent genotype–phenotype correlation. The function of the gene product is incompletely understood; experimental data suggest a role in the maturation of ribosomes, in stabilization of mitotic spindles, in pH regulation of vacuoles, and in DNA metabolism.

The islet cells and much of the ductal architecture remain preserved. It is the second most common cause of exocrine pancreatic insufficiency in children, with an estimated incidence of about 1 : 100,000.

The main hematological features of SDS are intermittent or permanent neutropenia, anemia, thrombocytopenia, or pancytopenia. Infants are at risk of severe bacterial infections. More than 10% of patients develop myelodysplastic syndrome or acute myeloid or lymphatic leukemia. Other clinical features of SDS are skeletal or dental anomalies (metaphyseal chondrodysplasia, short ribs, pathological fractures, delayed second dentition, increased tooth decay), short stature, and mild mental retardation. Some patients develop hepatomegaly and elevated liver enzymes (see Chapter 22).

The diagnosis is made by the clinical presentation and respective laboratory parameters:

- Exocrine pancreatic insufficiency (fecal elastase).
- Blood cell counts (repeated measurements because cytopenia might be cyclic); these should be confirmed by genetic analysis (gold standard).
- Skeletal X-ray.
- Abdominal ultrasound or MRI (diffuse fatty infiltration of the pancreas).

Differential diagnoses includes cystic fibrosis, other causes of neutropenia such Diamond–Blackfan anemia or Fanconi anemia, and congenital dyskeratosis.

Therapy is symptomatic and consists of substitution of pancreatic enzymes and antibiotics for infections. For neutropenic patients with recurrent or severe infections, granulocyte colony-stimulating factor (G-CSF) or bone marrow transplantation may be considered [16]. Exocrine dysfunction improves with age and 50% of patients do not require enzyme replacement therapy from their second decade of life.

Johanson–Blizzard syndrome

Congenital EPI with severe failure to thrive in conjunction with hypoplasia or aplasia of the nasal wings are the hallmarks of this autosomal recessive inherited syndrome. The incidence is around 1 : 250,00 live births. The syndrome is caused by mutations in the *UBR1* gene encoding an E3 ubiquitin ligase which is part of the proteasome system and involved in the degradation of intracellular proteins.

Other symptoms (listed in decreasing frequency) with varying manifestations include oligodontia of the permanent teeth, sensineural hearing loss, congenital scalp defects, growth and psychomotor retardation, hypothyroidism, heart defects, anal atresia, and renal and urogenital malformations [17]. Young adults might develop diabetes mellitus.

Diagnosis is made by the typical constellation of symptoms and can be confirmed by *UBR1* sequencing. Differential diagnoses are other forms of congenital EPI such as cystic fibrosis or SDS.

Therapy is symptomatic and consists of pancreatic enzyme supplementation and, when indicated, substitution of thyroid hormones, supply of hearing devices, and surgical correction of malformations.

Prognosis depends on the associated malformations. Lethal malformations such as bilateral kidney dysplasia are rare. Mental retardation is variable, but approximately one-third of affected subjects show normal intelligence.

Pearson syndrome

Mutations of mitochondrial DNA lead to the extremely rare and fatal Pearson marrow–pancreas syndrome. Onset is usually in early infancy. Key characteristics are macrocytic anemia with ringed sideroblasts and vacuolization of progenitor cells in the bone marrow and exocrine pancreatic dysfunction with acinar atrophy, fibrosis of the pancreatic parenchyma, and failure to thrive.

In contrast to SDS, there is no pancreatic lipomatosis. Other features of Pearson syndrome are lactate acidosis, renal tubulopathy, liver failure, diarrhea, endocrinological dysfunction, and splenic atrophy. Some patients develop ocular and muscular symptoms as seen in the Kearns–Sayre syndrome, which is also caused by deletions of mitochondrial DNA. In laboratory examinations, complex organic aciduria with 3-methylglutaconic aciduria is noted. Therapy is symptomatically and in selected cases bone marrow transplantation can be considered. The syndrome has a poor prognosis and patients usually die in early childhood due to sepsis or liver failure.

Congenital enzyme defects

Inherited defects of pancreatic enzymes or duodenal enteropeptidase (enterokinase) are very rare. So far, isolated deficiencies of lipase, co-lipase, amylase, trypsin, and enteropeptidase as well as combined deficiencies of lipase and co-lipase have been reported. Of clinical relevance are mainly deficiencies of trypsin and enteropeptidase, which manifest with diarrhea, hypoproteinemia, and edema. Therapy consists of enzyme substitution.

Systemic congenital anomalies affecting the pancreas

Beckwith–Wiedemann syndrome (BWS) is a congenital disorder associated with overgrowth. It is characterized by the classic triad of omphalocele, macroglossia, and gigantism. Visceromegaly can affect the pancreas, resulting in massive

enlargement and cystic dysplasia. Patients with BWS are at a greater risk for development of abdominal malignancies such as pancreatoblastoma [18].

Von Hippel–Lindau disease (VHL) is an autosomal dominant inherited disorder characterized by retinal angiomas and by hemangioblastomas of the central nervous system. Pancreatic lesions in VHL include multiple cysts, serous cystadenoma, islet cell tumors, and ampullary and pancreatic carcinoma. Pancreatic cysts are common in VHL; they can be single or multiple, virtually replacing the pancreas.

Pancreatic trauma

The most common mechanisms of accidental injuries are road traffic accidents or bicycle handlebar injuries. Pancreatic injury accounts for 2% of all abdominal trauma in children, which are mostly due to blunt injuries. Pancreatic ductal injuries are even rarer, with a reported incidence of approximately 0.12–0.4%.

Diagnosis is often delayed due to a lack of reliable clinical signs and poor sensitivity of imaging modalities used during the initial assessment. Clinical symptoms are often non-specific abdominal pain, vomiting, or fever and should raise suspicion in the setting of a blunt abdominal injury. Serum lipase or amylase levels are diagnostic in 70% of cases [19]. The role of ultrasound is limited and CT is the investigation of choice in the trauma setting. A negative CT at initial assessment does not exclude pancreatic injury and follow-up CT is indicated if there is a clinical suspicion. ERCP is a valuable tool in evaluating patients with equivocal CT findings.

The grading of pancreatic injuries according to the American Association for the Surgery of Trauma is shown in Table 29.5.

Management

Management depends on the location and severity of the injury, the presence of other injuries, and the expertise of the surgical and interventional teams. The majority of blunt injuries are minor (grade 1 and 2) and can be successfully managed by a conservative approach. Patients with ductal injuries need aggressive management, and the absence of ductal injury is the single most important predictor of success with conservative management.

The optimal management of trauma with ductal injuries is controversial. Endoscopically placed stents have been occasionally used for the definitive management of high-grade isolated injuries to the pancreas. Operative management is indicated in patients with peritonitis, hemodynamic instability, or evidence of pancreatic duct disruption on CT [19].

A spleen-preserving distal pancreatectomy may be considered for distal injuries, especially in systemically unwell patients early within the first few days. Grade 4 and 5 injuries are complex and are associated with high mortality. Surgical intervention in the form of an anterior Roux-en-Y pancreatico-jejunostomy or pancreaticoduodenectomy is sometimes indicated. The treatment strategy should be individualized using approaches and techniques that are feasible in individual units.

Late complications include pancreatic fistulae, pancreatitis and pancreatic pseudocysts, or pancreatic ascites. Treatment options include ERCP placed stents and surgical or endoscopic drainage of cysts that present several weeks after injury.

Table 29.5 The grading of pancreatic injuries.

Grade	Injury
1	Minor contusion without duct injury or superficial laceration
2	Major contusion or laceration without duct injury or tissue loss
3	Distal transection or parenchymal injury with duct injury
4	Proximal transection or parenchymal injury involving the ampulla
5	Massive disruption of the pancreatic head

Pancreatic tumors

Malignant tumors of the pancreas are exceedingly rare. According to the SEER (Surveillance, Epidemiology, and End Results) program data, the estimated age-adjusted annual incidence of pancreatic malignancy is 0.191 per million pediatric population. There are no standardized treatment protocols due to limited experience, and much of the available information in the literature is anecdotal. The tumors have a wide histological spectrum and in general tend to have a better prognosis than in adults [20].

Tumors are classified into epithelial and non-epithelial (Table 29.6). Epithelial tumors are further divided into exocrine or endocrine tumors.

Table 29.6 Pancreatic neoplasms.

Epithelial		
Exocrine	**Endocrine**	**Non-epithelial**
Ductal cell origin: Ductal adenocarcinoma Acinar cell origin: Pancreatoblastoma Acinar cell carcinoma Uncertain origin: Solid pseudopapillary tumor	Functioning Non-functioning	Sarcoma Rhabdomyosarcoma Burkitt lymphoma Hemangioendothelioma

Exocrine tumors

Pancreaticoblastoma

Pancreaticoblastoma or infantile pancreatic cancer is a malignant embryonal tumor that is commonly seen in young children. Horie *et al.* used the term pancreaticoblastoma to describe infantile carcinoma of the pancreas in 1977 and, so far, only 75 cases have been reported [21].

Clinical features

It is most commonly seen during the first decade of life with a mean age of 4.5 years and a male predominance. Congenital cases have been described in association with Beckwith–Wiedemann syndrome and familial adenomatous polyposis. Most children become symptomatic with large abdominal mass, abdominal pain, fatigue, anorexia, and weight loss. Jaundice or gastric outlet obstruction are uncommon features.

Diagnosis

Alpha-fetoprotein (AFP) is a useful tumor marker in pancreaticoblastoma; elevated levels have been reported in up 68% of cases. Ultrasound, CT, or MRI will achieve a diagnosis. The tumor, node, metastasis (TNM) classification is usually applied for staging.

Management

Radical surgical resection is the best treatment modality. The prognosis is better in children and a 50% 5-year survival has been reported in large series [22]. The tumors are generally large and localized to the head of the pancreas. They can compress the adjacent vascular structures but invasion into the superior mesenteric artery or vein is rare. Metastases to the liver and abdominal lymph nodes are found in 35% at presentation.

Complete surgical resection in the form of a pancreaticoduodenectomy or duodenum-sparing pancreatectomy is offered to patients with no evidence of metastatic disease or locally advanced disease. Chemotherapy and radiotherapy have a role in recurrent, residual, unresectable, and metastatic disease. Several chemotherapy agents have been reported in the literature, however the TERP project (Tumori Rari in Eta` Pediatrica (Rare Tumors in Pediatric Age)) guideline recommends the PLADO (cisplatin and doxorubicin) chemotherapy regimen used for hepatoblastoma.

A clinical follow-up with measurement of serum AFP levels is recommended to identify recurrence. Prolonged survival is reported in patients presenting recurrent disease in the liver.

Solid pseudopapillary tumor

This is a unique tumor with a low malignant potential. They are commonly misdiagnosed as non-functioning islet cell tumors, adenocarcinomas, cystadenomas, or pseudocysts.

Clinical features

These tumors are commonly seen in adolescent girls and young women; 22–53% of patients diagnosed with such tumors are in the pediatric age group. Presenting symptoms are non-specific, with abdominal discomfort or pain and a mass. The tumors are usually solitary and have a predilection to the tail of pancreas. Ultrasound, CT, or MRI can demonstrate a characteristic, well-circumscribed, capsulated lesion with variable solid and cystic components (Figure 29.8). EUS-guided fine needle aspiration biopsy can be used in patients with equivocal imaging findings.

Management

Solid pseudopapillary tumors are usually slow growing with a potential for aggressive behavior. A complete cure can be achieved by surgical resection in 95% of patients with such tumors localized to the pancreas. Enucleation or pancreas-preserving surgery should be offered depending on the location of the tumor. Metastasis is rare in children, most commonly found in the liver or peritoneum, and is not a contraindication for surgery. Prolonged survival can be achieved following surgical resection of metastases or surgical debulking of large inoperable tumors. Adjuvant treatments are rarely needed [23].

Acinar cell tumor

Acinar cell tumors represent 1–2% of pancreatic exocrine tumors and are, in contrast to adults, much more common than ductal adenocarcinomas. The most common clinical presentation is abdominal pain. The tumors are often large and have no preferential location within the gland. CT or MRI are the imaging modalities for diagnosis and tumor staging. They are pathologically similar to pancreatoblastomas and a histological differentiation is often difficult. Surgery is the treatment

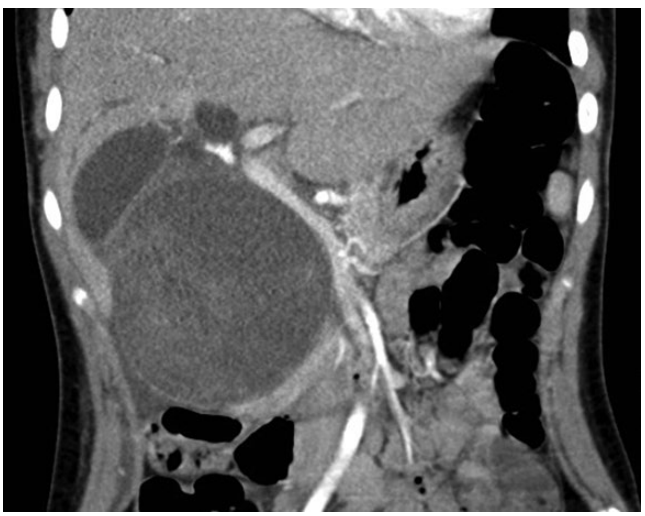

Figure 29.8 Contrast CT (coronal section) showing a solid pseudopapillary tumor lesion in the head of the pancreas.

of choice; the prognosis is slightly better compared with ductal adenocarcinoma. A median overall survival of 36 months is reported.

Ductal adenocarcinoma

In adults, this entity accounts for 80–95% of pancreatic cancers, but is uncommon under the age of 40 and convincing cases in children are extraordinarily rare. The occurrence in young patients usually is associated with special circumstances or familial predisposition. Genetic conditions associated with increased risk of pancreatic carcinoma include hereditary pancreatitis, hereditary pancreatic cancer syndrome, hereditary non-polyposis colon carcinoma, Peutz–Jeghers syndrome, familial atypical multiple mole melanoma, and *BRCA2* gene mutations.

The clinical presentation includes vague abdominal pain or jaundice. CT, MRI, or EUS are useful for diagnosis and staging. Surgical resection is the treatment of choice in the absence of metastatic disease. The disease is associated with poor prognosis – a 15–20% 5-year survival can be achieved with radical resection [24].

Endocrine tumors

Endocrine neoplams of the pancreas are slow-growing tumors with a malignant potential. They are commonly seen during the fourth to sixth decades of life, but occasionally may be encountered in older children. A study from the Mayo Clinic reported insulinoma in 4.9% of patients in the pediatric age group over a 60-year period [25]. Endocrine tumors are further divided into functioning and non-functioning.

The most common functioning tumor is insulinoma (47% of functioning islet cell tumors), followed by gastrinoma (30%). Other types are exceedingly rare or have never been reported in children. The presentation varies according to the type of hormone produced by the tumor.

Clinical symptoms

Insulinoma is the tumor of β-cells and causes fasting hyperinsulinemic hypoglycemia. In children, hypoglycemia can be manifested as behavioral problems, seizures, or coma. The Whipple triad of fasting hypoglycemia, symptoms of hypoglycemia, and immediate resolution of symptoms with the administration of glucose is a common manifestation. Recurrent episodes of untreated hypoglycemia could result in impaired neurological development.

Gastrinomas are composed of G cells and cause the Zollinger–Ellison syndrome. Gastroesophageal reflux or diarrhea is a common symptom and patients frequently have multiple or recurrent peptic ulcers in uncommon locations.

Adrenal corticotropic hormone-secreting islet cell tumor (ACTHoma) is a rare cause of Cushing syndrome. VIPoma is composed of D1 cells, secretes vasoactive intestinal peptide (VIP), and causes diarrhea, hypokalemia, and achlorhydria. VIP-producing tumors in children usually do not originate from the pancreas. Patients with multiple pancreatic neuroendocrine tumors should be evaluated for a possible multiple endocrine neoplasia type 1 syndrome.

Insulinomas usually involve the body and tail of the pancreas (65%), while gastrinomas have a predilection for the head (71%).

Diagnosis

A low fasting serum glucose level in conjunction with a high serum insulin level and an elevated serum C-peptide level is diagnostic. A 72 h fasting study has a sensitivity of 98% and may be performed if there is a clinical uncertainty.

Serum gastrin levels are often markedly elevated in patients with gastrinoma. The secretin stimulation test is indicated in patients with normal or mildly elevated serum gastrin levels. CT and MRI can localize tumors in 85–100% of cases. EUS or operative exploration with intraoperative ultrsound is indicated in some cases (Figure 29.9). Somatostatin receptor scintigraphy is particularly useful to localize intra- and extrapancreatic gastrinomas or their metastases.

Management

Functioning endocrine tumors of the pancreas have low malignant potential and often have an indolent course. The common site of metastases is the liver, however long-term survival can be achieved even in patients with metastatic disease. Surgery is the treatment of choice and a partial pancreatectomy or enucleation is performed depending on the tumor characteristics.

Poor prognostic factors for well-differentiated tumors include a size >2–4 cm, vascular or neural invasion, a high mitotic rate, and a high Ki-67 index. Poorly differentiated endocrine carcinomas (also referred to as high-grade endocrine carcinomas) account for less than 2–3% of all pancreatic endocrine tumors and are usually seen in middle aged or elderly men. Palliative treatment options include

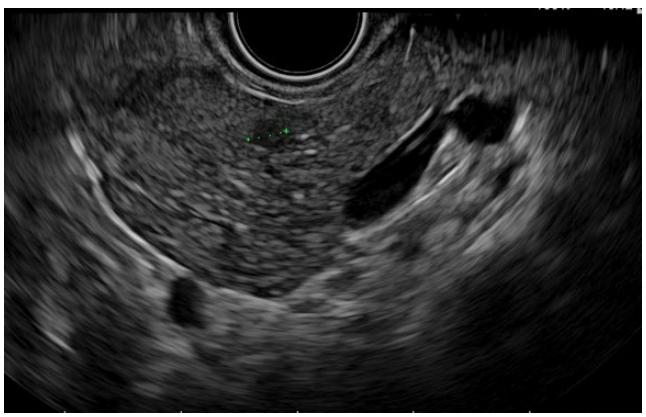

Figure 29.9 Linear EUS showing a 3.4 mm hypoechoic lesion in the midbody of the pancreas. Fine needle aspiration biopsy confirmed a neuroendocrine tumor.

medical therapy, chemotherapy, surgical debulking, radiofrequency ablation, hepatic artery chemoembolization, and radionuclide therapy.

Nesidioblastosis

Nesidioblastosis, also referred to as congenital hyperinsulinism or persistent hyperinsulinemic hypoglycemia of infancy (PPHI), is the most common cause of persistent hypoglycemia in infancy. It is a tumor-like disorder characterized by an extensive, often disorganized, formation of pancreatic islets. The overall incidence is 1 in 30,000–50,000 births, and is much higher in Saudi Arabians and Ashkenazi Jews. The disorder is commonly caused by mutations of subunits of the pancreatic ATP-sensitive potassium channel *KCNJ11* or *ABCC8*, respectively.

Clinical presentation

Symptoms are apparent within a few hours after birth, but can occasionally be delayed for a year. The diagnostic criteria include: (i) fasting and post-prandial hypoglycemia (2.5–3 mmol/L) with unsuppressed insulin secretion (plasma insulin concentrations >1 mU/L); (ii) a positive response with the administration of glucagon; (iii) negative ketone bodies in urine and plasma; and (iv) prolonged dependence on glucose treatment.

Two distinct pathological entities have been identified, with a difference in treatment and prognosis. The diffuse form accounts for two-thirds of cases and is characterized by pathological changes throughout the pancreas, whereas the pathology is limited to a mass lesion in the focal form.

The radiological diagnosis of the diffuse entity is challenging as it causes no imaging alterations. US, CT, and MRI may identify a heterogeneous mass in the focal form. ^{18}F-fluoro-L-dihydroxyphenylalanine positron emission tomography (PET) is emerging as a simple and effective test to differentiate the diffuse and focal forms.

Prognosis and management

Initial treatment is aimed at preventing hypoglycemia with enriched feeds, glucose, and glucagon infusion in severe cases. Specific treatment includes oral diazoxide or octreotide injections in children not responding to diazoxide. Surgery is offered as second-line treatment. The focal form can be cured by a partial pancreatectomy; however a near-total pancreatectomy is needed to treat the diffuse form. Surgery is often complicated by exocrine and endocrine insufficiency and the decision should take into account the expertise of the medical team and the psychological or ancillary support services available.

Other tumor types

Non-Hodgkin lymphoma (NHL) is the most commonly diagnosed non-epithelial childhood pancreatic tumor. Lymphoma can arise primarily in the pancreas or infiltrate as mass lesions originating from the peripancreatic nodes. Large-cell or Burkitt lymphoma are the common subtypes of NHL involving the pancreas. The mass lesions can be either solitary, multiple, or diffusely infiltrating on radiological imaging (MRI/CT/ultrasound). The presence of multiple nodal masses can distinguish lymphoma from other tumors, however an accurate diagnosis is not always possible on imaging. An EUS-guided fine needle aspiration/core biopsy can confirm the diagnosis. Treatment with multiagent chemotherapy is associated with good outcomes. A surgical resection is occasionally offered in some cases where the diagnosis is ambiguous with a suspicion of adenocarcinoma.

Benign and malignant *mesenchymal tumors* are very rare. Rhabdomyosarcoma is the most common malignant mesenchymal tumor of the pancreas in children. Sarcomas are highly malignant and frequently metastasize to the liver. Primitive neuroectodermal tumors most commonly arise in the soft tissue extremities. They are regarded as part of the Ewing sarcoma family of tumors and occasionally can originate from the retroperitoneum invading the pancreas. Secondary involvement of the pancreas by direct tumor invasion is commonly seen with neuroblastoma. Abdominal pain and jaundice are common symptoms. These are aggressive tumors with poor prognosis, and surgery is the treatment of choice

Conclusion

In summary, pediatric pancreatic tumors have a relatively better prognosis compared with those in adults. An understanding of the malignant potential is critical in planning and optimizing the treatment options. Curative surgical resections should be offered when possible; debulking or resection of metastases has a role in selected cases [26, 27].

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SECTION 12

Transplantation

CHAPTER 30

Anesthesia and Intensive Care in Pediatric Liver Disease

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Key points

- Anesthesia has physiological effects on many organ systems, most importantly on the cardiovascular and respiratory systems.
- The effects of anesthesia can interact with the effects of liver disease and co-morbidities.
- Maintaining physiological and biochemical stability close to normal values reduces the risk to the patient.
- Liver transplantation may cause severe derangements of hemodynamic and biochemical parameters.
- Intensive care support is focussed on maintaining organ systems to allow time for recovery.
- Timely intervention in the treatment of sepsis or other organ failure is crucial.
- The complexity of liver disease and interventions on children with liver disease makes multidisciplinary team working essential.

This chapter is not a detailed guide to the anesthetic and intensive care management of a child with liver disease, but an insight into the care of these challenging patients, so that hepatologists and pediatricians understand the specific problems liver patients face that increase the risks of anesthesia, perioperative care, and intensive care. A clear understanding of those risks is essential to calculating a realistic risk/benefit analysis to any proposed intervention, and that understanding can only come from direct experience of patient outcomes, from the reported experience of others, or from insights gleaned from colleagues in other specialties who will evaluate the situation from different perspectives. Only by taking full advantage of this range of resources, including the expertise of the multidisciplinary team in managing complex patients, can the specialist develop appropriate treatment plans and ensure the best outcome for the patient.

In order to understand the risks of anesthesia, it is essential to be familiar with the effect of anesthesia on physiological systems, which is an extensive topic and beyond the remit of this book. The effects of anesthesia vary with the use of different agents or techniques, but we summarize the main effects of commonly used agents and techniques. It is likely that other factors will aggravate or reduce these effects, for example giving intravenous fluids, using positive pressure ventilation, and ensuring active warming of the patient, which may all be part of routine anesthetic management. We will start by outlining the most relevant issues, and then focus on the impact of liver disease in children on the conduct of anesthesia.

We will then explain the process of anesthesia for liver transplantation, as that is arguably the most complex procedure, in the course of which many factors must be considered, and it illustrates a range of interventions which are possible, and important aspects of intraoperative and intensive care management.

Anesthesia in liver disease

Anesthesia is only one part of what is happening to the patient during a procedure or investigation; these procedures have their own effects and risks, and it is not always clear whether the anesthesia or the procedure itself is responsible for a particular outcome. The main purpose of anesthetic care, apart from providing the obvious reversible loss of consciousness and post-procedure pain relief, is to mitigate the dangers of the procedure itself, and therefore anesthesia needs to be

Diseases of the Liver and Biliary System in Children, Fourth Edition. Edited by Deirdre A. Kelly.

Table 30.1 Major effects of anesthesia.

Body system	Anesthetic effect
Cardiovascular	Fall in cardiac output due to negative inotropic effect Vasodilation and fall in systemic vascular resistance Variable change in heart rate Loss of autoregulation of blood flow to organs Possible promotion of right-to-left shunting of blood flow
Respiratory	Depression of respiratory drive Weakening of respiratory muscle strength Fall in tidal volume and minute volume Ventilation/perfusion mismatch Fall in functional residual capacity Airway collapse/atelectasis Loss of ciliary function
Renal	Loss of renal perfusion pressure Fluid shifts and endocrine responses Direct nephrotoxicity
Liver and gastrointestinal tract	Fall in splanchnic blood flow Gastroparesis and ileus
Neurological	Loss of consciousness Prolonged sedation Disturbance of cerebral blood flow Rise in intracranial pressure Direct neurotoxicity
Metabolic/endocrine	Systemic stress response Loss of glucose homeostasis Antidiuresis Fluid and electrolyte shifts
Immunological	Increased susceptibility to infection (multifactorial)
Temperature	Body cooling

carried out with care and expertise. The potential major effects of anesthesia, using the commonest agents and techniques are given in Table 30.1. Given all the risks, it is a tribute to the careful research, serendipitous discoveries, experience, skill, and diligence of generations of anesthetists that anesthesia is as safe as it is today.

Effect of liver disease on body systems relevant to anesthesia

Liver disease has the potential to affect every physiological system, making management both complex and interesting. Although the target of anesthesia is to reversibly obliterate consciousness and to relieve pain, current anesthetic agents and techniques are imprecise, so there may be unintended effects on other systems, and hence the possibility of interaction with the effects of liver disease.

Cardiovascular

The circulation in chronic liver disease is a high-output, low-resistance state. In acute liver failure this is even more pronounced. The circulation is often volume loaded, so echocardiography will often show atrial dilation. To some extent this state protects the patient from the effects of anesthesia; some loss of output in a high-output state still leaves plenty of output, and if the system is maximally dilated, this will reduce the tendency of anesthesia to cause additional vasodilation. The disadvantage is that it may leave the patient vulnerable to the effects of volume depletion, as the ability to compensate for intravascular loss by vasoconstriction is impaired, and the maintenance of perfusion then depends on an elevated heart rate. So it is of particular importance to avoid hypovolemia, which can arise from bleeding, dehydration, or diuretic therapy.

It is not always obvious to non-anesthetists that volume loading does not necessarily indicate cardiac failure.

The presence of congenital cardiac anomalies complicates management. There are many different anomalies, and those that may impair the patient's ability to provide a high cardiac output can lead to serious deleterious effects. If there is a right-to-left connection in the circulation there is a risk that during liver transplantation or major surgery when large blood loss is a possibility, that the effects of large volumes of fluid or inotropic support can convert a balanced or left-to-right shunt into a right-to-left shunt with concomitant hypoxia. If there is pulmonary hypertension, circulatory failure may develop which could fail to respond to usual measures.

The objectives and interpretation of cardiovascular assessment before possible liver transplantation or major hepatobiliary surgery is essential and clarity is required so that the objectives of both anesthetists and their colleagues are understood.

The expected cardiovascular effect of reperfusing a liver graft is that the cardiac output will increase (often by around 50% over the baseline value, which in liver disease is often higher than normal), the mean arterial pressure will drop because of a fall in systemic vascular resistance, and the pulmonary vascular resistance will rise: the "post-reperfusion syndrome" [1]. If the patient cannot achieve an appropriate increase in cardiac output or tolerate the other effects, then there is a serious risk that the graft will not perfuse well or that there will be circulatory failure or insufficiency, which might lead to a poor outcome, including death. So it is important to try to ascertain whether the patient has sufficient cardiac reserve to survive the potential stresses and cardiovascular changes that might be expected during the proposed procedure. Preoperative optimization of the patient's condition may require interventional cardiology or cardiac surgery procedures even if the patient is hemodynamically stable. Cardiac reserve as it applies to the complicated intraoperative situation may be difficult to assess meaningfully, but can to some extent be predicted by looking at resting values of heart rate, contractility, ejection fraction, and all those parameters that allow a cardiologist to evaluate ventricular function. A stress test with inotropic agents may add more information, but is itself rather complex and has its own risks.

Respiratory

The presence of ascites and organomegaly causes diaphragmatic splinting. A child with significant ascites who is coping well on the ward may not be able to cope under anesthesia. Hypoxia can occur alarmingly quickly and it may not be possible to restore spontaneous respiration afterwards. Pleural effusions may also be a factor in causing respiratory difficulties under anesthesia.

Hepatopulmonary syndrome, which is an indication for transplantation, causes hypoxia as the result of a true right-to-left shunt through the lungs, and a degree of ventilation/perfusion mismatch. Investigations will define the degree of true shunt, but what is important to the anesthetist is the extent to which the hypoxia corrects with a high inspired oxygen fraction – a true shunt cannot be corrected in this way, but in many cases it is possible to achieve reasonable oxygenation in this simple manner, so that it is possible to support the patient through a liver transplant operation, which is the definitive treatment for the condition. Weaning from mechanical ventilation postoperatively may not be so straightforward, and prolonged support may be required.

Renal

Renal impairment is a fairly common feature in liver disease. The main concerns for the anesthetist are the potential for:

- Electrolyte and acid/base disturbance, which can be dangerous during anesthesia.
- The inability to deal with a fluid load.
- Drug handling with both renal and hepatic impairment.
- The impairment of platelet function.

Until the renal impairment becomes severe enough to require renal support, anesthesia is usually relatively straightforward. Derangements in serum potassium, which have the potential to cause dysrhythmias under anesthesia, are the most commonly encountered problem. Acute correction of serum potassium may cause rapid shifts in serum potassium and cause imbalances between intracellular and extracellular levels, with unpredictable effects on excitable membranes.

Both hyper- and hyponatremia are potentially hazardous and careful correction is required. Central pontine myelinolysis is a well-known consequence of rapid sodium rise, but is quite rare in children [2]. Fluctuations in sodium have the potential to cause rapid fluid shifts between compartments and this may be a major problem, especially in conditions where fluid stability is important, for example where there is raised intracranial pressure.

Calcium, magnesium, and phosphorus also have important effects:

- Hypocalcemia can cause neuronal instability leading to paresthesia, muscle spasms, tetany, and seizures. It can contribute to heart failure, hypotension, insensitivity to adrenergic agonists, and a prolonged QT interval. There may also be platelet dysfunction and coagulopathy.
- Hypomagnesemia can cause membrane instability, prolonged PR and QT intervals, and is prone to cause ventricular arrhythmias. There may be weakness, confusion, ataxia, and seizures, and it can aggravate hypocalcemia.
- Hypophosphatemia may cause respiratory muscle dysfunction and respiratory failure, myocardial dysfunction with decreased contractility, arrhythmia and acute failure, and paresthesia, encephalopathy, seizures, platelet dysfunction, and hemolysis.

The anesthetist therefore needs to be informed of derangements in these ions, and efforts should be made to keep levels within safe limits.

It is important to maintain a relatively normal acid–base balance to preserve the functioning of vital systems. The anesthetist is able to manipulate this system to some extent by using ventilation to control CO_2 levels, and by instituting renal support.

The role of renal function in volume regulation during anesthesia is less than might be expected, as normal homeostatic mechanisms are too slow to have much impact in the rapidly changing situation during surgery. In major procedures in sick patients, volume status will be managed with central venous pressure monitoring and judicious fluid or blood product administration to ensure the most favorable conditions, which for liver patients generally means "full," to ensure the ideal end-diastolic volume. It is important not to overload the patient with fluid, and an experienced anesthetist will take care to balance these requirements.

The hepatologist can assist in preparing the patient for anesthesia by maintaining the patient in as stable a condition as possible, with volume electrolyte and acid/base values as near normal as can reasonably be achieved. Problems sometimes arise with a patient on intermittent hemodialysis. Patients tend to come off dialysis somewhat dehydrated and may have experienced rapid changes in electrolytes, so it is best to allow sufficient time for the patient to stabilize before anesthesia or surgery. This needs to be balanced against the hazards of anesthetizing a patient who is significantly hyperkalemic or acidotic (volume overload is more easily corrected, as some fluid loss is more or less inevitable during surgery).

Venovenous hemodiafiltration may be the best way to manage this precarious situation, either started before surgery if time permits, or intraoperatively. The main advantage over intermittent hemodialysis is that the fluid and electrolyte shifts are not so rapid. The disadvantage is that it requires additional large-bore venous access, and an experienced technician to run the filtration system, which may cause logistical problems. Fortunately, few patients are sick enough to need it.

Coagulopathy

Coagulopathy is a familiar problem to anyone caring for liver patients. There are obvious implications for anyone planning an operation or invasive procedure. Greatly prolonged clotting times need to be corrected, but it is rarely necessary to

normalize them. A prothrombin time twice normal is adequate for surgical hemostasis in most cases, especially if there is an adequate platelet count, above 50×10^9/L. Attempting to overcorrect coagulation risks volume overload, may cause thrombotic complications, and is wasteful of valuable blood bank resources, effort, and time.

Encephalopathy

Encephalopathy is a potentially fatal complication of end-stage liver disease that may be exacerbated by anesthesia. Cerebral perfusion in the presence of cerebral edema is governed by the difference between the mean arterial pressure and the intracerebral pressure (ICP). Anesthesia tends to cause the mean arterial pressure to fall without affecting the ICP, thereby worsening perfusion. Respiratory depression may cause PCO_2 to rise, which will cause ICP to increase. In addition, the sedative effects of anesthesia are added to the effects of encephalopathy, so even a short general anesthetic can cause a dangerous fall in conscious level. Careful consideration must therefore be given to whether a patient can be safely woken up post-procedure or whether transfer to the intensive care unit may be required.

The anesthetist's opinion about the patient's condition and prognosis following intervention makes anesthetic and intensive care input to decision making crucial in planning the care of the critically ill or deteriorating liver patient. An anesthetic presence in the multidisciplinary team has many benefits, not only in assessing the risks of anesthesia, but also in enhancing the confidence of other specialists, carers, families, and patients themselves that all aspects of disease management have been considered. Anesthetists will be involved in patient care not only during transplantation or other major surgical procedures, but also during investigations and procedures such as radiological examinations, liver biopsies, and endoscopy. They are key to providing supportive care of the sick patient, advising on or performing vascular access procedures, supervising pain control, and much else, so close communication and working within teams is very much in the patients' interest.

Anesthesia for liver transplantation

Specific procedures and challenges pertinent to the anesthetist during liver transplantation are described below.

Central venous access

Central venous catheters are essential for liver transplantation because they allow central venous pressure monitoring, the administration of inotropes and other drugs, and the rapid transfusion of fluids and blood products. The catheters are non-tunneled devices that can remain in place for up to 2 weeks and are made of polyurethane; often an additional large-bore introducer sheath is placed to facilitate rapid fluid infusion. Long-term silastic catheters are generally unsuitable for intraoperative use.

The insertion of central venous access devices is associated with significant morbidity including infection, pneumothorax, hemothorax, and pericardial tamponade [3]. Hemorrhagic complications are particularly likely in the patient with liver disease. The use of real-time ultrasound guidance has been demonstrated to increase the success rate of cannulation of the internal jugular vein and to reduce carotid artery puncture rate compared with the anatomical landmark technique, and is supported by expert guidance [4, 5].

Thrombosis of the central veins is common in children requiring long-term central venous access for parenteral nutrition or hemodialysis, making the placement of central venous catheters particularly challenging and occasionally impossible by conventional techniques. An assessment of the central venous anatomy with Doppler ultrasound, computed tomography (CT) venography, or magnetic resonance venography before listing for transplant is useful.

Temperature maintenance

Children develop hypothermia during liver transplantation for a number of reasons. The consequences of hypothermia during liver transplantation include cardiac arrhythmia, impaired coagulation, and poor platelet function, so core temperature is monitored continuously throughout the procedure.

Strategies to reduce the drop in temperature include increasing the ambient temperature, warming surgical irrigation fluid following implantation, and placing clear plastic drapes around the child to reduce convective heat loss and prevent pooling of fluid. Devices such as the Hotline™ or Level1™ warm intravenous fluids and blood products efficiently during rapid infusion. Warming mattresses and warm air convection devices are effective in maintaining normothermia.

Hemodynamic monitoring

The profound changes in the circulation seen during pediatric liver transplantation necessitate close hemodynamic monitoring with continuous electrocardiogram, oxygen saturation, central venous pressure, and invasive arterial pressure monitoring.

Cardiac output monitoring remains more challenging in infants and small children compared with adults. Methods include thermodilution using a pulmonary artery (Swan–Ganz) catheter, pulse contour analysis using pulse contour cardiac output (PiCCO) [6] or lithium dilution cardiac output (LiDCO), and the measurement of stroke volume by Simpson's method with transesophageal echocardiography. All the techniques offer benefits and potential errors, and carry significant risks, so some do not monitor cardiac output at all, instead inferring it from the interpretation of standard hemodynamic parameters.

Assessment of coagulation

Coagulation changes rapidly, usually for the worse, during liver transplantation for a variety of reasons including hypothermia, dilutional coagulopathy, liver dysfunction, and graft reperfusion. An array of point-of-care coagulation monitors has found popularity in an attempt to quickly identify coagulation factor and platelet deficiencies and assess therapeutic interventions.

The thromboelastograph (TEG®) measures the shearing forces transmitted from a sample of whole blood at 37°C rotating back and forth to an immersed metal pin; as the sample clots and ultimately lyzes, the measured strain is displayed graphically and numerically against time. In practice, recalcified citrated blood is used and tissue factor added to standardize initiation of the test. Characteristic thromboelastograph patterns are observed that may indicate a range of abnormalities such as dilutional coagulopathy, thrombocytopenia, disseminated intravascular coagulation (DIC), or hyperfibrinolysis, which are expected at different stages of the liver transplant (Figure 30.1). A series of standardized results such as reaction time (RT), α-angle, *K* time (KT), and maximum amplitude (MA) provide useful information about clotting factor abnormalities and guide product replacement. Clot breakdown can also be assessed.

Refinement of the standard sample measurement by the addition of heparinase provides information about the influence of heparin-like substances released at graft reperfusion [7]. Although lacking specificity for individual clotting factors, thromboelastography is a useful monitor of whole blood coagulation and has been shown to reduce blood component therapy in liver transplantation.

Thromboelastometry (ROTEM®) and the platelet function analyzer (PFA-100®) perform broadly similar tests with additional refinements that may offer some advantages.

The thromboelastograph trace should be used to guide rather than dictate correction of coagulopathy; there may be profuse bleeding from surgical incisions in the presence of normal thromboelastography, or a dry surgical field in the presence of a deranged thromboelastograph trace. The MA is commonly reduced in portal hypertension and during liver transplantation, and platelet infusion may correct this problem. An increased RT suggests deficiency of clotting factors early in the clotting process, which may indicate the transfusion of fresh frozen plasma, a particularly useful product as it helps intravascular volume replacement. A reduced α-angle and KT are suggestive of fibrinogen deficiency and may suggest the infusion of cryoprecipitate. It is important to remember the thromboelastograph trace is a picture of the whole clotting process – many different parts of the clotting cascade occur simultaneously, and it would be a mistake to interpret the findings of the test in a too rigidly compartmentalized way.

Thromboelastographic evidence of accelerated fibrinolysis has led to the popularity of antifibrinolytic agents in adult liver transplantation.

Major hemorrhage

Major blood loss in pediatric liver transplantation often greatly exceeds standard definitions and is particularly common in late re-transplantation and portal vein thrombosis, but can occur in any case. Successful management relies upon preparation, adequate wide-bore (central) venous

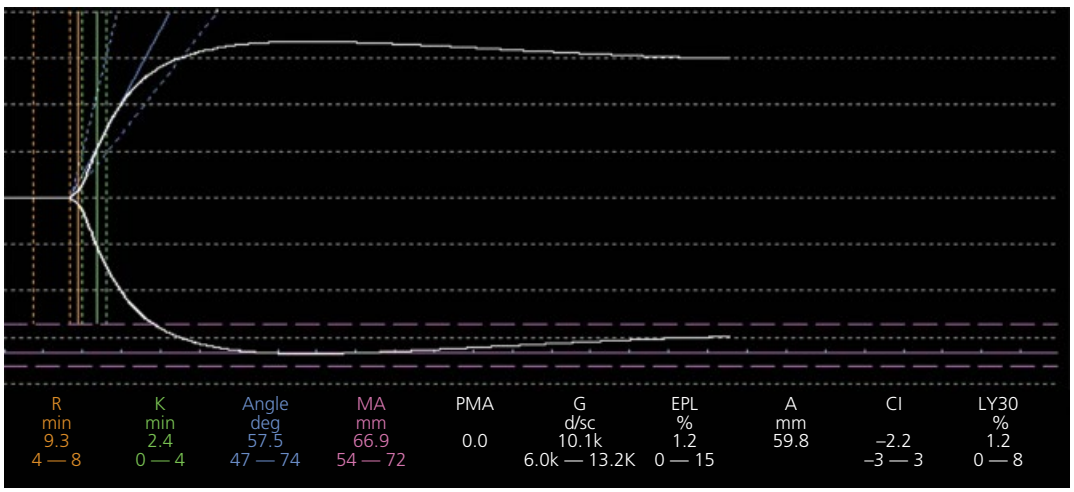

Figure 30.1 A standardized thromboelastograph trace. A typical trace is displayed on a thromboelastograph monitor. The various derived values are displayed. *R* (solid orange line) denotes the reaction time, from the start of the test to the first appearance of a detectable clot. *K* (solid green line) is the time from clot initiation to the thromboelastograph trace achieving a width of 20 mm. The α-angle (solid blue line) is the angle between the tangent drawn from the point of clot initiation to the curve of the trace and the *x*-axis; it reflects the rate of clot formation. Maximum amplitude (MA) is the width of the trace at its widest and reflects maximum clot strength.

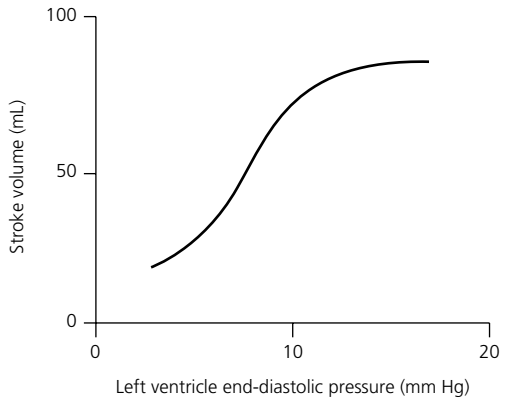

Figure 30.2 Pressure–volume relationship of the left ventricle relating to preload filling (end-diastolic volume).

access, suitable rapid infusion equipment, careful monitoring, an efficient blood bank, as well as good communication and team work – and experience helps!

Regular blood gas and electrolyte analysis is important, often revealing electrolyte abnormalities and progressive metabolic acidosis. Hypocalcemia and hypomagnesemia are likely and require correction [8]. Correction of acidosis is more contentious, with some reserving treatment with sodium bicarbonate for cases of profound circulatory failure.

Hyperfibrinolysis may be treated with tranexamic acid or aprotinin. Tranexamic acid inhibits plasminogen activation and has a relatively good safety profile. Aprotinin, a serine protease inhibitor, remains under suspicion due to side effects including renal failure observed following cardiac surgery; however it may still have a role to play in cases of hemorrhage associated with fibrinolysis. Recombinant factor VII is usually reserved for intractable bleeding associated with severe coagulopathy.

The mainstay of management remains the transfusion of blood and blood products; close liaison with the blood bank is of crucial importance. Intraoperative red cell salvage may help reduce the amount of banked blood required.

Major hemorrhage has the potential for rapid deterioration and death; staffing levels of anesthetists and support staff during the operation must reflect this.

Intraoperative red cell salvage

Reducing the use of donor blood, and thus the risks and costs of transfusion, make intraoperative red cell salvage attractive [9]. Technological improvements in cell salvage devices have led to a reduction in the volume of salvaged blood necessary to process red cells for autotransfusion, making it suitable for children.

Intraoperative red cell salvage remains controversial in transplantation for liver tumors, despite the use of white cell filters, due to concerns over the reinfusion of malignant cells. The technique has been used in liver transplantation for hepatocellular carcinoma in adults with a significant reduction in donor blood transfusion and no evidence of increased tumor recurrence [10].

Reperfusion

Reperfusion of the donor liver graft with blood is a challenging time for the anesthetist and marks the start of the reperfusion phase. The preceding anhepatic phase is associated with reduced venous return and cardiac output due to clamping of the inferior vena cava (IVC) – any resulting hypotension is usually overcome with the judicious infusion of inotropes and intravenous fluids. Portal vein clamping leads to reduced venous return and intestinal congestion, which may be ameliorated by the surgical construction of a temporary portacaval shunt. Throughout the anhepatic phase there is a progressive metabolic acidosis, and hypoglycemia and hypocalcemia are commonplace. Towards the end of the anhepatic phase, the donor liver is flushed with room temperature crystalloid or other solution to remove preservation fluid, metabolites, air, and inflammatory mediators; the portal vein and IVC anastamoses are then completed.

Reperfusion is almost always via the portal vein and is usually followed rapidly by systemic arterial hypotension, the severity of which may fulfill criteria for the various definitions of the post-reperfusion syndrome [1]. In practice some degree of systemic arterial hypotension is inevitable; indeed its absence suggests poor portal vein perfusion. Peripheral vasodilation and myocardial depression are frequently seen; cardiac arrhythmias may result from hyperkalemia and hypothermia. The circulation is supported with inotropes, usually epinephrine and norepinephrine, and judicious administration of intravenous fluid. Hemorrhage from the collateral vessels or the cut surface of a split liver graft may be troublesome.

When a degree of cardiovascular stability and hemostasis is achieved the surgical team proceed to arterialize the engrafted liver, which may involve the construction of an aortic conduit. Severely altered hemodynamics are unusual with arterial reperfusion. With adequate graft perfusion (and time) the hemodynamics usually slowly improve sufficiently to allow a cautious reduction of inotropic support.

When adequate hemostasis is achieved the surgical team will establish biliary drainage, place abdominal drains, and attempt abdominal closure.

Re-transplantation

Early re-transplantation for graft non-function can be a relatively straightforward surgical procedure due to the lack of adhesions, although the patient can be in a precarious state with many organ systems requiring intensive support. Conversely, late re-transplantation can be surgically extremely challenging due to profuse bleeding from multiple vascularized intra-abdominal adhesions and the presence of sepsis in the diseased liver.

Anesthesia after liver transplantation

Pediatric liver transplant recipients often require general anesthesia, usually for related procedures such as endoscopic examinations of the gastrointestinal tract and liver biopsies. Careful assessment is necessary to identify liver dysfunction and the effects of immunosuppressant medications.

Such children often display anxiety regarding anesthesia and benefit from friendly reassurance, encouragement, and the presence of parents at induction; gaseous induction is offered to those with needle phobia, or simply as a preference. Play therapy and distraction techniques are very helpful; additionally a sedative anxiolytic such as oral midazolam may be necessary prior to arrival in theater. More sophisticated cognitive behavioral therapy techniques are occasionally required.

Pain relief

Intravenous morphine remains the commonest analgesic following liver transplantation, initially in combination with midazolam to provide sedation in the pediatric intensive care unit. Patient or nurse-controlled analgesia regimens, often including background infusions, are popular following extubation and a return to general ward care. Paracetamol is a useful adjunct; avoidance of potentially hepatotoxic dosing schedules is essential. Non-steroidal anti-inflammatory drugs are generally avoided due to their potential for gastrointestinal hemorrhage and other side effects. Central neuraxial blockade (epidural, etc.) is rarely used because of concerns regarding thrombocytopenia and coagulopathy, although it may be used in other forms of liver surgery.

There is some evidence that the implementation of non-pharmacological pain therapies as part of a broader comprehensive postoperative pain management program resulted in improved pain scores in children receiving liver transplants [11].

End of life care

Unfortunately a successful outcome cannot be guaranteed; rarely a child dies during the liver transplant procedure despite the best efforts of the teams in the operating theater, usually as a result of uncontrollable hemorrhage or profound cardiovascular collapse. It is likely that this follows reperfusion of the liver and thus the liver graft is unsuitable for another recipient. Senior clinicians are required to identify those recipients suffering severe intraoperative instability who are unlikely to survive, and swiftly adopt a process of end of life care. This should not necessarily involve a reduction or withdrawal of support, indeed vasopressor infusions and fluid transfusion may be continued to "buy time" and allow parents some precious time with their dying child. It is important that this process is conducted with sensitivity to the family's emotional state. Transfer to a quiet room in the intensive care unit may be preferable to the theater environment, however if this is impossible replacing blood-stained drapes with clean sheets, cleaning the theater, and the removal of surgical equipment can go some way to reducing distress. Members of the theater team may also need considerable emotional support in the ensuing days following such an event.

Anesthesia for hepatobiliary surgery

Hepatobiliary surgical operations can be divided into laparoscopic procedures, procedures involving the gallbladder and biliary tree, and those involving the liver itself. Laparoscopic cholecystectomy for the treatment of gallstone disease offers a number of advantages over open cholecystectomy including less postoperative pain, a smaller incision, quicker recovery, and reduced incidence of wound infection. The anesthetic challenges are largely a consequence of pneumoperitoneum and head-up tilt: respiratory compromise, impaired venous return, and reduced cardiac output. Conversion to an open procedure may be necessary for technical indications, usually the presence of intraperitoneal adhesions or excessive bleeding. Postoperative pain following laparoscopic cholecystectomy is usually well controlled with simple analgesics such as paracetamol and non-steroidal anti-inflammatory drugs (although shoulder tip pain can be severe and resistant to treatment). Discharge home on the day of surgery is nonetheless often possible.

Biliary surgical procedures, such as the Kasai portoenterostomy, are characterized by a right upper quadrant incision, retraction of the liver, and dissection of the biliary tree. Anesthetic challenges include bleeding, sepsis, and impaired venous return. Adequate venous access, often using a central vein, is necessary to ensure adequate fluid and blood product replacement.

Postoperative pain relief can be challenging. Epidural analgesia, if not contraindicated, can offer excellent pain relief; conversely morphine infusion regimens by either patient- or nurse-controlled analgesia programs are often satisfactory too. Recently there has been much interest in the transversus abdominalis plane (TAP) block, which involves the delivery of local anesthetic agents into the plane between the transversus abdominalis and the internal oblique muscles to provide analgesia to the anterior and lateral abdominal wall. The local anesthetic agents may be directed into the fascial plane using an anatomical landmark technique, or more usually with ultrasound guidance, and may be infused continuously postoperatively through catheters to prolong pain relief.

Surgical procedures involving the liver parenchyma are performed for tumor excision, most commonly hepatoblastoma, either as a localized tumor resection or hemihepatectomy

following the segmental anatomy of the liver vasculature. Particular risks include impaired venous return secondary to manipulation of the IVC, air embolus, and severe hemorrhage. The conduct of the anesthetic shares many similarities with liver transplantation. A notable difference is that allowing the central venous pressure to fall a bit while the liver resection is in progress may help to reduce bleeding and avoid engorgement of the liver, making access and visibility better for the surgical team. Excessively low filling pressures should be avoided as there can be adverse hemodynamic consequences; there may be renal impairment and the empty large veins can entrain air and cause air embolus. Once the resection is completed, filling pressures can be restored. During the resection, perfusion of the residual liver can be impaired by surgical interference with the liver vessels, so signs of hepatic insufficiency can appear even if there is plenty of liver tissue remaining. The liver vessels may be deliberately occluded by the surgical team to improve visibility and limit bleeding (the Pringle maneuver), which will necessarily render the liver ischemic, and so the time it is applied for must be strictly limited (15 min is a guideline maximum). The maneuver can be repeated, but there is obvious potential for cumulative liver damage. Resection of a large volume of liver tissue can result in inadequate liver function, the "small for size" syndrome', which may be suggested intra-operatively by hypoglycemia, prolonged prothrombin time and progressive metabolic acidosis. In such cases hemodynamic manipulation may be necessary to minimize further injury and support regeneration of the liver; this should be continued postoperatively on the intensive care unit.

The World Health Organization's surgical safety checklist has been widely implemented in an attempt to reduce perioperative morbidity and mortality during all forms of operating department activity, especially those resulting from human errors. A safety questionnaire completed before induction of anesthesia is known as "sign in." Prior to the start of surgery the team takes "time out" to identify the patient, reconfirm the operation, and raise any relevant issues. Following surgery, swab, needle and instrument counts are checked, the postoperative plan agreed, and concerns voiced – the "sign out." Modified checklists are being adopted by transplant teams in the hope of improving the safety of liver transplantation and related surgery.

Critical care support

Patients with hepatobiliary disease may require critical care support for initial stabilization, diagnostic investigation processes, perioperative care, or as a consequence of evolving multiorgan failure. The target of critical care is to treat amenable underlying conditions, to provide support through the liver transplantation process if required, and importantly to alleviate pain and suffering. There are some patients who will not have conditions amenable to treatment, nor be suitable for liver transplantation, in which case the coordination and delivery of comfort or palliative care is of the utmost importance [12].

Infants, children, and young people may present to critical care services with hepatobiliary conditions at any time from the neonatal period to transition to adult care. A systematic approach in critical care is essential irrespective of the age at presentation. The "ABC approach" used by resuscitation services worldwide is appropriate for use both in patient care and in the communication of information between care providers or transport services (Table 30.2). It is not always clear when a patient should be referred to critical care services. An early referral can enable a collaborative approach to the decision making with the intensive care physician.

Table 30.2 The ABC approach for communication of information.

Heading	Components
Referrer information	Name, grade, hospital, contact number, responsible physician
Patient information	Name, date of birth, gender, location, hospital identifier, weight
A – airway	Airway symptoms, stridor, secretion clearance, airway protection reflexes, dysmorphism, details of intubation
B – breathing	Breathing symptoms, effort, respiratory rate, pulse oximetry reading, oxygen supports, ventilation supports, chest signs
C – cardiac/circulation	Cardiac symptoms, heart rate, perfusion, blood pressure, pulse volume, femoral pulses, urine output
D – neurology	Neurological symptoms, level of consciousness (responsiveness scale "alert, voice, pain, unresponsive" (AVPU) or Glasgow Coma Score (GCS)), focal neurological signs, seizures, pupil responses
E – other symptoms and general examination findings	Overall clinical state, abdominal signs, fluid hydration status, blood glucose control, infection markers (fever, rashes, contact history)
Medication and allergies	Medications exposure and administration
Other relevant background	Social, family, maternal/birth history, immunization
Investigations	Chest X-ray, blood gas analysis, blood tests (include hematology, coagulation, and biochemistry), microbiology results, specialist imaging (e.g., CT, MRI, ultrasound)

Hepatobiliary disease and the underlying processes can lead to various organ systems failure for which support may be available.

Respiratory system

Abdominal distension, ascites, and fluid overload often lead to respiratory distress developing due to low lung volumes, pulmonary edema, and impaired diaphragm excursion. Tachypnea may be the first sign of impaired respiratory function, and may be followed by a need for supplemental oxygen to maintain normal pulse oximetry readings. Signs that a patient may benefit from positive pressure breathing support include recession and expiratory grunting – these signs show impaired lung compliance and the need for a distending pressure to reduce atelectasis. Early intervention with high-flow nasal cannula oxygen, continuous positive airway pressure (CPAP), or non-invasive ventilation (NIV) may prevent the need for intubation and invasive ventilation.

For some patients these non-invasive optimizing strategies do not improve the situation, and a carefully planned intubation is the most appropriate intervention. Blood gas analysis is often used to check for the patient developing respiratory failure, and the elevation of a carbon dioxide level with acidosis is a concerning but often late sign of deterioration. The clinical markers for work of breathing should be given priority. A blood gas analysis is a useful investigation as it provides a baseline for the respiratory values, and adds information regarding the blood lactate, electrolytes, glucose, and non-respiratory acidosis components.

Circulatory system

Patients may develop cardiovascular instability in the context of fulminant liver failure or septic shock. The progression to a life-threatening situation can be rapid and the risk should not be underestimated. The international Surviving Sepsis Campaign and more recently a bundle called "sepsis 6" can be implemented as interventions to improve survival in patients with sepsis [13, 14]. In a patient with hepatobiliary disease, it is important to be vigilant for shock and make regular observations of heart rate, blood pressure, and end organ function (urine output, level of consciousness, skin perfusion, and core–peripheral temperature gap). In patients showing impaired cardiac output, resuscitation should be initiated and the response to treatment monitored. The critical care team will use the central venous blood gas (lactate and venous oxygen saturation levels) to guide resuscitation.

The causes of shock include:

- Inadequate preload (hypovolemia, bleeding).
- Cardiogenic (pump failure).
- Sepsis (peripheral vasodilation or vasoconstriction, capillary leak, cardiac dysfunction).
- Neurogenic (peripheral vasodilation).
- Anaphylaxis (peripheral vasodilation and cardiac dysfunction).

In the case of inadequate preload, fluid boluses are the first-line treatment to be given in conjunction with early administration of broad-spectrum antimicrobials. In response to a fluid challenge, it should be seen that the central venous pressure, or preload, of the heart increases. In the majority of situations, this will result in an improved cardiac output in accordance with the recognized pressure–volume relationship of the left ventricle. In a situation where the fluid bolus has had little or no effect, then it may be that the heart itself has impaired function or fluid losses are ongoing (e.g., bleeding, capillary leak). In a situation where there is no significant improvement with these initial measures, a referral for critical care support should be made.

In the post-transplant situation, it will be of particular importance to consider hypovolemia secondary to bleeding as the cause of hypotension. If this is the case, there is a need to resuscitate with fluid volume including activation of a major hemorrhage protocol, which may be required to ensure provision of blood products (packed red blood cells, platelets, fresh frozen plasma), to investigate (abdominal ultrasound with Doppler flow analysis of the graft), and to escalate concerns promptly to the liver surgical team to decide if repeat laparotomy is indicated.

In the case of impaired cardiac function, or failure to respond to initial fluid bolus therapy, inotropic and vasoactive medication administration should be considered. The heart can be driven to improve cardiac output by increasing the heart rate (tachycardia is the usually physiological reaction to shock) or by increasing the stroke volume (as improved by preload filling and the force and depth of contraction). The inotropic agents used in critical care will tend to increase the force of contraction, the heart rate, and modify the afterload (peripheral vascular resistance) (Table 30.3). These medications can usually be delivered temporarily by intraosseous vascular access or in a dilute form through a peripheral venous line. The definitive route of administration is through a central venous line.

Manipulation of the systemic vascular resistance will depend on the clinical situation. For the heart to generate an adequate blood pressure, it must have some resistance against which to push (mean blood pressure = cardiac output × systemic vascular resistance). In some situations (e.g., sepsis, liver failure, anaphylaxis, neurogenic shock) the systemic vascular resistance is low due to vasodilation – in this setting, a vasoconstrictor medication (norepinephrine or vasopressin) in addition to fluid and inotropic support may be required. In other situations (e.g., sepsis, "cold shock") the systemic vascular resistance is high and despite adequate preload and cardiac contractility, the cardiac output is impaired – in this setting a vasodilator medication (milrinone or dobutamine) may be required. The most dangerous situation is when

Table 30.3 Inotropes and vasoactive agents used in critical care.

Medication	Effect
Dobutamine	Agonist for both β1- and β2-receptors, leading to an increase in heart rate, stroke volume, and vasodilation with a reduction in systemic vascular resistance
Dopamine	Agonist at low dose on dopaminergic receptors and leads to vasodilation; at higher doses it can act as a β1- and α1-agonist, which leads to increased contractility, stroke volume, and a rise in systemic vascular resistance
Epinephrine	β1-Agonist leading to an increase in heart rate and stroke volume; at higher dose infusion rates it will act as an α1-agonist and lead to vasoconstriction
Milrinone	Phosphodiesterase III inhibitor with effects to improve cardiac diastolic relaxation, myocardial contractility, and peripheral vasodilation leading to a reduced systemic vascular resistance
Norepinephrine	α1-Agonist leading to vasoconstriction and elevates systemic vascular resistance
Vasopressin	Endogenous hormone with actions to cause peripheral vasoconstriction leading to an increased systemic vascular resistance

cardiac contractility is impaired and the cardiac output is low despite afterload reduction with vasodilation. In this situation, the heart may fail further if additional vasoconstriction is applied in an effort to elevate the blood pressure. Determining the right vasoactive or inotropic agent can be difficult, and utilizing the expertise of the critical care team is crucial.

Steroid supplementation is considered in those patients with shock known to be on long-term steroid supplementation or if inotropes/vasoactive medication remains ineffective. The role of plasma exchange in sepsis is debated, but in patients with fulminant liver failure, the use of therapeutic plasma exchange can be used to remove unmeasured vasoactive or cardiodepressive toxins and cytokines, improve coagulopathy, and lower ammonia levels [15].

In patients with persistent shock despite escalation of intensive care support, a discussion with the regional extracorporeal life support (ECLS) provider should be considered. ECLS may be able to provide temporary cardiorespiratory support whilst awaiting recovery of the underlying condition. The use of ECLS in pediatric fulminant liver failure with associated cardiorespiratory failure has been successfully applied in at least one case as a bridge to liver transplantation.

Hepatorenal system

Secondary organ damage from liver dysfunction, toxic metabolic compounds (e.g., ammonia), episodes of hypotension, and hypoglycemia often occur. Patients may require high concentrations of glucose solutions and medications and large volumes of intravenous blood products, and this can lead to fluid overload. Diuretic therapy, typically furosemide, is instituted to improve urine output and maintain a neutral fluid balance when possible. Renal failure is a frequent occurrence and when diuretic therapy is inadequate, the use of continuous venovenous hemofiltration or diafiltration (CVVH or CVVHDF) is particularly useful. A high turnover can be used to facilitate clearance of ammonia, the removal of unmeasured toxins (as seen in impaired hepatic detoxification of the portal circulating neurotoxins), and for the optimization of fluid balance status in patients.

The choice to use continuous hemofiltration rather than intermittent hemodialysis is usually due to the better tolerance of the patient's cardiovascular system with the slower blood flow rates in this mode [16]. Patients with liver failure-associated hyperammonemia may have their levels of ammonia reduced significantly within 12 h but the therapy may have to continue until liver function improves or transplantation is performed. Renal replacement therapy (CVVH, CVVHDF) may be required in the post-transplant patient to support homeostasis whilst renal recovery from the primary insult occurs.

Neurological system

It is crucial that a patient with hepatic insufficiency has close neurological monitoring – this should include an assessment of consciousness, airway protection, and potential for seizures. It should not be assumed that an impaired conscious state is due to hepatic encephalopathy; the possibility of raised intracranial pressure (ICP), intracranial bleeding, hypoglycemia, or subclinical status epilepticus should be considered.

ICP can be measured directly by the placement of an intraparenchymal bolt, but the application of ICP monitoring in this patient population requires more evidence to determine benefit [17]. There is a risk of hemorrhage with this procedure, and the pressure reading may prompt aggressive osmotic therapy use with unclear clinical benefit. Less invasive markers of raised ICP include the fundoscopic appearance, pupillary reactivity index (pupillometer measure), and transcranial Doppler resistance index. Imaging with CT to exclude hemorrhage is important, especially in patients with asymmetrical neurological findings. MRI may be helpful in diagnosing cerebral ischemia, edema, or other underlying conditions (e.g., mitochondrial disease).

In the post-transplant period, confusion and encephalopathy may be due to the immune suppression-associated posterior reversible encephalitis syndrome (PRES), which is demonstrated on MRI and electroencephalography (EEG). The syndrome responds to a reduction in immunosuppression, but the effects may be delayed [18].

Cranial ultrasound imaging may be used in infants with an open fontanelle, but the presence of extra-axial bleeding will be unreliably detected. Continuous EEG monitoring should be used to check for subclinical seizure activity and the degree of encephalopathy, as it may both show important treatable conditions and offer prognostic information [19]. Anticonvulsant use in the liver patient will usually initially

be with a benzodiazepine (e.g., midazolam, lorazepam). Consultation with a pediatric neurologist is recommended for patients having persistent or recurrent seizures. Treatments indicated to target underlying causes are essential – including glucose supplementation, osmotic therapy, electrolyte normalization, and clearance of ammonia.

Postoperative transplant care

The meticulous attention to detail required by anesthetists to maintain patient homeostasis during the challenges of liver transplant surgery is again required in critical care. Respiratory support by ventilation is almost universally required for the first postoperative night. Some patients will make good progress in tolerating a reduction in their support levels over this time in preparation for extubation. Those patients with previous lung disease, reduced respiratory reserve, lower age/body weight, or complications of the transplant (fluid overload, massive transfusion, transfusion-associated lung injury, abdominal distension, or diaphragmatic splinting) may require ventilator support for days, or even weeks in some cases. A standardized protocol of care for these patients will include guidance on antirejection medication, antimicrobial agents, fluid and feed management, graft assessment, and early detection of complications including rejection/non-function, bleeding, biliary leak, and infection. The primary goal of the critical care support is to enable time for patient recovery and successful engraftment. This requires the maintenance of adequate physiological homeostasis and the prevention of secondary organ dysfunction.

Conclusion

Patients with hepatobiliary disease may require critical care support. Prompt assessment, resuscitation, stabilization, and referral will enable the patient to access these supports. Early interventions (e.g., with aggressive sepsis treatments, reversal of hyperammonemia, and normalization of ICP) are performed to limit secondary organ damage. It must be remembered that critical care aims to care holistically for the patient and their family – treating the treatable, supporting them through the liver transplantation process if applicable, and, importantly, to alleviate pain and suffering. Whilst always working to achieve a good outcome for the patient, there are some patients who will not have conditions amenable to treatment. In all cases, collaborative working with the patient, family, and other care teams is essential.

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CHAPTER 31

Liver Transplantation

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Key points

- Pediatric liver transplantation has significantly improved the prognosis for many infants and children with liver failure and metabolic disease.
- The main indications for liver transplantation are: biliary atresia, metabolic liver disease, and acute liver failure.
- Innovative surgical techniques including live-related transplantation have increased the limited organ donor pool for children.
- The main complications post-transplant are technical problems immediately post-transplant, rejection, and infection.
- Survival rates have improved to 90% at 1 year and 75% at 15–20 years with good quality of life.
- Transition to adult services remains a significant challenge for long-term survivors.

The successful development of pediatric liver transplantation has dramatically changed the prognosis for many infants and children dying of end-stage liver failure and is now accepted therapy for this condition.

Key elements in improving survival post-liver transplantation have been:

- Better preoperative management of hepatic complications and nutritional support.
- Innovative surgical techniques to expand the donor pool.
- Improvements in postoperative immunosuppression
- Management of long-term graft injury.

The improvement in survival rate has extended the range of indications for liver transplantation in children to include semi-elective liver replacement, transplantation for metabolic liver disease, and unresectable hepatic tumors. As short-term survival has improved, interest has focussed on quality of life and long-term survival.

Indications for liver transplantation

Liver transplantation is standard therapy for acute or chronic liver failure (Box 31.1 and Figure 31.1A,B) [1–3].

Chronic liver disease

Neonatal liver disease

Biliary atresia remains the commonest indication for liver transplantation in children, accounting for 74% of children undergoing transplant below the age of 2 years [1]. Despite the professional emphasis on early diagnosis and management of this condition, in practice children are often referred too late to benefit from a palliative Kasai portoenterostomy (see Chapters 8 and 25). Urgent transplantation is required for those children who have an unsuccessful Kasai portoenterostomy or who develop nutritional or hepatic complications.

Cholestatic liver disease

The outcome of cholestatic liver disease in infancy, such as in Alagille syndrome or progressive familial intrahepatic cholestasis, is variable. Liver transplantation is indicated for the development of cirrhosis and portal hypertension, development of malnutrition or growth failure unresponsive to nutritional support, or intractable pruritus which is resistant to maximum medical therapy or biliary diversion.

Inherited metabolic liver disease

α1-Antitrypsin deficiency is the commonest form of inherited metabolic liver disease presenting in childhood in Europe. Although 50–70% of children may develop persistent liver disease progressing to cirrhosis, only 20–30% require transplantation in childhood.

The management of tyrosinemia type I has changed dramatically since the introduction of nitisinone – NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexenedione) – which prevents the formation of toxic metabolites and

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Box 31.1 Indications for liver transplantation.

Chronic cholestatic liver failure

- Biliary atresia
- Alagille syndrome
- Progressive familial intrahepatic cholestasis (PFIC1–4)

Inherited metabolic liver disease

- α1-Antitrypsin deficiency
- Cystic fibrosis
- Glycogen storage types III and IV
- Tyrosinemia type I
- Wilson disease

Chronic hepatitis

- Autoimmune types 1 and 2
- Sclerosing cholangitis
- Postviral (hepatitis B, C, other)
- Immunodeficiency

Other

- Fibropolycystic liver disease ± Caroli syndrome

Acute liver failure

- Fulminant hepatitis
  - autoimmune hepatitis types 1 and 2
  - paracetamol poisoning
  - viral hepatitis (A, B, C, E, or seronegative)
- Metabolic liver disease
  - fatty acid oxidation defects
  - neonatal hemochromatosis (gestational alloimmune liver disease)
  - tyrosinemia type I
  - Wilson disease

Inborn errors of metabolism with extrahepatic disease

- Crigler–Najjar syndrome type I
- Familial hypercholesterolemia
- Primary hyperoxaluria
- Organic acidemia
- Urea cycle defects

Liver tumors

- Benign tumors
- Unresectable malignant tumors

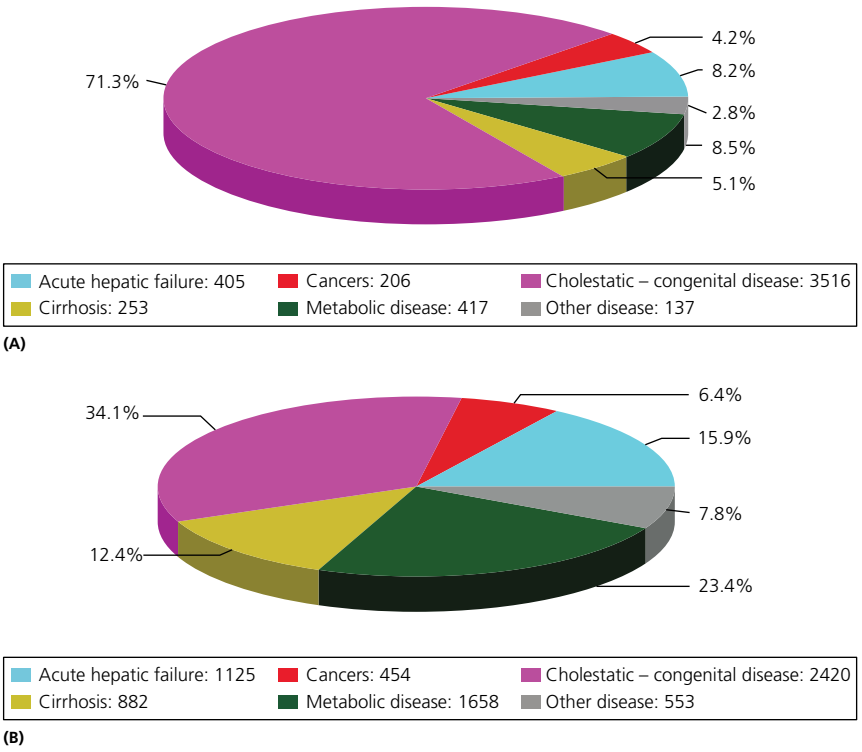


Figure 31.1 Primary indication for liver transplantation in pediatric patients: (A) 0–2 years; and (B) 2–18 years, 1988–2015 (European Liver Transplant Registry). (Source: www.eltr.org; last accessed July 2016. Reproduced with permission of European Liver Transplant Registry.)

produces rapid clinical improvement. Use of this drug has altered the natural history of the disease and the indications for transplantation. Prior to the introduction of nitisinone, liver transplantation was indicated for acute or chronic liver failure, the development of hepatic dysplasia, or hepatocellular carcinoma (HCC). Liver transplantation is now only indicated for those children who have a poor quality of life, do not respond to therapy, or in whom hepatic malignancy is thought

to have developed. Routine monitoring of children with tyrosinemia type I being treated with nitisinone includes ultrasound and magnetic resonance imaging (MRI) to detect the development of nodules and/or early HCC in association with regular α-fetoprotein levels. A persistent or sustained rise of α-fetoprotein may indicate the early development of HCC.

Wilson disease is a rare indication for liver transplantation in childhood (see Chapter 20) but is indicated for those children who present with advanced liver disease (Wilson score >6), fulminant liver failure, or who have progressive hepatic disease despite penicillamine therapy.

As long-term survival improves in children with cystic fibrosis (CF; see Chapter 16), liver transplantation is now the commonest indication for transplantation in adolescents in some centers. Hepatic decompensation is a late feature of CF liver disease, but portal hypertension is common and bleeding from esophageal varices is a recurrent problem. Selection for liver transplantation is indicated only for those children with hepatic decompensation (falling serum albumin, prolonged coagulation unresponsive to vitamin K), severe malnutrition, or complications of portal hypertension unresponsive to medical management – ascites or uncontrolled variceal bleeding. Careful assessment of pulmonary function is required as severe lung disease (<50% of lung function) may indicate the necessity for a heart, lung, and liver transplant. Thus, early liver transplantation is indicated for those children with moderate lung disease prior to the development of significant irreversible lung disease. Preoperative management of respiratory disease is important and should include vigorous physiotherapy, intravenous antibiotics, and treatment with deoxyribonuclease (DNase). Postoperative antibiotics should be based on known bacterial colonization and antibiotic sensitivity.

The majority of children with glycogen storage disease type I should respond to appropriate medical and nutritional management. Transplantation is indicated only for those children who develop multiple hepatic adenomata or in whom metabolic control has a significant effect on quality of life. Glycogen storage disease types III and IV may progress to cirrhosis and this may be an indication for transplantation because of hepatic dysfunction.

Chronic hepatitis

Autoimmune liver disease types I and II. The majority of children with autoimmune liver disease types I or II respond to immunosuppression with prednisolone or azathioprine (see Chapter 11). Liver transplantation is indicated for those children who have advanced portal hypertension or do not respond to immunosuppression despite the use of second-line drugs such as ciclosporin A, tacrolimus, and mycophenolate mofetil, or those who present with fulminant hepatic failure. Children with autoimmune hepatitis type II are more likely to present in fulminant hepatic failure and have an increased requirement for liver transplantation (see Chapter 11).

Chronic hepatitis B or C. Most children with chronic hepatitis B or C are asymptomatic carriers in whom the development of cirrhosis, portal hypertension, and/or HCC may evolve over 20–30 years and might now be prevented with modern therapy, especially the new direct acting antivirals (see Chapter 13). Recurrence of hepatitis B or C post-transplantation is likely in 90% of patients without prophylactic therapy.

Fibropolycystic liver disease

Fibropolycystic liver disease is a rare indication for liver transplantation in childhood, as liver function usually remains normal for many years in these children even if they develop severe portal hypertension. Liver replacement is only indicated if hepatic decompensation occurs in association with portal hypertension or hepatic enlargement interferes with quality of life. The disease may be associated with infantile polycystic kidney disease and both liver and kidney replacement will be required, usually at the time of renal replacement (see Chapter 11).

Primary immunodeficiency

Many children with primary immunodeficiencies have associated liver disease (see Chapter 22). The most common immunodeficiency requiring liver transplantation is CD40 ligand deficiency (hyper immunoglobulin M (IgM) syndrome), in which recurrent cryptosporidial infection of the gut and biliary tree lead to sclerosing cholangitis. In this group of children it is important to carry out bone marrow transplantation before the development of significant liver disease or to consider combined liver and bone marrow transplantation if necessary.

Timing of transplantation for children with chronic liver failure

Many children with cirrhosis and portal hypertension have well-compensated liver function, so the timing of liver transplantation is difficult to predict. In practice, the need for liver transplantation is indicated by a persistent rise in total bilirubin >150 mmol/L, prolongation of prothrombin ratio (international normalized ratio (INR) >1.4) and a fall in serum albumin <35 g/L. These parameters have been utilized to develop the pediatric end-stage liver disease (PELD) score to predict death and have confirmed their accuracy in predicting need for liver transplantation.

As protein-energy malnutrition is a known complication of chronic liver disease in the developing child, serial evaluation of nutritional parameters may be a useful guide to early hepatic decompensation. Progressive reduction of fat stores (triceps skinfold) or protein stores (mid-arm muscle area) despite nutritional support are an indication for transplantation.

Children with hepatic complications such as chronic hepatic encephalopathy, refractory ascites, intractable

pruritus, or recurrent variceal bleeding despite optimum management require prompt referral for transplantation. Variceal hemorrhage which is not controlled by variceal banding or endoscopic sclerotherapy may be temporarily managed by the insertion of a transjugular intrahepatic portosystemic shunt (see Chapters 5, 21, and 27). This technique reduces portal vein pressure and prevents variceal hemorrhage, providing a "bridge to transplantation."

Children with chronic liver disease may have delayed psychosocial development with reduction in motor skills, which is reversed following liver transplantation if performed early enough. Thus, any significant delay in developmental parameters is an indication for referral for liver transplantation.

It is essential that children with chronic liver disease should be referred for transplantation before the complications of their liver disease adversely impair the quality of their lives and before growth and development are retarded.

Acute liver failure

The indications for liver transplantation for acute liver failure vary depending on whether the disease process is due to fulminant hepatitis or secondary to an inborn error of metabolism (see Chapters 9, 18, and 19). In general, children with acute liver failure should be referred early to a specialist unit with facilities for transplantation in order to provide time for stabilization and to find an appropriate donor organ.

Fulminant hepatitis

When considering transplantation for children with fulminant hepatitis it is essential to:

- Assess prognosis for spontaneous recovery.
- Prevent or treat hepatic complications while awaiting a donor organ/regeneration of native liver.
- Provide hepatic support.
- Provide psychosocial support and information for parents.

Poor prognostic factors for children with fulminant hepatitis which require listing for liver transplantation are as follows:

- Seronegative hepatitis.
- Rapid onset of coma with progression to grade III or IV hepatic coma.
- Diminishing liver size.
- Falling transaminases.
- Increasing bilirubin (>300 mmol/L).
- Persistent coagulopathy (PT >40 s, INR >4).

Unlike adults, children with fulminant hepatitis may have severe coagulopathy but mild encephalopathy and therefore both are not required prior to listing for liver transplantation.

All children with grade III hepatic coma, or those who have a persistent coagulopathy (prothrombin time (PT) >40, INR >4) and have no evidence of irreversible brain damage from cerebral edema or hypoglycemia should be listed for transplantation. As medical management for cerebral edema is unsatisfactory and methods of determining irreversible brain damage unreliable, this may be a difficult decision. Cerebral computed tomography (CT) scans may detect gross cerebral edema, hemorrhage, or infarction which may be contraindications for transplantation. Monitoring of cerebral edema by measurement of intracranial pressure improved the selection of recipients but is rarely used now.

Electroencephalography (EEG) may demonstrate a reduction in electrical activity and ultimately brain death, although these results must be interpreted cautiously in ventilated patients or those treated with thiopentone as the EEG tracing is affected by sedation and anesthetic drugs.

Paracetamol (acetaminophen) poisoning

The incidence of poisoning with paracetamol has fallen in the UK since the introduction of limited blister packs. Children and adolescents have a lower incidence of liver failure with paracetamol overdose than adults, possibly because of the effect of hepatic maturation and glutathione production. Transplantation is more likely if the overdose was taken with another drug (e.g. lysergic acid diethylamide (LSD), Ecstasy) or with alcohol.

Children should be considered for liver transplantation if there is a persistent coagulopathy (INR >4), metabolic acidosis (pH <7.3), an elevated creatinine (>300 mmol/L), or rapid progression to hepatic coma grade III. In some children cerebral edema may persist despite evidence of hepatic regeneration and recovery, and influence their postoperative recovery.

Metabolic liver disease

Acute liver failure may be the presenting feature of inherited metabolic liver disease such as Wilson disease and tyrosinemia type I (see Chapters 9, 14, 19, and 20). The clinical presentation is likely to be subacute and liver failure occurs in the presence of underlying cirrhosis. Selection for liver transplantation is on the basis of non-response to medication or severe coagulopathy as jaundice and encephalopathy may not be obvious. Diminishing liver size does not occur because of the underlying cirrhosis.

Infants with neonatal hemochromatosis or gestational alloimmune liver disease (GALD) presenting within days or weeks of birth with severe coagulopathy and encephalopathy may be candidates for liver transplantation if medical management using immune globulin and plasma exchange has failed (see Chapters 9 and 10).

Inborn errors of metabolism

Certain inborn errors of metabolism are secondary to hepatic enzyme deficiencies (see Chapters 9 and 19). Liver transplantation is indicated for these conditions if the hepatic enzyme deficiency leads to:

- Irreversible liver disease/liver failure and/or hepatoma.
- Severe extrahepatic disease.

Diseases in which the inborn error of metabolism leads to liver failure (tyrosinemia type I, Wilson disease, or α1-antitrypsin deficiency; see earlier) are managed as acute or chronic liver failure.

Severe extrahepatic disease

In these diseases (see Box 31.1) the liver functions normally but the missing hepatic enzyme leads to severe extrahepatic disease such as kernicterus in Crigler–Najjar syndrome type I, coronary artery disease in familial hypercholesterolemia, and systemic oxalosis in primary hyperoxaluria.

Selection for transplantation is difficult. It is important to evaluate the quality of life of the child on medical management and to consider the potential mortality and morbidity of the primary disease in comparison with the risks, complications, and outcome following liver transplantation.

The timing of transplantation in these disorders depends on:

- The rate of progression of the disease.
- Quality of life of the affected child.
- The development of severe irreversible extrahepatic disease.

Crigler–Najjar syndrome type I

The timing of transplantation depends on:

- The quality of the child's life, i.e., how many hours of phototherapy per day are required to control the unconjugated bilirubin levels.
- The potential development of irreversible structural brain damage secondary to kernicterus.

It is appropriate to transplant these children between the ages of 3 and 5 years in order to reduce disruption to their education. The most appropriate transplant operation for these children is now auxiliary liver transplantation (see later) or hepatocyte transplantation.

Organic acidemia

Children with propionic acidemia or methylmalonic acidemia are at lifelong risk of recurrent metabolic acidosis and long-term brain damage. Liver replacement is considered palliative treatment for these conditions as the enzyme deficiency affects all body tissue. It should be considered early for children who have a particularly severe phenotype or family history (see Chapter 9). Very careful preoperative management, including preoperative dialysis and perioperative hemofiltration to control acidosis, is essential to ensure good operative control. Until recently, orthotopic liver replacement has been considered necessary to provide adequate enzyme supplementation. It is possible that auxiliary liver transplantation may be sufficient for mildly affected patients.

Familial hypercholesterolemia

Children who are homozygous are prone to premature development of coronary artery disease and thus should be transplanted before coronary artery disease is irreversible. In view of recent progress with gene therapy for this condition, auxiliary liver transplantation or gene therapy may become appropriate treatment strategies.

Primary hyperoxaluria

Liver replacement in this condition should be prior to the development of severe irreversible renal failure. As this is often not possible, liver and kidney replacement may be required simultaneously. As deficiency of the enzyme alanine glycoxylate aminotransferase results in an overproduction of oxalate, these children are not suitable for auxiliary liver transplantation.

Liver tumors

Potential indications for liver tumors include unresectable benign tumors causing hepatic dysfunction, and unresectable malignant tumors (hepatoblastoma or HCC) which are refractory to chemotherapy without evidence of extrahepatic metastases (see Chapter 28).

Preoperative evaluation should include:

- A meticulous search for extrahepatic metastases, with CT scanning of the chest and abdomen and regular monitoring of serum α-fetoprotein to detect relapse or recurrence outside the liver.
- Careful assessment of cardiac function because of the cardiotoxic effects of drugs such as danorubicin.

The timing of transplantation is crucial and is best planned electively during the course of chemotherapy or at completion. Live-related donation is useful as the operation can be planned appropriately Bone marrow suppression at the time of transplantation is supported with administration of granulocyte-stimulating factors.

Children with rhabdomyosarcomas are usually unsuitable for transplantation because of the extent of the tumor and the presence of extrahepatic metastases (see Chapter 28).

Pre-transplant evaluation (Box 31.2)

Evaluation of the patient before transplantation should:

- Assess the severity of the liver disease and the presence or absence of hepatic complications.
- Establish the urgency for transplantation.
- Assess whether the operation is technically feasible.
- Consider any significant contraindications to successful transplantation.
- Consider whether the transplant operation is appropriate for child and family.
- Prepare the child and family psychologically.

This systematic approach is easier to complete for children with chronic liver disease and is more challenging in children with acute liver failure. It is essential to evaluate whether liver transplantation will improve the quality of life for both child and family. Living-related donation should be explored with the family.

Box 31.2 Pre-transplant assessment.

- Nutritional status
  - height, weight, triceps skinfold, mid-arm muscle area
- Identification of hepatic complications
  - ascites, hepatosplenomegaly, varices on endoscopy
- Cardiac assessment
  - ECG, echo, chest X-ray (cardiac catheterization if required)
- Respiratory function
  - oxygen saturation*, ventilation perfusion scan*, lung function tests†
- Neurological and developmental assessment
  - EEG, Bayley developmental scales, Stanford–Binet intelligence scales
- Renal function
  - urea, creatinine, electrolytes
  - urinary protein/creatinine ratio
  - chromium EDTA
- Dental assessment
- Radiology
  - ultrasound of liver and spleen for vascular anatomy
  - wrist X-ray for bone age and rickets
  - MRI/angiography‡
- Serology
  - cytomegalovirus
  - Epstein–Barr virus
  - varicella-zoster virus
  - herpes simplex
  - hepatitis A, B, C
  - HIV
  - measles
- Hematology
  - full blood count, platelets, blood group

* If cyanosed.
† In cystic fibrosis.
‡ If portal vein anatomy equivocal.
ECG, electrocardiogram; EDTA, ethylenediamine tetraacetic acid; EEG, electroencephalogram; HIV, human immunodeficiency virus; MRI, magnetic resonance imaging.

Pre-transplant assessment of severity of liver disease

The indications for transplantation should be critically evaluated, the diagnosis should be reviewed, the prognosis should be considered, and alternative medical or surgical therapy should be evaluated.

Hepatic function

The histological diagnosis should be reviewed and if necessary liver histology should be obtained. The decision to list for transplantation is usually based on serial deterioration in hepatic function as indicated by:

- Albumin (<35 g/L).
- Coagulation time (INR >1.4).
- Rising bilirubin (>150 mmol/L).
- Presence of encephalopathy (acute or chronic).

The extent of portal hypertension may be estimated by establishing the size of the portal vein on ultrasound, and by visualizing esophageal and gastric varices by gastrointestinal endoscopy, which also establishes the presence of gastritis and/or peptic ulceration.

Renal function

The main abnormalities of renal function in children with either acute or chronic liver failure include renal tubular acidosis, glomerulonephritis, acute tubular necrosis, and hepatorenal syndrome. Careful assessment of renal function, including chromium ethylenediaminetetraacetate (EDTA) assessment, is necessary in order to modify the potentially nephrotoxic effects of post-transplant immunosuppression and to assess the necessity for perioperative renal support. Children with pre-transplant exposure to nephrotoxic drugs (e.g. CF, hepatoblastoma) are particularly at risk.

Hematology

Full blood count, platelets, coagulation indices, and blood group are obtained. Human leukocyte antigen (HLA) matching is not required for liver transplantation alone, but is useful information in case of difficult post-transplant rejection.

Serology

It is important to establish immunity to previous infection (see Box 31.2). As donor grafts are matched for cytomegalovirus (CMV) status if possible, assessment of previous infection with CMV is important. Children who are Epstein–Barr virus (EBV) negative are more likely to develop a primary infection post-transplant and have a higher risk of developing post-transplant lymphoproliferative disease. In some parts of the world (see Chapter 34), many donors and recipients will be hepatitis B positive, and this needs to be taken into consideration. Human immunodeficiency virus (HIV) is no longer a contraindication for transplant.

Radiology

The most important technical information required is the vascular anatomy and patency of the hepatic vessels. Most of the necessary information is obtained by color flow Doppler ultrasound examination of the liver and spleen. MRI or conventional angiography may be required to visualize abnormal anatomy such as the hypovascular syndrome or to determine the extent of portal vein thrombosis.

Evidence of hepatofugal flow and/or a small portal vein (<4 mm at the porta hepatis) suggests severe portal hypertension and increases the urgency for liver transplantation.

Children with congenital liver disease, such as biliary atresia, may have an increased incidence of abnormal vasculature. The hypovascular syndrome consists of an absent

inferior vena cava, preduodenal or absent portal vein, azygous drainage from the liver, and the polysplenia syndrome. It may be associated with situs inversus, dextrocardia, or left atrial isomerism. CT angiography is advised to determine the position and size of these abnormal vessels.

Liver transplantation causes important hemodynamic changes during the operative and anhepatic phases. It is essential therefore to have baseline information on cardiac and respiration function. Most of the information required will be obvious from echocardiogram (ECG) or oxygen saturation.

Cardiac assessment

Particular attention should be paid to children with congenital cardiac disease, for example atrial and ventricular septal defects, which are associated with biliary atresia. Peripheral pulmonary stenosis is a known feature of Alagille syndrome. Cardiomyopathy may develop secondary to tyrosinemia type I and the organic acidemias or as a result of chemotherapy of malignant tumors.

Cardiac catheterization may be necessary to determine whether:

- Cardiac function is adequate to withstand the hemodynamic changes during the operation.
- Corrective surgery is required preoperatively.
- The cardiac defect is inoperable and liver transplantation is contraindicated.

Respiratory assessment

A minority of children with end-stage liver disease develop intrapulmonary shunts (hepatopulmonary syndrome). This potentially reversible complication of liver disease needs early consideration for liver transplantation. The clinical signs of cyanosis and digital clubbing and reduced oxygen saturation indicate the need for pulmonary function studies, ventilation-perfusion scans, bubble ECG, and/or cardiac catheterization.

Neurodevelopmental assessment

The aim of liver transplantation is to improve quality of life post-transplant. Thus, it is necessary to identify any existing neurological or psychological defects which may not be reversible post-transplantation. The psychological and developmental assessment of children with clinical liver disease may be performed using standard tests such as: the Griffiths developmental scale (for children under the age of 5 years) or the Bayley developmental scales or Stanford–Binet intelligence scales (children of all ages).

Dental assessment

Chronic liver disease has an adverse effect on the growth and development of young children, including their dentition. Clinical problems pre-transplant include hypoplasia with staining of the teeth and gingival hyperplasia related to poor hygiene. As gingival hyperplasia may be a significant problem post-transplant secondary to ciclosporin immunosuppression, it is important to establish good methods of dental hygiene prior to transplantation (see Chapter 24).

Contraindications for transplantation

As surgical skills have improved there are fewer contraindications to liver transplantation based on technical restrictions. Portal vein thrombosis, age, and size are no longer contraindications for transplantation. The contraindications for liver transplantation include:

- The presence of severe systemic sepsis, particularly fungal sepsis, at the time of operation. Infection based in the liver is not a contraindication to liver replacement.
- Malignant hepatic tumors with extrahepatic spread, because of rapid recurrence.
- Severe extrahepatic disease which is not reversible following liver transplantation, e.g., severe cardiopulmonary disease for which corrective surgery is not possible, or severe structural brain damage.
- Severe systemic oxalosis with cardiac involvement, as these children develop significant hypotension and may not withstand the hemodynamic disturbances post-transplant.
- Mitochondrial disorders with multisystem involvement.
- Alper disease and valproate toxicity because of the progression of neurodegeneration.
- Giant cell hepatitis with autoimmune hemolytic syndrome because of disease recurrence.

Initially, HIV positivity was a contradiction to transplantation, but with current effective treatment regimes, HIV-positive children can now be considered. Although hepatitis B and C recur post-transplant, postoperative therapy is possible and thus transplantation is acceptable.

Children with a higher risk at surgery include those with:

- Previous upper abdominal surgery, because of technical difficulties with adhesions and potential small bowel perforation.
- Portal vein thrombosis, because vein grafts or alternative techniques (portocaval hemitransposition, renoportal anastomosis) may be required.
- Graft failure awaiting re-transplantation.

Preparation for transplantation

Immunization

Although recent studies suggest that some live vaccines can be safely given post-transplantation, live vaccines are usually contraindicated in the immunosuppressed child for at least 12 months post-transplant. It is important to ensure that routine immunizations are complete, for example diphtheria, pertussis, tetanus and polio, Prevnar® or Pneumovax® for

protection from streptococcal pneumonia, and Hib vaccine for protection against *Haemophilus influenzae*. In children older than 6 months, measles, mumps, rubella, and varicella vaccination should be offered. Ideally hepatitis A and B vaccination should be prescribed pre-transplant. As influenza, and rotavirus frequently affect post-transplant patients, addition of these vaccines if available should be considered although this is not routine.

Management of hepatic complications

The treatment of hepatic complications is an important part of preoperative management. Variceal bleeding should be managed as described elsewhere (see Chapters 21 and 27) with esophageal banding or sclerotherapy, vasopressin, or octreotide infusion.

Esophageal banding is preferred to injection sclerotherapy for children on the active liver transplant list as the inevitable development of post-sclerotherapy variceal ulcers may be adversely affected by post-transplant immunosuppression. In children with uncontrolled variceal bleeding, the insertion of TIPSS (transjugular intrahepatic portosystemic stent-shunt) has proved an effective management strategy in older children (see Chapters 21 and 27).

Sepsis, particularly ascending cholangitis and spontaneous bacterial peritonitis, requires effective treatment with appropriate broad-spectrum antibiotics. Tazocin® (90 mg/kg/dose i.v. t.d.s.); is a useful first-line drug until bacterial cultures are positive. In children with acute liver failure, prophylactic antifungal therapy with either fluconazole or liposomal amphotericin is essential. Children should be suspended from the transplant list during episodes of significant sepsis.

Salt and water retention leading to ascites and cardiac failure should be effectively managed with diuretics and salt and water restriction. It is essential to consider intervention with hemodialysis and/or hemofiltration if acute renal failure or hepatorenal failure develop. Hemodialysis is rarely required in chronic liver failure unless there is acute decompensation, but hemofiltration may be necessary in acute liver failure to control cerebral edema and/or coagulopathy. Preoperative hemodialysis and perioperative hemofiltration are essential for children undergoing transplantation for organic acidemia.

Nutritional support

Intensive nutritional support is key to improving outcome post-transplant. A high-calorie protein feed, 150–200% of the estimated average requirement (EAR) for energy intake, is required (Table 31.1). It is now possible to provide this high-energy intake with modified commercial feeds (see Chapter 6) even in fluid-restricted children, although a modular feed may occasionally be required. It is usually necessary to provide these feeds by nocturnal nasogastric enteral feeding or continuous feeding. If enteral feeding is not tolerated, due to ascites, variceal bleeding, or recurrent hepatic complications, parenteral nutrition in normal amounts is required.

Psychological preparation

The most important aspect of the transplant assessment is the psychological counseling and preparation of the child and family. A skilled multidisciplinary team, including play therapist and psychologist, is essential to the success of this preparation. Parents and appropriate relatives must be fully informed of the necessity for liver transplantation in their child and of the risks, complications, and long-term implications of the operation. Psychological preparation in children older than 2 years is essential and may be successfully achieved through innovative play therapy and toys and books suitable for children (see Chapter 6).

Particularly careful counseling is necessary for parents of children who are being considered for liver transplantation because of an inborn error of metabolism. As their children are not dying from liver disease, these parents may find it more difficult to accept the risks and complications of the operation, the potential mortality, and the necessity for long-term immunosuppression. Parents of children who require transplantation for acute liver failure may be too distressed fully to appreciate the significance and implications of liver transplantation and will require ongoing counseling and education postoperatively. Children who survive the liver transplant operation for acute liver failure should have post-operative counseling and play therapy to help them come to terms with their transplant.

Families considering live-related donation require a separate process and independent psychological assessment.

Table 31.1 Nutritional support in infants and children undergoing liver transplantation.*

Nutrient	Preoperative	Postoperative
Carbohydrate (g/kg/day)	Glucose polymer (15–20)	Glucose polymer (6–8)
Protein (g/kg/day)	Low salt protein (3–4)	Whole protein (2.5–3)
Fat (g/kg/day)	50–70% medium-chain triglycerides (8)	80–90% long-chain triglycerides (5–6)
Energy intake (estimated average requirement)	120–150%	120%

*Best provided as a modular feed in infants and as calorie supplements in older children.

On the waiting list

Many families find the waiting time pre-transplant very stressful and continued support from the multidisciplinary team is required. Mortality on the waiting list used to be as high as 25% prior to the development of reduction hepatectomy and split liver transplantation [4–6]. There are continued problems with donor shortages, particularly for adolescents who are competing with adults for grafts.

Liver transplant surgery

Liver transplantation involves three operations – the donor operation, the back-table operation, and the recipient operation. The logistics of coordinating these operations together with transplantation of other organs from the same multiorgan donor are complex, and the surgeon relies heavily upon the services of the transplant coordinators. Liver grafts can be retrieved from heart-beating cadavers, from non heart-beating donors, or from live donors (see later).

Role of the transplant coordinator

Potential cadaveric organs are notified to the procurement coordinator, who is responsible for establishing their preliminary suitability for transplantation, coordinating the multidisciplinary procurement team, and making arrangements at the donor hospital. Other duties include promotion of organ donation, education of health service professionals, and donor family support. The recipient coordinator is responsible for organizing the recipient operation, including travel arrangements for the patient, organization of theaters, anesthesia, blood bank, and intensive care, care of the recipient's family during the operation and postoperative follow-up. The live donor coordinator is responsible for coordinating the live donation assessment process and transplant procedure.

Matching the liver graft

The recipient of a cadaveric graft is selected on the basis of a compatible blood group, size matching, medical urgency, and time on the waiting list. Occasionally, blood group O may be given to blood groups A or B, if medically necessary.

An alternative option is to use an ABO incompatible (ABOi) graft. Isohemagglutins develop with age, suggesting that ABO matching is not required in children <1 year and these children tolerate these grafts without problems. The risk of complications after ABOi liver transplant is higher, but using rituximab has improved patient and graft survival [7].

When possible, grafts from CMV-positive donors are not given to CMV-negative recipients, but medical urgency may dictate otherwise. Unlike the kidney, there is no benefit from HLA matching, and hyperacute rejection is exceptionally rare in liver transplantation even in the presence of a positive cytotoxic crossmatch.

Liver grafts from cadaveric donors

Most livers are retrieved from cadaveric donors that have fulfilled the criteria of brainstem death. However, livers can also be used from donors after circulatory arrest (DCD) whose heart has stopped beating and have suffered a period of warm ischemia. These have become a progressively more important part of the cadaveric donor pool in the past decade, reaching up to 40% of transplantable grafts in some countries. The DCD process can be uncontrolled or controlled. Whilst the former has not been relevant for transplantation in children, the latter has been utilized in pediatric recipients and implies withdrawal of life support on an intensive care unit or in an operating theater. Good results have been reported using selected livers from young DCD with good liver function, limited functional warm ischemia, and short cold ischemia times [8, 9].

DCD livers are, however, associated with a greater risk of delayed graft function and of the development of ischemic-type biliary strictures and should be utilized in children only in selected circumstances.

Proper care of the donor is essential to maintain good-quality organs from donors after brain death (DBD). Brainstem death results in loss of central regulatory mechanisms that control the cardiovascular, respiratory, and endocrine systems. Donor resuscitation is directed at optimizing tissue perfusion and oxygenation, maintaining normal blood glucose and body temperature, and controlling sepsis. With the increasing demands for cadaver organs, previous criteria of donor suitability have been expanded. There is no absolute age limit, although younger donors are preferable for pediatric recipients. There are few absolute contraindications to organ donation for pediatric transplantation: death without a clear diagnosis, malignancy (excluding primary cerebral tumors), active systemic infection, hepatitis B and C and HIV infection, chronic liver disease, and prion infections such as Creutzfeldt–Jakob disease.

Abnormalities of routine liver function tests do not by themselves predict graft function and survival. A reliable method of assessing a potential liver graft is examination of its macroscopic appearance by an experienced transplant surgeon. Livers affected by cirrhosis, fibrosis, or severe steatosis are clearly unusable. Other marginal grafts may be considered in particular circumstances of urgency for pediatric recipients and in these cases histological examination of a frozen section biopsy of the liver may be helpful. Very recent experiences of normothermic graft perfusion and preservation may change selection procedures and improve the performance of livers prior to transplantation in the next decade. Currently the best livers from younger donors are considered for children in need of liver replacement.

Multiorgan donor operation

The liver is retrieved from a cadaver donor as part of a multiorgan operation in which the kidneys, pancreas, heart, lungs, bowel, and other viscera may all be removed simultaneously for transplantation. The donor after brain death is administered broad-spectrum antibiotics to prevent infection and maintained on a ventilator, in the operating theater until the moment of circulatory arrest. The organs are removed through a full-length midline incision. The iliac arteries/aorta and the superior/inferior mesenteric vein are prepared for cannulation. The liver is inspected and its arterial anatomy defined. The hepatoduodenal ligament is dissected, dividing the common bile duct close to the duodenum, whilst the common hepatic artery is traced to its origin from the celiac trunk. After the abdominal and cardiothoracic organs are also mobilized ventilation is discontinued, the abdominal organs are perfused with cold preservation solution through the aorta and portal vein, and the abdomen is packed with ice-slush to achieve rapid cooling. Once the cardiothoracic organs have been removed the liver dissection is completed. The hepatic artery is taken in continuity with a patch of aorta at the origin of the celiac trunk and of the superior mesenteric artery in case it gives origin to a right replaced or accessory artery. Similarly it is important to recognize and respect a potential accessory or replaced left artery originating from the left gastric artery. The portal vein is divided between the liver and the pancreas. The infrahepatic vena cava is divided just above the origin of the renal veins, and the suprahepatic vena cava is cut at its junction with the right atrium. After removal, the liver is brought to the back-table where the hepatic artery and portal vein are flushed again with preservation solution and the bile duct is extensively rinsed free of bile. The liver, immersed in cold preservation solution, is hermetically sealed in plastic bags, and transported in an icebox. Iliac, splenic, and superior mesenteric vessels are also retrieved for possible vascular reconstructions in the recipient (i.e., portal thrombosis or hypoplasia or in case of need of an arterial conduit), especially if the liver is split (see later).

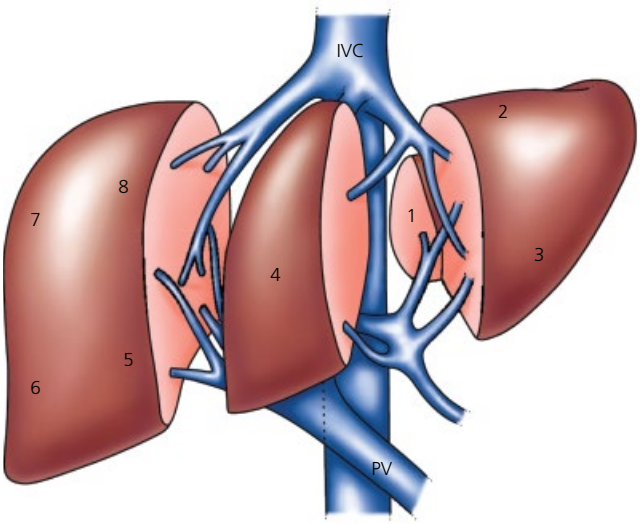


Figure 31.2 Schematic diagram of liver demonstrating eight segments. The left lateral segments, II and III, are most commonly used for reduction hepatectomy, split liver, living relation, or auxiliary liver transplantation.

Back-table operation, liver reduction, and liver splitting

The back-table operation is performed at the recipient hospital and is synchronized with the recipient operation. For a whole-liver graft, the back-table preparation requires clearance of diaphragmatic and lymphatic tissue from the graft and vascular pedicles and checking for vascular tributaries which require ligation. The majority of pediatric liver transplants require a split procedure to generate a size match segmental graft for a child and one for an adult recipient.

The majority of children needing liver transplantation are aged less than 5 years. In contrast, there are very few small pediatric organ donors. The shortage of suitable size-matched donor livers stimulated the development of techniques to produce smaller grafts suitable for children on the transplant waiting list [4, 5]. The principles of liver splitting are based upon the work of Couinaud, who described the segmental anatomy of the liver based upon blood supply and biliary drainage. The liver is divided into eight segments including four left lobe segments (I–IV) and four right lobe segments (V–VIII) [10] (Figure 31.2). The three main types of segmental liver grafts include the left lateral segment (segments II and III, 25–30% of the whole liver), which allows a size reduction from donor to recipient of up to 10 : 1. The left lobe (segments I–IV) provides a size reduction of 3 : 1 and the right lobe (segments V–VIII) of 1.5 : 1. Monosegments are seldom required for infants and provide a size reduction of up to 20 : 1. Recently a technique of monosegment II graft with thickness reduction has been introduced for transplantation in neonates [11].

Liver reduction was first used by Bismuth in Paris and proposed as a potential solution to the severe shortage of small pediatric livers. Although initial results were poor, the introduction of University of Wisconsin liver preservation solution, and technical modifications improved graft survival to match, and eventually surpass, results with whole livers in small children and infants. In the USA, partial liver grafts now account for 32% of all pediatric grafts and 56% of transplants in recipients aged less than 1 year, with excellent results. In particular, segmental grafts have a lower incidence of hepatic artery thrombosis compared with equivalent-sized whole liver grafts in small children because of larger size arteries and reduced vascular resistance. Pichlmayr in 1988 introduced the concept of split liver, so that the right lobe would not be discarded and a single liver could be used for two recipients (Figure 31.3).

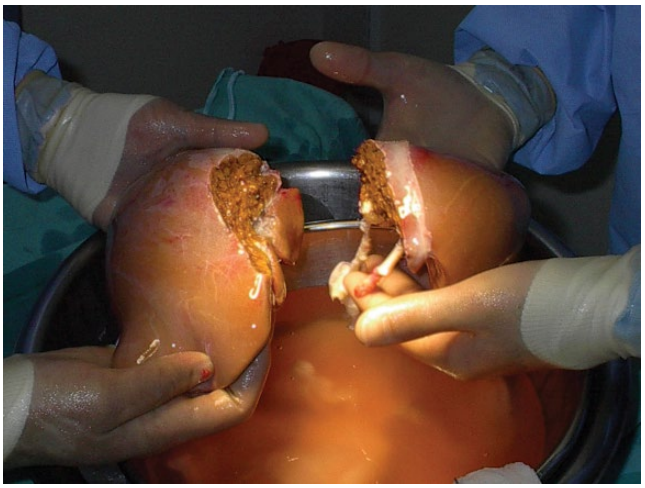


Figure 31.3 Conventional right extended lobe (segments I and IV–VIII) and left lateral segment (segments II and III) ex situ split in the preservation fluid bowl on the back-table.

In the conventional split the liver is divided along the plane of the falciform ligament to provide a left lateral segment graft (segments II and III) for pediatric transplantation, and an extended right lobe graft (segments IV–VIII) for transplantation into a larger child or adult. The common bile duct, portal vein, and the inferior vena cava are usually left to the right graft. The left lateral segment graft is drained by the left hepatic vein. The artery can be left to the left or right graft depending on different circumstances. Microvascular techniques may be used on the bench to lengthen short arterial stumps or during implantation to perform small arterial anastomoses. Biliary reconstruction is obtained with a Roux loop. This may be already present in a large percentage of children awaiting transplantation for biliary atresia and who have undergone a Kasai operation. For split grafts the liver may also be divided along the principal plane to provide full left and right lobes for older children.

The plane of the conventional split is a subject of debate as some surgeons perform the split 1 cm to the right of the falciform ligament and some directly on the left pedicle. Liver splitting can be performed either ex situ, on the back-table, or in situ as part of the donor operation. The ex situ procedure needs fewer resources and causes less inconvenience for the donor hospital, as the retrieval is shorter.

In situ splitting reduces the cold ischemic time, avoids potential back-table warming, makes it easier to share grafts with other centers, and provides superior cut surface hemostasis in the recipient. In situ split requires the availability of an expert surgeon and adds about 1–2 h to the operating time at the donor hospital with the potential to compromise other donor organs, the heart in particular. Long-term outcomes from ex situ and in situ split techniques have been similar and it seems more important the volume of splitting that a transplant center delivers than the technique itself.

Recipient operation

In the early days of liver transplantation, the operation was frequently complicated by extensive bleeding from raw surfaces, particularly in patients with adhesions from previous upper abdominal surgery. Patients with advanced liver disease have portal hypertension, thrombocytopenia, and deranged coagulation. During a long operation in a patient with poor liver function, hypothermia exacerbates the coagulopathy. In more recent times, a better understanding of coagulation disorders, improved monitoring, and more sophisticated hemostatic techniques have greatly reduced transfusion requirements.

Anesthetic monitoring includes serial measurements of blood gases, electrolytes, hemoglobin, and platelet count together with coagulation indices including thromboelastography. The thromboelastograph gives a pictorial representation of blood clotting and its interpretation allows the anesthetist to determine whether there is a defect in clotting factors or platelets, or whether antifibrinolytic drugs such as aprotinin are indicated.

The recipient liver transplant operation can be carried out through a bilateral subcostal incision; often with an upper midline extension or through a right hockey stick incision which spares the division of the left rectus muscle. The operation can be divided into three phases: hepatectomy in which the old liver is removed, the liver graft implantation, and the hemostatic phase.

Total hepatectomy

In the virgin abdomen, hepatectomy is usually straightforward. However, if there has been previous upper abdominal surgery such as a Kasai portoenterostomy or a previous liver transplant, then the dissection may be much more difficult due to the presence of vascular adhesions in the context of portal hypertension. The liver is mobilized from the diaphragm and the ligaments. The porta hepatis is dissected and the bile duct (or Kasai portoenterostomy), hepatic artery, and portal vein divided. The liver is then filleted from the vena cava and removed by dividing the hepatic veins. Alternatively, the old liver can be removed en bloc with the retrohepatic vena cava, particularly in cases of liver malignancy, i.e., hepatoblastoma, to achieve a radical excision of the tumor.

Graft implantation

Implantation of small grafts, which can rewarm quickly, needs to be performed rapidly to minimize warm ischemic injury. For a whole graft the vena cava and portal vein are anastomosed to the equivalent recipient vessels, allowing a "growth factor," to allow the anastomosis to expand, when tying the sutures. For a left lateral segment graft, the left hepatic vein and the confluence of the recipient's hepatic veins with the vena cava are both shaped in a triangular fashion for anastomosis. This avoids torsion and outflow problems of the left hepatic vein. Before reperfusion the liver

is flushed with albumin or normal saline to rinse the preservation fluid, and the venous clamps removed. After reperfusion of the graft with blood from the portal vein, the arterial anastomosis is carried out using microvascular techniques and magnifying loupes.

Conduits using donor iliac artery anastomosed to the infrarenal or supraceliac aorta are utilized as arterial inflow in small children and in those with an unsuitable native hepatic artery. A hypoplastic portal vein (commonly associated with biliary atresia) can be corrected with a venoplasty or replaced interposing donor iliac vein.

Hemostasis

Prior to completion of implantation it is essential to provide accurate and systematic hemostasis with sutures, diathermy, argon beam, and a range of hemostatic products including fibrin glue, thrombin-based preparations, and cellulose. Likely bleeding sites include the bare areas left by the hepatectomy, the anastomotic suture lines, or from the graft. A hemostatic pause may also be useful as it provides time for the anesthetist to assess and correct clotting defects. As hemostasis implies repeated manipulation of the liver, which would disrupt a fine biliary anastomosis, it is important to wait for the surgical field to be "dry" before proceeding with the final anastomosis: the biliary reconstruction. In adults and larger children without previous biliary pathology the donor and recipient common bile ducts are anastomosed together. Children with left lateral segment grafts and those with a previous Kasai operation require a hepaticojejunostomy for biliary drainage using a Roux-en-Y loop. Biliary complications are more common in small children, due to the small caliber and to the weaker blood supply to the bile duct of a segmental liver.

After completion of all the anastomoses the operative field is checked once again for hemostasis. Cardiovascular stability, spontaneous improvement of metabolic acidosis and clotting, and production of bile are all early signs that the graft is working. In case these signs are slow to appear and there is suspicion of graft dysfunction *N*-acetyl-cystein (NAC) is given intravenously. Intraoperative Doppler ultrasound is routinely performed prior to closure to exclude any inflow or outflow problems. In approximately one-fourth of the transplants for small children the liver graft may be too large and full abdominal closure would compromise hepatic venous outflow and ventilation. In such circumstances partial closure is recommended using a temporary silastic or a permanent biological mesh to widen the muscle layer before closing the skin. Large grafts shrink after a few days and the silastic mesh can then be removed allowing for full closure.

Live-donor liver transplantation

The first report of a live-donor liver transplant (LDLT) using a left lateral segment was by Raia *et al.* in 1989 followed by Strong *et al.* in 1990. The technique involves the resection of a part of the liver of a parent and transplanting it into their child. LDLT was further developed in the late 1980s at the same time in Japan and Chicago to overcome the shortage of suitable donors [12].

There are clear benefits of LDLT. It can be performed as a planned elective procedure, thus reducing waiting times. There is no deterioration or mortality on the waiting list. The quality of liver graft is excellent and the preservation time is minimal. Thus patients are transplanted in better clinical condition with lower probability of complications.

The main disadvantage is the risk of causing harm to the donor, who is an otherwise healthy individual. There have been reports of several donor deaths; the reported donor mortality is between 0.1% and 0.5% depending upon the extent of the hepatectomy. Donor complications include hemorrhage from the cut surface, bile leak, infection, and incisional hernia. There is also a high incidence of gastritis and peptic ulceration. Postoperative pain is difficult to control despite most centers making routine use of epidural analgesia.

Parents considering live donation must be fully informed of the risks of the procedure and of the prospects of finding a cadaver graft before making a decision. LDLT may be a life-saving choice for children with acute liver failure when/if a suitable cadaveric graft is not available, in children with severe decompensation of their chronic liver disease or in the narrow window post-chemotherapy in children with hepatoblastoma, when it is essential to choose the ideal time point for transplantation.

The technical and organizational aspects of the procedure are complex.

A careful assessment process is essential to ensure that potential donors can provide a suitable graft to the recipient in terms of size, quality, and blood group maintaining the highest levels of safety for the donor. Donation must be altruistic, and coercion by other family members or friends must be ruled out. All donors must be counseled about the perioperative risks and possible long-term complications of the procedure and should also have a formal psychological assessment.

Preoperative investigations include liver function tests, serology, and imaging to assess the size of the intended segmental graft as well as details of its vascular and biliary anatomy. Imaging and FibroScan® may indicate abnormal graft parenchyma, in particular fatty change; a liver biopsy may be necessary to confirm the suitability of the liver as a graft. CT scans may be processed with specific software to estimate the volume of the potential graft and of the future liver remnant and also the contribution of each segment to the graft size. Transplantation from a healthy donor requires a liver graft that weighs at least 0.8% of recipient body weight.

For a child up to 25 kg, the liver graft from a live donor is usually a left lateral segment (segments II and III), which provides 150–450 g of liver mass. However a full left (segments I–IV) or right lobe (segments V–VIII) graft may

be necessary for larger children. Recipients receiving a relatively small graft may develop small-for-size syndrome, characterized by synthetic dysfunction, high serum transaminases, prolonged cholestasis ascites, and vulnerability to infections.

The left lateral donor operation has similarities to the in situ split procedure and can be performed through a xypho-umbilical midline incision. The left branches of the hepatic artery, portal vein and bile duct, and the left hepatic vein are identified and the liver parenchyma divided approximately 1 cm to the right of the falciform ligament. Once the parenchyma is transected, the left bile duct is divided with part of the hilar plate. The vascular structures are then clamped and divided and the graft moved to the back-table where it is flushed with cold preservation. Currently most centers use histidine–tryptophan–ketoglutarate (HTK) solution, which is less viscous than UW® solution and does not need rinsing prior to reperfusion.

Implantation is identical to that for the cadaver left lateral segmental grafts (see earlier). Microsurgical techniques and optical loupes are required for the arterial anastomosis to reduce the risk of postoperative thrombosis.

Laparoscopic left lateral segment for adult to child LDLT yields similar short-term donor outcomes as laparoscopic donor nephrectomy. It is likely that in the near future the laparoscopic approach will be considered a new standard practice for retrieval of left lateral segment liver grafts as it is today for kidney donation [13].

In the USA approximately one-third of pediatric liver transplants are now performed from living donors and results compare favorably with those obtained using cadaver grafts. In the UK a mandatory policy on splitting livers from donors after brain death of less than 40 years of age has maintained the need for live-donor transplantation in children.

Auxiliary liver transplantation

Auxiliary liver transplantation is an accepted treatment option for select children with acute liver failure and metabolic liver disorders. It involves transplanting part of or a whole liver graft leaving a portion of the native liver [14]. In children with acute liver failure, auxiliary partial orthotopic liver transplantation involves transplanting a relatively large mass of liver graft which allows native liver recovery and avoidance of lifelong immunosuppression and its consequences. In those patients who demonstrate recovery, the auxiliary graft can support the patient until the native liver has adequately regenerated, after which time immunosuppression can be gradually discontinued and the allograft allowed to slowly reject and atrophy.

Criteria used to select potential auxiliary transplant recipients included availability of a graft of excellent quality from a young donor, young age of the recipient, and stable cardiovascular and neurological condition preoperatively.

The native liver resection needs to be extensive to minimize the toxic liver syndrome. The mass of the graft needs to be significant to avoid small-for-size syndrome. The procedure has not gained widespread popularity due to its complexity and the risks of complications including renal failure and bleeding.

Regeneration of the native liver is evaluated by cross-sectional and nuclear medicine imaging, and histology at intervals of 3–6 months and then yearly. Paracetamol- or drug-induced liver failure and seronegative hepatitis are all recognized indications. Acute liver failure secondary to autoimmune hepatitis or Wilson disease are relative contraindications to auxiliary transplantation and require a full liver replacement. Patient survival has been reported at 85% at 1 and 10 years [15]. Complete immunosuppression withdrawal is achievable in more than 70% of children. Therefore auxiliary partial orthotopic liver transplantation should be considered in children with acute liver failure and meeting criteria for emergency liver transplantation.

The other indication for partial auxiliary liver transplantation is non-cirrhotic metabolic liver disease, where the liver structure is normal but a defective enzyme causes severe and life-threatening extrahepatic complications. Part of the native liver will be replaced with an auxiliary graft to replace the deficient enzyme (e.g., Crigler–Najjar syndrome type I). In this situation only a small amount of normal liver (approximately 5% from experimental studies) is needed to compensate for the metabolic defect, and it usually is sufficient to replace the left lateral segments (II and III) with an equivalent donor graft. Reports suggest that this is successful in reducing the levels of unconjugated bilirubin in Crigler–Najjar syndrome type I.

The advantages of auxiliary transplantation in this setting are two-fold: in case of graft failure the native liver remains as a safety net and the patient will be back to his pre-transplant status. The graft can be removed and immunosuppression stopped.

The second benefit is that of retaining the potential for weaning immunosuppression and graft removal in recipients with a long life expectancy if the gene affected can be replaced. Recent advances have been made in liver-directed gene therapy, or by hepatocyte transplantation.

The main disadvantage with auxiliary partial orthotopic liver transplantation is the functional competition between livers with frequent progressive atrophy of the graft. This may require native portal vein embolization or lead to re-transplantation.

Metabolic liver diseases which are unsuitable for auxiliary partial orthotopic liver transplantation include primary oxalosis in which the enzyme deficiency contributes to an excess of oxalate production, and cirrhotic metabolic diseases such as Wilson disease or tyrosinemia in which there is a risk of malignancy in the retained liver.

Postoperative management

Ventilation

Patients are initially managed in the intensive care unit (ICU) (see Chapter 30) until their condition has stabilized. It is good practice to ensure that liver function is satisfactory and that there is good hepatic artery and portal vein flow on Doppler ultrasound before discontinuing ventilation. Large upper abdominal incisions are painful in the early postoperative period, necessitating epidural analgesia, intravenous morphine (according to body weight), or alfentanil (0.5 mg/kg/min), which may depress respiration. Young infants with severe malnutrition due to chronic liver disease or patients in hepatic coma due to fulminant liver failure may spend a prolonged period in ICU, but the majority of patients return to the ward within 24–48 h postoperatively.

Fluid management

The principles of fluid management are to maintain circulating volume by providing two-thirds of maintenance fluids with crystalloid, while half-replacing wound drain losses with 4.5% albumin as long as urine output is in excess of 1 mL/kg/h and central venous pressure is satisfactory (>5–6 mmHg). Patients are often vasoconstricted and relatively hypovolemic on return from theater due to fluid losses, especially if there has been preoperative ascites, hypothermia, and the use of intraoperative inotropes. Extra colloid fluid replacement with 4.5% albumin and inotropes, such as dopamine (2–5 mg/kg/min) may be necessary. Hemoglobin should be maintained between 8 and 10 g/L. Excessive blood transfusion is contraindicated as a postoperative hemoglobin of <10 g/L reduces the risk of hepatic artery thrombosis. Venesection is recommended for any patient with a hemoglobin >11 g/L during the first 2 postoperative weeks (Box 31.3).

Immunosuppression

Adequate immunosuppression is required to support graft function and should be balanced against the risks of side effects and over-immunosuppression. There is no agreed standard of care for immunosuppression choice and dose. Most centers use calcineurin inhibitor (CNI)-based regimens, mainly tacrolimus to avoid the gingival hyperplasia and hirsutism associated with ciclosporin. Addition of mycophenolate mofetil or m-TOR inhibitors such as sirolimus or everolimus may reduce the need for steroids and high-dose CNI in the long term.

Current protocols consist of the effective CNIs with or without anti-interleukin 2 receptor antibodies (IL-2 antibodies) (basilimax) [16] for induction and renal-sparing drugs (such as mycophenolate mofetil or sirolimus for maintenance. Steroid-free immunosuppression both at induction and for maintenance are used by some centers.

Protocols (Table 31.2) consist of:

- Ciclosporin microemulsion (Neoral®), prednisolone, and azathioprine (which is now rarely used).
- Tacrolimus with prednisolone.
- Basilimax with tacrolimus, with or without mycophenolate mofetil (steroid free).

The majority of units use steroids at induction, gradually reducing them over the first 2 weeks. Some units withdraw or reduce to alternate-day therapy after 3 months to improve growth. Azathioprine, if used, is usually discontinued after 1 year. Ciclosporin or tacrolimus are continued for life. Immunosuppressant drug monitoring is based on trough levels (see Table 31.2).

Box 31.3 Postoperative management of liver transplantation.

Fluid management

- Maintain:
  - CVP >6 cm H_2O
  - urine output >1 mL/kg/h with 4.5 or 20% albumin or 5–10% dextrose
  - Hb <11 g/L

Prophylactic antibiotics

- Tazocin 90 mg/kg/dose t.d.s. i.v. (if penicillin allergic: ciprofloxacin 5 mg/kg/dose b.d.) for 48 h or until after the abdominal drain is removed
- Metronidazole 8 mg/kg/dose t.d.s. over 1 h for 48 h (or rectally)
- Nystatin: 50,000 units orally q.d.s. if <10 kg; 100,000 units orally q.d.s. if >10 kg
- Amphotericin 1 mL/day orally
- If CMV-positive donor, aciclovir 500 mg/m^2/i.v. dose t.d.s. over 1 h
- Co-trimoxazole <5 years 240 mg o.d. p.o.; >5 years 480 mg o.d. p.o. for 6 months
- Amphotericin 3 mg/kg o.d. i.v. for 7–10 days for patients with acute liver failure, re-transplants

Antiplatelet therapy

- Aspirin 3 mg/kg/day per rectum or nasogastric tube (maximum 75 mg)
- Dipyridamole: if <10 kg, 25 mg t.d.s. orally for 3 months; if >10 kg, 50 mg t.d.s. orally
- Anticoagulation (if necessary)
- Heparin (60–120 units/kg/day) to maintain PT 20–30 s

Antacids

- Ranitidine 3 mg/kg/dose t.d.s.
- If gastric pH <5, omeprazole 10–20 mg i.v. b.d.

Antihypertensives

- Acute
  - labetalol 1–3 mg/kg/h
  - nifedipine 0.25–0.5 mg/kg/dose, max. 10 mg/dose sublingually PRN
  - sodium nitroprusside: 0.5–5 µg/kg/min
- Chronic
  - nifedipine 5–10 mg t.i.d.
  - atenolol 25–50 mg/day

Table 31.2 Immunosuppression with interleukin (IL)-2 antibodies.

	Trough levels (ng/mL)	
	Tacrolimus	Tacrolimus with mycophenolate mofetil
Day 1–14	11–14	N/A
Day 15–56	7–11	3–5
>8–12 weeks	8–12	4–7
3–12 months	3–8	3–5
>12 months	3–5	2–4
	Prednisolone: 2 mg/kg ± Azathioprine: 1–2 mg/kg for 12 months	

Tacrolimus: initial dose 0.2 mg/kg, then 0.05 mg/kg b.d.
Basiliximab (10–20 mg/kg): intraoperatively and day 4
Mycophenolate mofetil: 15 mg/kg b.d.

Tacrolimus is available as granules for infants (Modigraf®), capsules (Prograf®), and Advagraf® (a long-acting form of tacrolimus).

Mycophenolate mofetil 10–40 mg/kg is an antiproliferative agent which is similar in action to azathioprine and may depress the bone marrow. It is effective induction when combined with a CNI drug and an IL-2 antibody and may be used as monotherapy for maintenance if renal dysfunction is an issue. There are significant gastrointestinal and hematological side effects, particularly immediately postoperatively and it is not recommended in pregnancy because of reported teratogenic effects. However, it has no cosmetic side effects, is renal sparing, and does not require drug monitoring, although it is possible to measure mycophenolic acid (MPA) pharmacokinetics, there are little data on effective therapeutic levels.

Anti-IL-2 receptor antibodies are monoclonal antibodies, which selectively target the IL-2 receptors on activated T cells, which is a key step in the development of cell-mediated immunity. Only one antibody is commercially available, basiliximab, which is renal sparing and provides effective induction immunosuppression post-transplant in combination with a CNI.

Sirolimus is a macrocyclic triene antibiotic which prevents T-cell proliferation by inhibiting cytokine production and does not inhibit calcineurin. Sirolimus has been evaluated as both primary and rescue immunosuppression for liver transplant recipients and has the advantage of being both renal sparing and reducing the need for high-dose steroids [17]. Significant side effects include delayed wound healing, hyperlipidemia, and an increase in the rate of hepatic artery thrombosis, and it should not be used immediately post-transplant but may be useful for chronic rejection. Everolimus, which is similar to Sirolimus is being evaluated in children for maintenance therapy.

Prophylactic antibiotics

Broad-spectrum antibiotics are prescribed for 48 h unless there is continuing infection (see Box 31.3). Systemic antifungals, fluconazole, or liposomal amphotericin should be continued for 14 days in children with acute liver failure or those undergoing a second laparotomy for complications. Whilst patients are on steroids it is advisable to give low-dose co-trimoxazole or trimethoprim as prophylaxis against *Pneumocystis carinii* infection. Oral nystatin and amphotericin to prevent oral and esophageal candidiasis may be continued for 6–12 months.

Prophylaxis for CMV infection is required for CMV-negative recipients of a CMV-positive donor. Aciclovir (1500 mg/m^2/day i.v. or 200–400 mg/dose q.d.s. orally) or ganciclovir (5 mg/kg) prevents infection in the short term when immunosuppression is intense. There is no satisfactory prophylaxis for EBV, although some units use aciclovir or ganciclovir.

Other medications

The incidence of stress ulcers and excess gastric secretion used to be a problem in children recovering from liver transplantation, particularly those on high doses of steroids, but is reduced with steroid-free regimes. It is important to prevent steroid-induced peptic ulceration with ranitidine (3 mg/kg/dose t.d.s.) or omeprazole (10–20 mg i.v. b.d.).

Antiplatelet drugs, aspirin, and dipyridamole are prescribed to prevent vascular thrombosis and discontinued at 3 months. Intravenous heparin and/or warfarin may be indicated for children with a high risk of thrombosis.

Antihypertensive medication is usually required because of the effects of the immunosuppressive therapy. Nifedipine (5–10 mg/dose) and/or atenolol (25–50 mg/dose) are usually adequate for immediate use.

Postoperative complications

Early postoperative complications

Complications in the early postoperative period may be due to:

- The preoperative condition of the recipient (e.g., malnutrition, sepsis, renal failure).
- The quality of the graft (e.g., primary non-function, acidosis, coagulopathy).
- Surgical complications (e.g., intra-abdominal hemorrhage, vascular thrombosis, venous outflow obstruction).
- Side effects from drugs (e.g., CNI renal failure, hyperglycemia or neurotoxicity.

A number of factors may predispose to postoperative renal dysfunction. Some patients have impaired renal function preoperatively which may be aggravated by intraoperative cardiovascular instability requiring inotrope support. In such patients, induction therapy with IL-2 antibodies allows the use of low-dose CNI or a delay in their introduction. Alternatively, renal-sparing drugs such as mycophenolate mofetil, sirolimus could be administered once the initial postoperative period is past.

Oliguria is common and should be managed by ensuring adequate fluid replacement or furosemide challenge (1–2 mg/kg i.v., or infusion 0.25 mg/kg/h). Anuria with a rising urea, creatinine, or potassium requires renal hemodialysis or filtration and may be associated with graft dysfunction.

The main causes of graft loss in the first week include:

- Primary non-function (PNF).
- Hepatic artery or portal vein thrombosis.
- Systemic sepsis.
- Hyperacute rejection which is now rare and occurs on day 4–5.

Primary non-function is a serious complication which requires immediate re-transplantation. It may be suspected if there is persistent coagulopathy, acidosis, a high potassium, and high transaminases.

If secondary to hyperacute rejection, the diagnosis can only be made by liver biopsy (which may be impractical) or by identification of raised immunoglobulins. Treatment consists of re-transplantation or an increase in immunosuppression.

Hepatic artery thrombosis occurs in 10% of children. The incidence has fallen following the introduction of reduction hepatectomy and split liver grafts with the use of larger donor blood vessels (Figure 31.4). Medical prevention of hepatic artery thrombosis is by maintaining a low hematocrit (<10 g/hemoglobin) and the use of antiplatelet agents such as aspirin (3 mg/kg/day) and dipyridamole (25–50 mg t.d.s.).

Portal vein thrombosis is less common. The diagnosis of hepatic artery or portal vein thrombosis is made by Doppler ultrasound and confirmed by angiography or MRI angiography. Treatment includes:

- Immediate laparotomy with thrombectomy or thrombolysis.
- Anticoagulantion: intravenous heparin initially, followed by oral warfarin
- Re-transplantation.

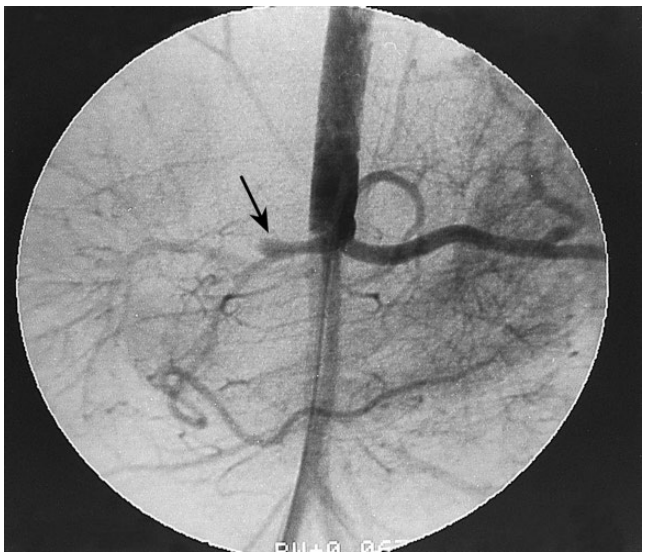


Figure 31.4 Hepatic angiogram indicating a patent celiac axis but absent hepatic artery secondary to hepatic artery thrombosis. The patient was re-transplanted successfully.

Re-transplantation is not always required as collateral blood vessels may develop. Late complications of hepatic artery thrombosis include biliary leaks and strictures or hepatic abscesses [16].

Systemic sepsis is treated as indicated with broad-spectrum antibiotics and antifungals. Re-transplantation is not indicated if sepsis leads to multiorgan failure and graft non-function.

Hemorrhage from the cut surface of the liver is a rare complication and is managed conservatively unless there is confirmed bleeding or hemodynamic instability. Abdominal tamponade may decrease renal blood flow causing renal failure. There is often a collection at the cut surface of the liver which usually resolves, but may need to be aspirated if large or a source of infection.

A major *ABO incompatibility* occurs when an organ from a patient of blood group A,B or AB has been transplanted into a patient without the corresponding A or B antigens, e.g., an 'A' organ into a group 'O' patient or an 'AB' organ into an 'A' patient. It may cause hyperacute rejection or antibody-mediated accelerated rejection of the allograft. The greatest risk is in the first hours and days after surgery. ABO incompatibility rarely occurs in children under 1 year [7].

Treatment consists of removing the antibodies by plasma exchange or suppressing them with mycophenolate, azathioprine or rituximab and intravenous immunoglobulins.

A minor incompatibility occurs when an organ from a patient who lacks A or B antigens has been transplanted into a patient with that antigen, e.g., an 'O' organ into an 'A' recipient. This rarely causes rejection.

Complications after the first postoperative week

Complications arising after the first postoperative week include:

- Acute rejection.
- Biliary leaks/strictures.
- Persistent wound drainage or ascites.
- Sepsis.
- Neurological abnormalities.

Acute rejection is less common in infants (20%) but increases to 50–60% in older children. The clinical signs and symptoms include fever, irritability, and abdominal discomfort. The diagnosis is confirmed by detecting a rise in bilirubin, alkaline phosphatase, aspartate and alanine transaminases, and γ-glutamyl transpeptidase (GGT). The onset of acute rejection may be delayed by the use of basilimax until 4–6 weeks post-transplant. It is always necessary to have histological confirmation.

Acute rejection is defined by the Banff criteria as the combination of a mixed inflammatory infiltrate in portal tracts with subendothelial lymphoid infiltration (endothelialitis) and

inflammatory infiltration of bile ducts (Figure 31.5). Treatment is with pulse methylprednisolone (20–40 mg/kg/day) intravenously over 2 or 3 days and an increase in baseline immunosuppression. If there is inadequate histological or biochemical response, treatment with methylprednisolone may be repeated but conversion to a more potent immunosuppressive drug such as sirolimus may be required.

Chronic rejection occurs in <10% of children at any time post-transplant. The diagnosis is suggested by the gradual onset of jaundice, pruritus, and pale stools which indicate biliary obstruction. Biochemical changes include a higher rise in bilirubin, alkaline phosphatase, and GGT than in transaminases. The Banff criteria define the histological features as biliary epithelial changes affecting a majority of bile ducts with or without duct loss; foam cell obliterative arteriopathy; or bile duct loss affecting >50% of portal tracts (Figure 31.6). Most children respond to an increase in immunosuppression, such as the addition of mycophenolate mofetil or conversion to tacrolimus or sirolimus, but some require re-transplantation.

The incidence of *biliary complications* has increased with the use of reduction hepatectomies and split liver grafts. Biliary strictures may be secondary to an anastomotic stricture, edema of the bile ducts or hepatic artery ischemia. Biliary leaks may be secondary to leakage from the cut surface of the liver or from hepatic artery ischemia. Most biliary leaks will settle with conservative management. Large leaks causing biliary peritonitis, biliary abscesses, or sepsis will require surgical drainage and reconstruction. The majority of intrahepatic biliary strictures are now managed medically with ursodeoxycholic acid (10–20 mg/kg) or radiologically using percutaneous transhepatic cholangiography (see Chapter 5). The dilated biliary tree is cannulated and external biliary drainage established (Figure 31.7A,B). Biliary dilatation may be performed using balloons and biliary stents. Surgical reconstruction is required for the management of extrahepatic biliary strictures or if interventional radiology fails.

Persistent drain losses may be due to preoperative ascites or secondary to rejection, sepsis, hepatic vein obstruction, or peritonitis. It may lead to acidosis and coagulopathy or an enhanced coagulable state due to loss of bicarbonate and coagulation factors. Treatment is of the primary cause, fluid restriction, and diuretics. Hepatic vein outflow obstruction may be related to the use of the "piggy-back" technique for anastomosis. It may be difficult to detect on ultrasound alone and may require venography and stenting.

Sepsis is still the commonest complication following liver transplantation (60–70%) [3]. The majority of infections are bacterial infections related to central line insertion

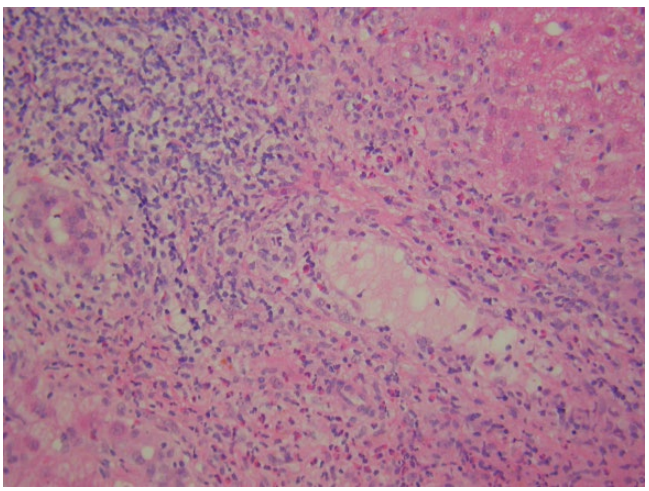
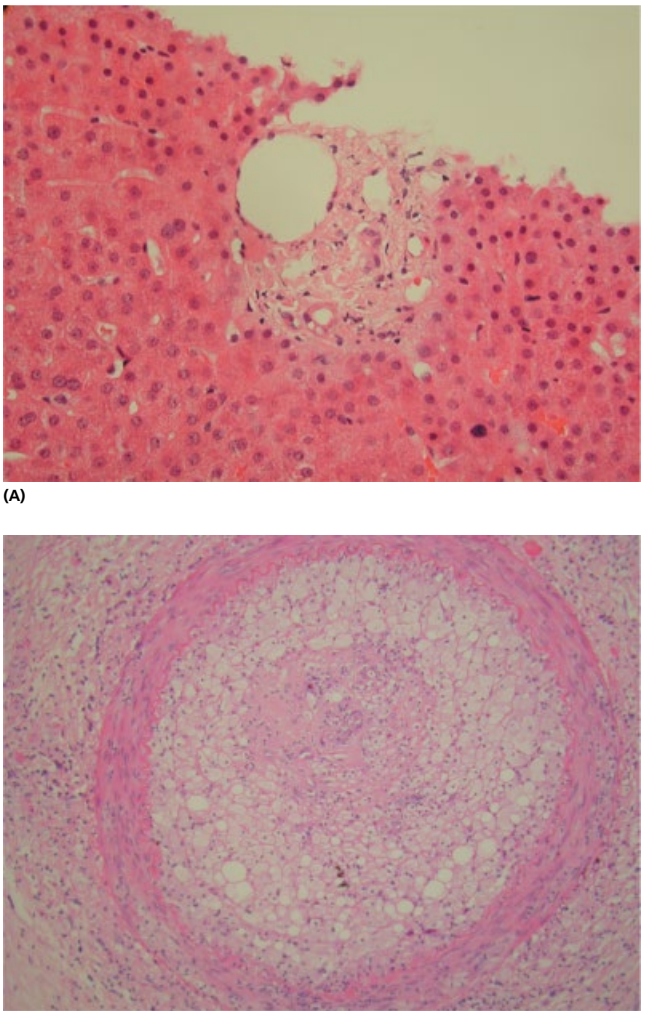


Figure 31.5 Liver histology demonstrating acute rejection. There is a mixed inflammatory infiltrate of the portal tract, with eosinophils, endotheliitis, lymphoid infiltration, and inflammation of the bile ducts. There is some hepatocyte loss. (H&E, ×200.)



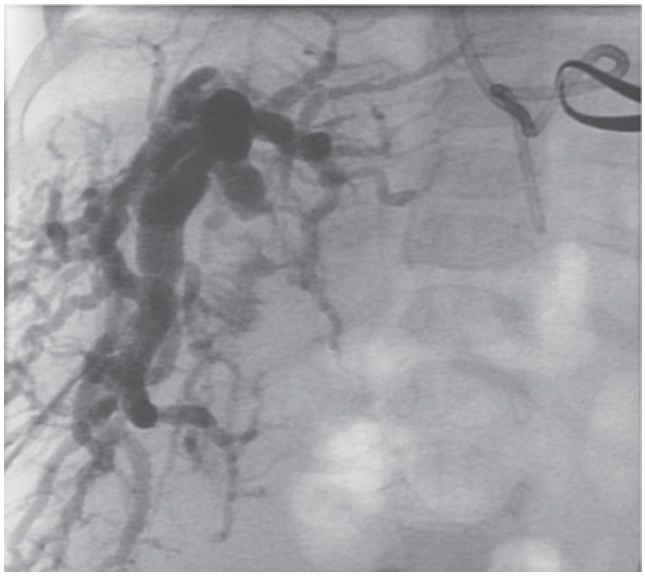
(A)

(B)

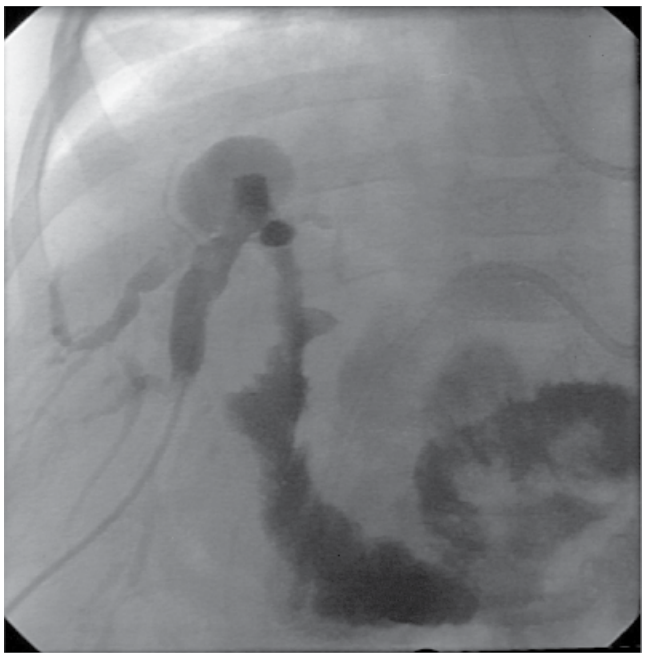
Figure 31.6 Liver histology demonstrating chronic rejection. There is an inflammatory infiltrate in the portal tract with loss of bile ducts "vanishing bile duct syndrome" (A) (H&E, ×200). In the later stages there is a foam cell arteriopathy (B) (H&E, ×100).

(*Streptococcus faecalis* and *S. viridans*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). Fungal infections with *Candida albicans* and *Aspergillus* spp. are documented in approximately 20% of patients, especially in patients with fulminant hepatitis with acute hepatic necrosis pre-transplant. Vancomycin-resistant *Enterococcus* (VRE) is a common gut pathogen, but systemic infection requires treatment with Synercid® or Linezolid®.

Neurological side effects, such as convulsions were common following the use of intravenous ciclosporin or high-dose tacrolimus. The development of convulsions is still reported as is the development of a reversible posterior leukoencephalopathy related to toxic CNI levels.



(A)



(B)

Figure 31.7 Biliary obstruction occurs in 20% of children post-liver transplant due to hepatic artery ischemia or a biliary anastomotic stricture (A). Following balloon dilatation of the stricture, using a percutaneous transhepatic approach, there is good drainage of contrast from the intrahepatic bile ducts across the biliary anastomosis into the jejunal Roux loop (B).

Late complications post-liver transplant

Late complications (after 3 weeks) may occur at any time post transplant. They include:

- Side effects of immunosuppression.
- CMV or EBV infection.
- Post-transplant lymphoproliferative disease (PTLD).
- Late biliary stricture.
- Late hepatic artery or portal vein thrombosis.
- Acute or chronic rejection.

There are numerous side effects of immunosuppressive therapy (Table 31.3). Some are short term, such as hypertension secondary to steroids, while stunting, nephrotoxicity, and increased risk of viral infection are lifelong. Hirsutism and gingival hyperplasia are side effects of ciclosporin which, although cosmetic, have an important effect on quality of life, and so ciclosporin is rarely used now. With careful monitoring of immunosuppression to ensure adequate trough levels, nephrotoxicity should be minimized.

CMV infection occurs 5–6 weeks post-transplant despite prophylaxis with aciclovir or ganciclovir. It is more common in children than adults, reflecting the number of CMV-negative children undergoing liver transplantation. The risk of CMV disease as opposed to CMV infection is indirectly related to receiving a transplant from a CMV-positive donor.

Primary CMV infection has the highest morbidity and mortality, while reactivation of latent infection or superinfection

Table 31.3 Immunosuppressive complications post-transplantation.

Drug	Complications
Steroids	Stunting Hypertension Cushingoid facies
Ciclosporin A	Hirsutism Gingival hyperplasia Hyperlipidemia
Ciclosporin/tacrolimus	Renal dysfunction Hypertension Neurotoxicity Hyperglycemia
Tacrolimus	Cardiomyopathy Food allergy
Sirolimus Everolimus	Wound healing/thrombosis Infection/mouth ulcers

with a new CMV strain is a milder illness. CMV disease presents with fever and hematological abnormalities (leukopenia, atypical lymphocytosis, and thrombocytopenia. Tissue-invasive CMV disease is associated with visceral organ involvement (gastrointestinal tract, liver, lungs). CMV has also been associated with rejection, fungal infection, and late patient and graft loss. Treatment is with intravenous ganciclovir (5 mg/kg) and hyperimmune CMV globulin dose.

The development of *primary EBV* is an important cause of morbidity and mortality. Monitoring by polymerase chain reaction (PCR) of EBV levels and subsequent immunomodulation has significantly reduced mortality.

Approximately 65% of children undergoing liver transplantation will be EBV-negative pre-transplant and 75% of this group will have a primary EBV infection within 6 months of transplantation. It is important to diagnose primary EBV infection and reduce immunosuppression, if possible, in order to prevent further progression to lymphoproliferative disease.

There is a close relationship between primary EBV infection and the development of lymphoproliferative disease. The spectrum of B-lymphocyte proliferation ranges from benign hyperplasia to malignant lymphoma.

EBV has a wide spectrum of disease, from asymptomatic seroconversion to a non-specific viral illness and/or PTLD, usually during the first year post-transplant. Most patients have symptoms of infectious mononucleosis (fever, malaise, exudative pharyngitis, lymphadenopathy, hepatosplenomegaly, and atypical lymphocytosis) although hepatitis, pneumonitis, gastrointestinal symptoms and hematological manifestations (leukopenia, thrombocytopenia, hemolytic anemia, and hemophagocytosis) may also occur.

PTLD tends to affect organs of the reticuloendothelial system and/or the transplanted liver, although other tissues such as brain and iris have been reported.

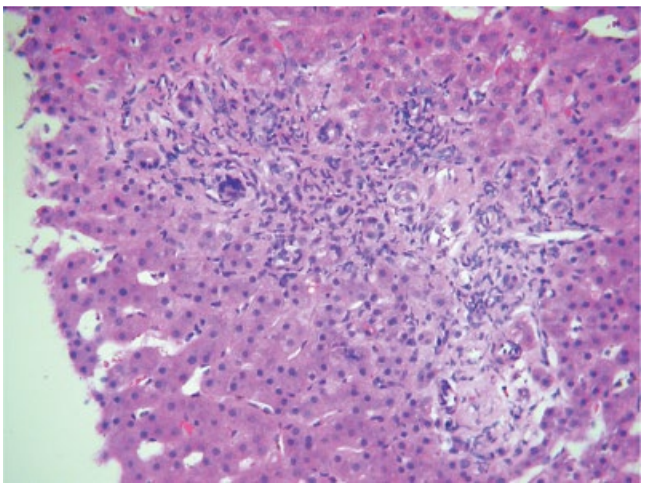
The diagnosis is based on identifying the characteristic histology from the affected tissue, which may demonstrate polymorphic B-cell proliferation or lymphomatous features of nuclear atypia and necrosis. EBER staining for EBV is positive (Figure 31.8A,B). Immunofluorescent staining of heavy-chain and light-chain immunoglobulins may differentiate monoclonal from polyclonal infiltrates. Almost any organ in the body may be affected, although the liver and gut are most commonly involved. There is no difference in incidence between tacrolimus and ciclosporin.

Treatment includes:

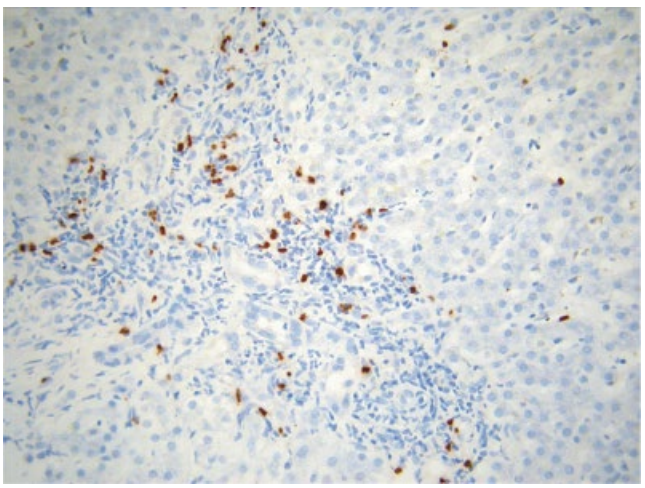
- Reduction of immunosuppression.
- Intravenous aciclovir (3 mg/m^2) or ganciclovir (6–10 mg/kg) may be effective.
- Intravenous rituximab (an anti-CD20 monoclonal antibody against B cells) 375 mg/m^2 body surface area repeated at weekly/monthly intervals with intravenous immunoglobulins.
- Infusion of autologous T cells directed against EBV cells.
- Low-dose chemotherapy with cyclophosphamide and prednisone.
- Chemotherapy which is tailored to the type of lymphoma is necessary if the lymphoproliferative disease becomes overtly malignant.
- If the reduction in immunosuppression leads to rejection, then balancing therapy is difficult.

The incidence of *late hepatic artery thrombosis* is between 3% and 10%. And may be difficult to detect as there may be collateral arterial flow into the transplant liver. Since blood supply to transplanted bile ducts is only from the hepatic artery, hepatic artery thrombosis at any time is associated with biliary complications leading to biliary fibrosis or cirrhosis. Hepatic abscesses may also develop secondary to ischemia. Treatment of late hepatic artery thrombosis is re-transplantation [16].

Late *portal venous thrombosis* occurs between 2% and 10%, but most cases are asymptomatic because collateral

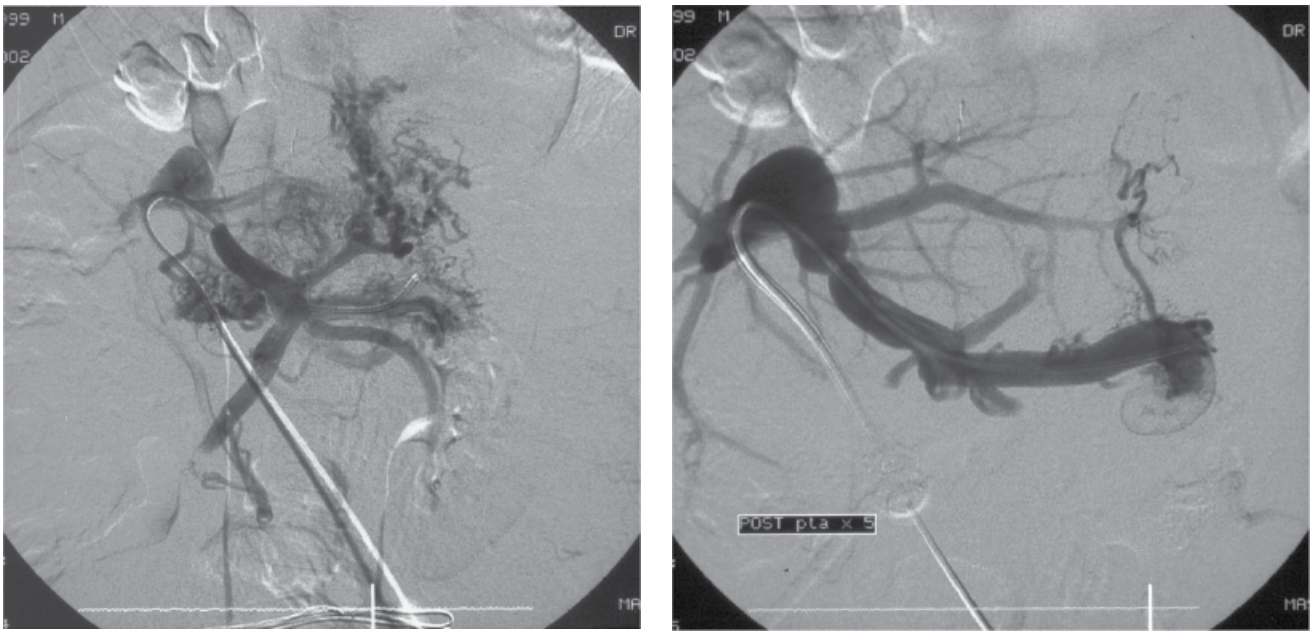


(A)



(B)

Figure 31.8 Children are more likely to develop a primary Epstein–Barr virus (EBV) infection post-transplantation because they are negative pre-transplant and receive a donor from an older (EBV-positive) donor. Histologically there is an inflammatory infiltrate, mainly with B cells (H&E, ×200) (A). Immunocytochemistry demonstrates positive EBV cells in the affected tissue (EBER, ×200) (B).



(A) (B)

Figure 31.9 (A) Percutaneous transhepatic portogram shows a critical stenosis of the portal vein anastomosis post-transplant with numerous varices in the upper abdomen. This was successfully treated with balloon dilatation (B) demonstrating no pressure gradient across the portal vein anastomosis and a reduction in variceal filling.

flow around the transplanted liver using natural shunts may compensate for many years. Portal vein anastomotic strictures develop in reduced or split grafts and may cause portal hypertension with varices. Graft function is not affected. It should be treated radiologically by venoplasty or surgical reconstruction or shunt (Figure 31.9).

The incidence of *late biliary strictures* ranges from 5% to 25%. The main cause is graft ischemia related to hepatic artery thrombosis. Ischemic biliary strictures are frequently multiple and affect all aspects of the biliary tree with histological evidence of biliary obstruction. They present with jaundice and pruritus, an elevated alkaline phosphatase and GGT values 5–10 times normal. Treatment of late biliary strictures includes therapeutic interventional radiology, surgery, or re-transplantation (see Figure 31.7A,B).

Late acute rejection develops in 50% of children within 1 year and 60% within 5 years. It has different histological features compared to those immediately post-transplant. These include a predominantly mononuclear portal inflammatory infiltrate, less conspicuous inflammation of bile ducts and venous endothelium, more prominent interface hepatitis, and more lobular inflammation, which is often confined to centrilobular regions ("central perivenulitis") [18].

Chronic rejection occurs in 8–32% of children at any time, especially if there is non-compliance. The clinical presentation is with jaundice, pruritus or biliary obstruction with elevated bilirubin, transaminases, alkaline phosphatase, and GGT. Histology is as above. It is the main reason for long-term graft dysfunction and fibrosis requiring late re-transplantation.

Survival following liver transplantation

Current results from international units indicate that 1-year survival after elective pediatric liver transplantation is 90% [19]. Long-term survival (15–20 years) ranges from 60 to 80% [20]. Patients receiving elective living-related liver transplantation may have a higher 1-year survival (94%) compared with those receiving cadaveric grafts (78%) (Figure 31.10).

Factors affecting survival

Pre-transplant factors

There are a number of factors which may influence survival. Age at transplantation has previously been considered a significant risk factor and transplantation was originally contraindicated in infants under 1year old. Technical developments such as reduction hepatectomy, split liver transplantation, and living-related transplantation have reduced waiting list mortality and extended liver transplantation to this young age group, even in infants less than 5 kg.

Protein malnutrition has a significant influence on morbidity and mortality post-liver transplantation, so that all efforts should be made to ensure adequate nutritional status pre-transplant.

The severity of liver disease has a significant effect on short-term survival as children transplanted electively have an improved survival compared with those transplanted for acute liver failure or fulminant hepatitis.

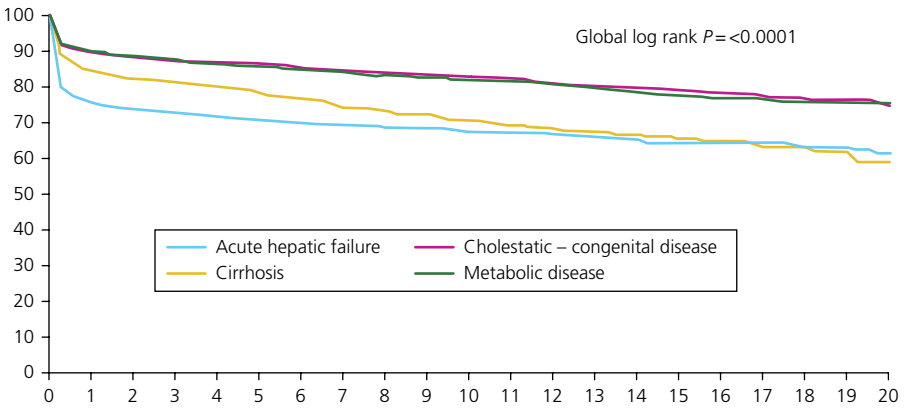


Figure 31.10 Twenty-year survival post-liver transplantation in children 2–18 years 1988–2015 (European Liver Transplant Registry). (Source: www.eltr.org; last accessed July 2016. Reproduced with permission of European Liver Transplant Registry.)

Hematological complications

Graft-versus-host disease is rare post-liver transplant, but may respond to increased immunosuppression. Both autoimmune hemolytic anemia and idiopathic thrombocytopenia are reported and may be immunological or drug related, particularly tacrolimus. Both respond to high-dose steroids or intravenous immunoglobulin.

Aplastic anemia may complicate seronegative hepatitis and present either before or post-transplant. Treatment is with ciclosporin or, if required, bone marrow transplantation.

Recurrent disease post-transplantation

In some instances survival may be affected by the recurrence of the original disease. Recurrence of hepatitis B virus (HBV infection) is almost 100% likely in those children who are HBV-DNA-positive or HBe-antigen-positive at the time of their operation without adequate prophylaxis or antiviral therapy (see Chapter 13). Recurrence of hepatitis B infection in children transplanted for fulminant hepatitis B is unusual.

Although chronic hepatitis C is an unusual indication for liver transplantation in childhood, reinfection of the graft is inevitable without antiviral therapy (see Chapter 13).

The recurrence of autoimmune liver disease is well documented in up to 25% of cases (see Chapter 11). These children should not have steroid-free immunosuppression as recurrence may then be up to 40%. The disease presents with abnormal liver biochemistry, raised immunoglobulin levels, and non-specific autoantibodies (ANA, SMA). The diagnosis of recurrent autoimmune hepatitis may be difficult to differentiate from rejection. Treatment is the same as for the original disease with prednisolone and azathioprine.

Sclerosing cholangitis recurs in ~10% of children, presenting a mean of 18 months post-transplantation, especially in those with inflammatory bowel disease. It is not known, whether colectomy pre-transplantation in children with primary sclerosing cholangitis and inflammatory bowel disease reduces recurrent primary sclerosing cholangitis (as seen in adults). It is particularly difficult to differentiate recurrent primary sclerosing cholangitis from rejection or biliary tract complications and requires both biliary tract imaging and histology. Treatment is unproven, but ursodeoxycholic acid is usually given.

Giant cell hepatitis in association with autoimmune hemolytic anemia is a rare disease which has been shown to recur post-transplant.

Recurrence of disease has been recognized in children who were transplanted for progressive familial intrahepatic cholestasis type 2 in which there is a deficiency of the canalicular bile salt export pump (BSEP; ABCB11). Symptoms include jaundice and pruritus and may take up to 12 years to develop. Histological and immunological evaluations showed patients developed anti-BSEP antibodies towards the receptor in the donor liver. Treatment is for the medical condition and re-transplantation may be required.

The outcome for children transplanted for malignant hepatic tumors is related to the rate of recurrence of the primary tumor, and if no extrahepatic metastases were present at the time of surgery long-term outcome may be excellent (see Chapter 28).

De novo autoimmune hepatitis

A number of studies have documented the development of autoantibodies (ANA, SMA, and rarely LKM) post-transplant in both children and adults in recipients who did not have autoimmune disease pre-transplant. The incidence varies from 2% to 3% to 50%. Although the etiology is unknown, the hepatitis resolves with steroid therapy, or with azathioprine (Figure 31.11; see also Chapter 11).

Graft hepatitis and fibrosis

Histology from 5- and 10-year protocol biopsies in children have detected an increasing incidence of graft hepatitis and fibrosis which has been reported from many centers [21]. The commonest histological abnormality was chronic hepatitis which was up to 60% at 10 years. The incidence of fibrosis also increased with time, being present in 90% at 10

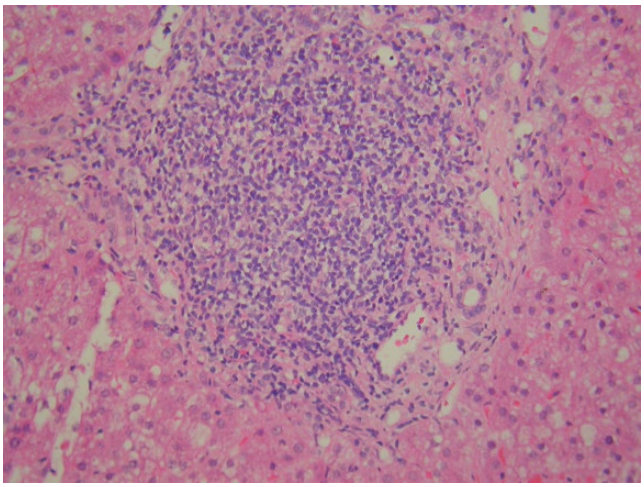


Figure 31.11 Chronic graft hepatitis develops 5–10 years post-transplant and may be related to chronic rejection or de novo hepatitis. In this section there is a mixed inflammatory infiltrate confined to the portal tract (H&E, ×200).

years. Fifteen percent had progressed to cirrhosis at 10 years. There was no evidence of viral infection and no association with immunosuppressive medication. On multivariate analysis, the only factor predictive of chronic hepatitis was autoantibody positivity (present in 13% and 10% of children with normal biopsies at 5 and 10 years, respectively, and 72% and 80% of those with chronic hepatitis at 5 and 10 years, respectively) (P <0.0001). It is not clear whether these histological changes represent a form of chronic rejection or de novo autoimmune hepatitis, as the graft hepatitis improved with increased immunosuppression.

Recent studies have identified hepatitis E as a cause of chronic graft hepatitis with progressive fibrosis in adults who are positive for hepatitis E, but similar findings have not yet been reported in children. Occasional cases of chronic hepatitis E virus infection have also been observed in pediatric liver allograft recipients [22].

Long-term renal function

The development of nephrotoxicity with both ciclosporin and tacrolimus was inevitable. There is a 30% reduction in renal function post-transplant which stabilizes or improves with low-dose maintenance immunosuppression or transfer to renal-sparing drugs such as mycophenolate mofetil or sirolimus. Only 4–5% of patients develop severe chronic renal failure long term requiring renal transplantation [16, 23].

Hypertension

Acute postoperative hypertension is seen in 65% of children, but only persists in 28%. Decreased glomerular filtration rate (GFR) and use of steroids were associated with hypertension. Treatment with Losartan®, an angiotensin-II antagonist (2 mg/kg/day; usually 25–50 mg/day) is effective for chronic hypertension and may be more effective than nifedipine (5–10 mg/dose) and/or atenolol (25–50 mg/dose [16].

Diabetes mellitus

The incidence of diabetes mellitus post-transplant is significantly less in children than in adults with an incidence of 11.2% at 5 years [16]. It was more common in children with CF, older age at transplant, and was associated with Hispanic race, early use of steroids, and CNI medication. The increase in obesity in the population may affect this incidence in future.

Hyperlipidemia

Ciclosporin and sirolimus both increase serum lipids, particularly cholesterol, which resolves on transfer to tacrolimus or mycophenolate mofetil. Studies of Pediatric Liver Transplant (SPLIT) registry data on 461 5-year survivors transplanted between 1991 and 2001 found that 12% were obese (weight exceeding 95th percentile) and 7% had hypercholesterolemia. Of 10-year survivors, 19% and 23% had increased cholesterol and triglycerides, respectively. None were on statins [16].

Cardiovascular disease

It is possible that cardiovascular disease might affect long-term survivors, especially because immunosuppressive medications increase risk for diabetes, hyperlipidemia, hypertension, obesity, and metabolic syndrome.

Obesity

The obesity epidemic has affected up to 30% of adult liver transplant recipients who also have diabetes (30%), hyperlipidemia (60%), and hypertension (60%). The impact and extent of the metabolic syndrome in pediatric liver transplant recipients is just being evaluated and appropriate advice on diet and exercise should be given [24].

Transplant tolerance

There is considerable interest in the development of transplant tolerance, and adult studies have demonstrated that approximately 20% of patients can be withdrawn from immunosuppression. Complete withdrawal of immunosuppression in children is anecdotal and related to case reports of anergy following post-transplant lymphoproliferative disease.

It has been hypothesized that immune engagement of the recipient immune system toward graft components could predispose to the development of operational or "prope" (= "almost") tolerance. These findings support the concept that gradual reduction of immunosuppression is a key component of strategies to induce spontaneous tolerance in liver transplantation.

More definitive data on the correct protocols to safely and reliably promote tolerance should become available through the Immune Tolerance Network in the US.

Quality of life post-transplant

Children who survive the initial 3 months post-transplant without major complications should achieve a normal lifestyle despite the necessity for continuous monitoring of immunosuppressive treatment.

Children who underwent transplant for metabolic liver disease have both phenotypic and functional recovery (α1-antitrypsin deficiency, Wilson disease, and tyrosinemia type I). Children with organic acidemias will only have palliation of their defect if the enzyme defect is not restricted to the liver (propionic acidemia or methylmalonic acidemia).

Growth post-transplant

Following a successful transplant with good nutritional management, most children should have good linear growth [25]. Catch-up growth is dependent on steroid usage and may not occur until steroids are discontinued or reduced.

There is a rapid return to normal of mid-arm muscle area and mid-arm fat area within 6–12 months post-transplant. Weight gain may initially be excessive due to the effects of steroids, appetite, and salt and water retention, but most children will regain normal weight within 12 months. Newer regimes which avoid or reduce steroids will benefit growth in the long term.

Growth failure post-transplant

The most important factors inhibiting growth post-transplant are:

- Excessive use of steroids.
- Preoperative stunting.
- Genetic disorders.
- Behavioral feeding problems.

Children who are particularly stunted pre-transplant (height SDS less than –1) initially have rapid catch-up growth but do not achieve their genetic potential, while children who are less stunted (height SDS greater than –1) have slower catch-up growth but will eventually achieve normal height.

Failure to thrive and stunting are intrinsic features of certain genetic disorders such as Alagille syndrome. Linear growth may improve post-transplantation for patients with Alagille syndrome, but approximately half of these children do not achieve normal height.

Behavioral feeding problems

Children with end-stage liver disease have associated anorexia and vomiting. They are often fed unpalatable feeds, sometimes by nasogastric tube. Many of these infants may never have fed normally prior to their transplant and thus will have missed their developmental milestones for chewing, swallowing, and normal feeding behavior. The perioperative emphasis on nutritional support often creates parental anxiety about feeding which further exacerbates these difficult behavioral problems. Prevention of behavioral feeding problems depends on a multidisciplinary approach with a dietitian, food psychologist, and a strict behavioral feeding regimen. In the minority of patients, however, nocturnal enteral feeding may be required for 1–2 years to maintain normal growth.

Endocrine development

End-stage liver disease may cause endocrine complications (growth failure, pubertal delay, hepatic osteodystrophy). Reduced production of sex hormones due to severe liver disease pre-transplant, particularly in adolescents, may affect growth and delay puberty which resolves post-transplant. Most recipients undergo normal puberty and have normal fertility [16].

Chronic liver disease is associated with menstrual abnormalities which resolve after successful transplantation. A study in 471 girls (age 10–20 years) with liver disease or post-transplant, found that 7.4% had menstrual problems (similar to the general population). No post-transplant girls had primary amenorrhea and if transplanted as a young child, the timing of menarche was normal.

Successful pregnancies have been reported. It is important that appropriate advice about fertility, contraception, and immunosuppressive therapy are provided (see Chapters 7 and 36).

In chronic liver disease, hepatic osteodystrophy leads to low bone mass, bone mineral density (BMD), fractures, rickets, and spine abnormalities. BMD remains low or may decrease before normalizing after 1 year. Of these patients, 12–38% have post-transplant fractures which include vertebral and non-vertebral fractures. Bisphosphonates may be required for low bone mass with a vertebral fracture.

Avascular necrosis (AVN) is an occasional complication of high-dose steroid treatment.

Hepatic protein synthesis, including insulin-like growth factor 1 (IGF-1) production, improves after transplant stimulating catch-up growth. Vitamin D (25-OHD) levels in children are low pre- and immediately post-orthotopic liver transplantation but improve in the first year. Vitamin D deficiency has been reported post-transplant and requires supplementation.

Psychosocial development

There is an initial deterioration in psychosocial development post-transplant as noted by deterioration in social skills, language development, and eye/hand coordination for up to 1 year post-transplant.

However, the majority of children will achieve normal psychosocial development post-transplant but the rate of improvement is related to the age of onset of liver disease and age at the time of transplant [16].

Many studies have documented that pediatric liver transplant patients have lower physical and psychosocial function compared to normal children although this is similar to other groups of chronically ill young people. A recent study of 800 recipients found psychosocial function was more affected than physical function, particularly if there was cognitive impairment or significant school absence [26], while 16% of adolescents reported symptoms consistent with post-traumatic stress disorder. The role in preoperative counseling in preventing this is not known.

Long-term studies have indicated that although within the normal range, transplant recipients fall in the lower IQ range, although it is not clear whether this is due to pre-transplant factors or post-transplant immunosuppression [27].

Neurocognitive function

Liver disease in infancy affects neurodevelopment, possibly because of malnutrition or encephalopathy while infants with metabolic diseases (such as urea cycle defects and tyrosinemia type 1) develop neurological damage, preventable by timely liver transplantation.

In some children with chronic liver disease, neurocognitive delay persists after physical rehabilitation post-transplant. Of recipients 10–15% have impaired intellectual ability, while 30% require special education.

In contrast, long-term data from Birmingham on 117 survivors >15 years demonstrated that 32% had been to university, 50% to college, 18% were still at school, and 35% were employed in full-time work [28].

Quality of life

Recent studies have suggested that health-related quality of life (HRQoL) post-transplant is suboptimal but comparable or better than children with chronic liver disease, chronic heart failure, diabetes, or undergoing cancer treatment. Emotional functioning is similar to healthy peers. Children who were younger at the time of transplant or who had longer follow-up and high self-esteem had better HRQoL [29].

Family functioning

The extreme stress experienced by families may lead to marital break-up and dysfunctional family behavior. HRQoL scores are less than for normal families as expected, but within the normal range.

Non-adherence with therapy

Non-adherence is the major cause of graft loss or rejection in adolescent transplant recipients, accounting for 17% of liver grafts (see Chapter 35). Additional risk factors for non-adherence are the age at which transplantation takes place, social and economic factors, and the process of transition to adult care. There is good evidence that non-adherence with medication, clinic visits, and medical advice increases following transfer to adult care, indicating the need for an appropriate transition policy. The management of non-adherence is difficult and relies on a non-judgemental approach, and efforts to improve education, social functioning, and behavioral strategies to encourage self-motivation and self-management (see also Chapter 35).

Outpatient monitoring

Initial post-transplant management includes frequent follow-up by the transplant center, usually at weekly intervals, extending with time to monthly, 3-monthly, and then 6-monthly intervals.

Monitoring should include:

- Assessment of nutritional status by measuring height, weight, triceps skinfold, mid-arm circumference, and mid-arm muscle area.
- Detection of potential complications (e.g., rejection, infection, hepatic artery thrombosis, biliary complications, development of graft hepatitis, and fibrosis) by performing regular liver function tests, serology (hepatitis B, C, and E, CMV, EBV) screening for autoantibodies (ANA, SMA, LKM) annually and 6-monthly, or annual abdominal ultrasound examinations.
- Monitoring immunosuppression to maintain adequate trough levels of ciclosporin, tacrolimus, or sirolimus to prevent rejection and reduce toxicity (see Box 31.3).
- Screening for PTLD by EBV PCR 3–6-monthly; and measuring serum albumin as a falling albumin may be an early sign of gut PTLD.
- Renal dysfunction is best detected by monitoring of serum creatinine. Although measuring GFR remains the preferred method, estimating GFR using the updated Schwartz formula (height in centimeters × 0.4/serum creatinine (mg/dL) is acceptable, ensuring GFR >70 mL/min/1.73 m^2 and reducing CNI immunosuppression as required. Transfer to a renal-sparing drug, or reduction in CNI drugs should be considered if the chromium EDTA values fall below 70 mL/min/1.73 m^2 as it is important to preserve renal function.
- Regular measurement of blood pressure and/or ambulatory blood pressure monitoring may be required if the blood pressure is elevated at clinic.
- Measuring calcium, phosphate, vitamin D, and parathyroid hormone levels at least twice a year with a dual energy X-ray absorptiometry (DEXA) scan as indicated to detect bone disease). It is necessary to ensure appropriate intake of calcium and phosphate, especially in children on immunosuppressants such as tacrolimus which can cause phosphate

loss. Vitamin D is given as cholecalciferol (vitamin D_3) or ergocalciferol (vitamin D_2) if required [30]. Children with pre-transplant osteopenia should be monitored for scoliosis and fractures. In children >5 years, DEXA scanning at liver transplantation and 12 and 24 months after may help, using appropriately size correction and lateral thoracic spine X-rays. As children become adolescents, appropriate advice about adolescent issues are required (see Chapter 26).

- Protocol liver biopsies: 1-, 5- and 10-year biopsies may detect chronic hepatitis and fibrosis in 25–50% of children which may be autoimmune in origin and requires alteration of immunosuppression.

It is essential to encourage both child and family to return to a normal life by reducing outpatient visits and encouraging return to school, nursery, and playgroup, and discouraging the parents from continuing to maintain their child in a sick role. Many families may find the transition from intensive management in specialist units to the more relaxed outpatient follow-up difficult to cope with, and need additional support and encouragement to regain a normal life. Out reach clinics held in the referring hospital are a good way of ensuing return to normality.

Future prospects

For the moment, liver transplantation is here to stay, although the rapid development of techniques to improve hepatocyte transplantation for acute liver failure or metabolic liver disease is encouraging. The continued advance in targeted less toxic immunosuppressive drugs can only improve the outcome for children undergoing liver transplantation.

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CHAPTER 32

Small-Bowel Transplantation in Children

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Key points

- Intestinal failure requires multidisciplinary care including pediatric hepatology, gastroenterology, pediatric surgery, and transplant to optimize outcomes.
- The incidence of intestinal failure-associated liver disease in children with intestinal failure is decreasing with current medical therapy focussed on lipid therapy optimization, control of sepsis, and aggressive attempts at intestinal adaptation.
- Intestinal transplantation, in isolation or combination with liver transplantation, can be a life-saving therapy when irreversible intestinal failure is accompanied by complications of parenteral nutrition dependency such as central venous line access or hepatic injury and portal hypertension.
- Early outcomes after intestinal transplantation have improved significantly but long-term outcomes have not proportionally improved.
- Long-term graft outcome improvement depends on optimizing immune monitoring and understanding the impact of donor-specific antibody on chronic rejection after intestine transplant.

Children with intestinal failure – which is defined as malabsorption due to the reduction of functional intestinal mass necessary for adequate digestion and absorption of nutrients and for meeting fluid and growth requirements – would have died of malnutrition in the 1960s.

Advances in the use of parenteral nutrition (PN), clinical surgery, and immunosuppressive management have made human intestinal transplantation a clinical reality. This chapter reviews the advances in PN, management of intestinal failure including the success of clinical pediatric intestinal and liver transplantations, and recent advances in the field of intestinal transplantation.

Intestinal failure

Intestinal failure is defined as a gastrointestinal condition of malabsorption due to the reduction of functional intestinal mass necessary for adequate digestion and absorption of nutrients and for meeting fluid and growth requirements.

There are many causes of intestinal failure, which can be divided into broad categories as outlined in Box 32.1.

Not all children with intestinal failure, dependent on PN will need intestinal transplantation. In most children PN is a bridge towards intestinal adaptation. Intestinal adaptation is a process whereby children can be weaned from PN onto enteral feeding by using various medical, dietetic, and non-transplant surgical techniques. Hence, intestinal failure is classified into reversible (PN can be stopped and the child weaned onto enteral feeds) and irreversible intestinal failure (complete dependence on PN). A multidisciplinary approach to intestinal failure management within an intestinal rehabilitation program facilitates the active management of intestinal failure, prevents complications, and enables the process of intestinal adaptation. Recognition of complications and optimization of PN and enteral management may stabilize patients' clinical syndromes and independence from total PN (TPN), thus avoiding transplantation.

Surgical causes

The commonest cause of intestinal failure in childhood is the short-bowel syndrome, due to surgical correction of congenital defects such as gastroschisis, midgut volvulus, intestinal atresia, or surgery for necrotizing enterocolitis (NEC; see

Diseases of the Liver and Biliary System in Children, Fourth Edition. Edited by Deirdre A. Kelly.

Box 32.1 Causes of intestinal failure.

Short-bowel syndrome

- Neonatal age
  - congenital malformations
  - necrotizing enterocolitis
  - volvulus
  - intestinal atresia
  - gastroschisis*
- Older child
  - Crohn disease
  - radiation enteritis
  - trauma
  - tumors

Intestinal dysmotility

- Intestinal pseudo-obstruction
- Intestinal aganglionosis (Hirschsprung disease)
- Megacystis microcolon hypoperistalsis syndrome
- Abnormalities of the interstitial cells of Cajal
- Neuronal intestinal dysplasia

Enterocyte absorptive impairment

- Microvillus inclusion disease (MyoVb mutation)
- Autoimmune or idiopathic enteropathy
- Phenotypic diarrhea of infancy
- Tufting enteropathy (EPCAM mutation)

Tumors

- Familial polyposis
- Inflammatory pseudotumor

* Gastroschisis may be associated with intestinal atresia, volvulus, necrotizing enterocolitis, or vascular occlusion.

Box 32.1). Patients with gastroschisis may have intestinal dysmotility in association with their abdominal wall defect.

The capacity for intestinal adaptation is influenced by many factors such as: the length and type of residual small bowel (whether it is ileum or jejunum); the site of intestinal resection; the presence or absence of the ileocecal valve; the length of remaining colon; and the age of the patient and experience of multidisciplinary team. In clinical practice, heterogeneity of cases with similar diagnosis and bowel length makes the prediction of factors influencing the intestinal adaptation process and potential to wean from PN very difficult. In general, ileum adapts better than jejunum. Hormones and growth factors have been evaluated in both animal and human studies to promote intestinal adaptation. Trials of growth hormone (GH) in combination with glucagons have shown contradictory results. There are no pediatric randomized controlled trials in children with the use of GLP-2 (teduglutide), but studies on adult short-bowel syndrome patients have shown that GLP-2 use can reduce PN requirements by 20%. A multicenter randomized control in the use of GLP-2 in pediatrics is underway at present.

Functional causes

Functional causes for intestinal failure include disorders of motility, e.g., chronic intestinal pseudo-obstruction (CIP), Hirschsprung disease, and aganglionosis; or mucosal disorders such as microvillous inclusion disease and protracted diarrhea of unknown cause.

Most children present in the newborn period, although a quarter develop symptoms after 1 year of age. This is a heterogeneous group of disorders in which functional rather than mechanical obstruction is present as a result of enteric neuronal diseases involving lengthy segments of gut. The underlying pathology may be a smooth muscle myopathy or enteric neuropathy. Associated malrotation, congenital shortening of the small intestinal length, or involvement of the urinary tract are common and this group of children may need long-term PN.

An important subgroup of patients with pseudo-obstruction have total intestinal aganglionosis (long segment Hirschprung disease), resulting from a failure of migration of new neural crest cells into the gut during embryonic life. It presents with severe pseudo-obstruction and without PN support is rapidly fatal. Whilst it is currently incurable, the ability to transplant the enteric nervous system is a realistic possibility in the distant future. In the experimental animal, autologous transplantation can be used to treat aganglionosis by implanting stem cells derived from the neural crest.

Mucosal causes

Microvillous inclusion disease is an inherited disorder secondary to the *MYOVB* mutation which is a common cause of severe protracted diarrhea beginning in the newborn period. It is an autosomal recessive disorder and characteristically presents with severe watery diarrhea in the first few days of life. In some cases, onset may be delayed to a few months of age (late-onset microvillous inclusion disease). Stool volumes are usually high (up to 300 mL/kg/day) with both an osmotic and a secretory component. The disorder is fatal without long-term PN.

Tufting enteropathy is secondary to the *EPCAM* mutation also presents in the first week of life with intractable watery diarrhea requiring long-term PN. It is an autosomal recessive condition and 40% of patients have consanguineous parents and/or affected siblings who died during the first few months of life with severe diarrhea. Histology is characteristic with variable villous atrophy, with disorganization of surface enterocytes producing so-called tufting. Crypts are often dilated and pseudocystic. Early biopsies may show villous atrophy only and therefore repeated duodenal biopsies are often needed to make the diagnosis.

Intestinal tumors

Unlike adult practice, malignancy is an unusual cause of intestinal failure. Diffuse intestinal polyposis requiring extensive surgery is occasionally encountered.

Complications of total parenteral nutrition

PN permits children with intestinal failure to survive for many years, but is associated with a number of life-threatening complications. These include catheter-related blood stream infection (CRBSI), metabolic disorders, loss of venous access from extensive venous thrombosis, pulmonary embolism, and the development of TPN-induced liver dysfunction, now termed "intestinal failure-associated liver disease" (IFALD). The term IFALD replaces the old terminology of PN-related liver disease because it is not due to PN alone but due to a combination of factors such as line infections, sepsis, lack of enteral feeding, small-bowel bacterial overgrowth (SBBO), and components of PN.

Intestinal failure-associated liver disease

IFALD varies in frequency depending on age, etiology of intestinal failure, duration of TPN use, and associated complications such as infection. This complication is more common in neonates who have suffered extensive loss of their intestine due to NEC or intestinal atresias. Many studies have noted that IFALD is more common in premature or low-birthweight infants, which may be related to the immaturity of the neonatal liver. Inability to establish enteral feeding is common in children requiring PN, and liver disease is more likely to develop in those children who are unable to tolerate any enteral feeding.

Recent clinical and experimental evidence suggested a role of soybean oil (main lipid source) based intralipid emulsions (ILE) in the onset of IFALD. This led to the development of alternative lipid emulsions including those containing olive oil and fish oil. A randomized controlled trial compared the short-term effect of an olive oil based ILE (ClinOleic®, Baxter) with the traditional soybean-based ILE in critically ill neonates and found no adverse effects. However no long-term data on the effect of ClinOleic on IFALD are available. More recently, fish oil has been introduced either as a supplement to soybean-based ILE, as an ingredient in a combination emulsion (SMOFlipid®, Fresenius Kabi, 30% soybean, 30% medium-chain triglycerides (MCT), 25% olive oil, and 15% fish oil), or as a monotherapy (Omegaven®, Fresenius Kabi). The presumed hypothesis for using fish oil based ILE is that the high concentration of the anti-inflammatory omega 3 fatty acids interfere with proinflammatory omega 6 pathway and the increased clearance of triglycerides and the reduction in lipogenesis may modulate hepatic injury in IFALD. Recent experience showed a significant reversal of cholestasis in children fed with Omegaven compared to soybean controls, but concerns remain about the development of essential fatty acid deficiency with fish oil based ILE monotherapy and the progression of fibrosis despite the use of fish oil based ILE. Randomized controlled trials are needed to obtain the long-term safety and efficacy data of fish oil based ILEs.

The severity of IFALD should be assessed in order to plan treatment options and referral for intestinal transplantation. Historically the classification of severity of IFALD has been based on the level of bilirubin. Bilirubin is no longer such a sensitive marker as the newer formulations of PN improve bilirubin level but may not prevent progression of fibrosis despite the absence of cholestasis.

Catheter-related blood stream infection

The role of the multidisciplinary team (MDT) in facilitating early discharge on home PN, preventing line infections, and improving overall outcome has been well documented in the literature. Patients with recurrent episodes of CRBSI have a 30% greater chance of developing IFALD and thus it is important to reduce the incidence of line infections. Studies have evaluated the use of lock therapy with vancomycin and gentamicin or with ethanol locks, both of which reduced bacteremia, sepsis, and catheter-related sepsis.

Taurolidine-based locks have shown promising results in the reduction of line infection but antibiotic-impregnated catheters have not received generalized acceptance because of the cost and they are only recommended for short-term use.

Exit site infections at the point of entry of the central line and skin should be aggressively managed with daily inspection as they can lead to colonization of the line and CRBSI. Octenesan washes are now available for children having persistent exit site line infection.

Children presenting with recurrent life-threatening episodes of catheter sepsis with metastatic infections, unusual pathogens, or multisystem organ failure should be referred for intestinal transplantation.

Vascular access

In practical terms, venous accessibility is limited to six sites in younger children and eight sites in older children – the pairs of internal jugular, subclavian, innominate, and iliac veins (in older children). The need for lifelong venous access in children on long-term PN means that vascular access should be managed by dedicated clinicians (pediatric surgeons, interventional radiologist, and experienced anesthetists). Impaired venous access (the loss of 50% of these sites) warrants consideration for intestinal transplantation.

Other contributing factors

Other contributing factors to the development of IFALD could be exposure of amino acids to light, di(2-ethylhexyl) phthalate (DEHP) materials in the PN infusion systems and aluminum contents in PN additives.

Hepatotoxicity

Hepatotoxicity may be related to the components of TPN, and a variety of precipitating factors have been suggested, such as the excessive provision of protein, carbohydrates, glycine, alanine, tryptophan, and flavonoids, associated with relative deficiencies in selenium, tocopherol, and taurine. Hypermanganesemia in children with TPN cholestasis was related to excessive manganese supplementation, suggesting that manganese toxicity exacerbated cholestasis in children with established liver disease.

Clinical features and diagnosis

Jaundice is a sensitive indicator of ongoing liver damage, although bilirubin may improve with treatment of sepsis or intestinal adaptation. Persistently elevated bilirubin (>100 mmol/L) carries a high 1-year mortality and is an indication for transplantation. Splenomegaly may develop early and is helpful in staging hepatic fibrosis. Clinical evidence of portal hypertension (history of bleeding esophageal varices, hepatosplenomegaly, and ascites) suggests irreversible liver disease.

Children with progressive IFALD may not display the characteristic signs of progressive primary liver disease and it is essential to remember this when assessing the severity of IFALD.

Abdominal ultrasound can be useful in detecting increased echogenicity in livers with steatosis, gallstones, and biliary sludge and in tracking an enlarging spleen in portal hypertension particularly in children, and it is good practice to carry out an annual abdominal ultrasound once per year when following patients on home TPN.

Endoscopic evaluation may not be helpful as varices develop at an ectopic site and are only seen at the esophageal level in advanced IFALD. Splenomegaly can occur without portal hypertension in children on long-term PN due to recurrent sepsis. Endoscopic ultrasound may be helpful in identifying submucosal esophageal and gastric varices and aid staging the disease and in making the choice between isolated intestinal and combined liver and bowel disease.

Early histological changes in the liver are related to centrilobular cholestasis without inflammation, necrosis, or fatty infiltration. Steatosis is relatively uncommon in infants, but can be secondary to hepatic accumulation of lipid or glycogen. More advanced liver disease includes portal fibrosis, pericellular fibrosis, and bile ductular proliferation. The histopathological changes can be patchy leading to incorrect interpretation of the severity of liver disease.

Non-invasive markers of liver fibrosis have not been reliable in assessing the severity of fibrosis in IFALD. Hepatic venous wedge pressure gradient measurement to assess the degree of portal hypertension may be an objective technique to assess the severity of IFALD; large multicenter studies are needed to validate this technique.

Other. Another approach to quantifying fibrosis without resorting to liver biopsy is the use of a specialized sound wave emitted by a FibroScan® which becomes attenuated in the presence of fatty tissue and fibrosis.

Both CT and MRI have been used to evaluate hepatic changes in IFALD. MRI avoids irradiation but the technique is still in the evaluation stage and may not come into routine use because it is relatively expensive and difficult to perform in young children.

Management

Management strategies to prevent the development and progression of IFALD are important in the management of children with chronic intestinal failure.

- It is important to prevent sepsis, particularly catheter sepsis. Children with dilated loops of bowel may have SBBO leading to bacterial translocation and CRBSI.
- Enteral nutrition plays an important part in encouraging intestinal adaptation and reducing gut stasis.
- The use of oral selective decontamination agents to prevent bacterial overgrowth as well as innovative non-transplant surgical techniques (e.g., the Bianchi procedure, the serial transverse enteroplasty (STEP) procedure) may successfully improve the motility of previously dilated segments of bowel. This improvement in function together with an increase in the surface area of the intestine may allow sufficient intestinal adaptation for TPN to be discontinued.
- Children with irreversible intestinal failure and progressive IFALD will need a rescue intestinal transplant with a liver inclusive graft. In children with progressive IFLAD but with an adequate length of small bowel and/or an ileocecal valve in whom there is potential for further intestinal adaptation, an isolated liver transplant may be preferred to a combined liver and intestinal transplant. Selection criteria for isolated liver transplant in children with short-bowel syndrome and progressive IFALD have been established (Box 32.2). This option needs to be performed in intestinal rehabilitation centers with considerable experience in the management of intestinal failure and established links with an intestinal transplant center.

Timing of referral for transplantation

The majority of children on home PN have excellent long-term survival reported from various intestinal rehabilitation centers [1], but a small minority develop complications related to PN. There is currently no consensus regarding appropriate timing of referral of children with complications related to long-term PN who should be referred to an intestinal transplant center. The lack of clear criteria for

Box 32.2 Selection criteria for isolated liver transplantation in children with short-bowel syndrome with a potential for enteral adaptation (to be considered in experienced intestinal rehabilitation centers).

- Established intestinal failure-associated liver disease (serum bilirubin >200 μmol/L, moderate/severe fibrosis, portal hypertension)
- At least 50 cm functional small bowel remaining intact in the absence of an ileocecal valve or 30 cm with an ileocecal valve
- At least 50% of the estimated daily calorie requirement was tolerated as enteral feeds for at least 4 weeks before the development of liver disease and was associated with an increase in weight
- In children with dilated and dysmotile bowel, minimal line infections (less than six in 12 months)

referral and attempts to wean children from PN with advancing liver disease has resulted historically in late referrals to the transplant centers.

The impact of late referral has been well described in the literature, but recently the impact of early referral and listing has been documented. A Canadian study using a Markov analytic model demonstrated that early listing was associated with advantages both in terms of life-years added and quality-adjusted life-years (13.16 versus 12.89 and 10.51 versus 9.75, respectively) [2]. *Pironi et al.* conducted a prospective survey of 300 patients on home PN and concluded that early referral to a transplant center is essential in the overall management of patients on long-term home PN [3].

Early referral to an intestinal transplant center before the development of severe life-threatening complications such as liver disease may serve the following purposes:

- Alternatives to intestinal transplantation (non-transplant surgery and intestinal rehabilitation options should be explored in conjunction with the referring team).
- Children with short-bowel syndrome and progressive IFALD could be considered for isolated intestinal transplantation which improves their potential to be transplanted.
- Children referred before the development of progressive IFALD can be considered for isolated intestinal transplantation. Significantly decreased mortality has been observed in children on transplant waiting lists awaiting isolated intestinal transplantation as compared to combined liver and bowel transplantation.
- Children transplanted in a stable condition have a better long-term outcome.
- The referring team needs to be aware that the most important limiting factor for intestinal transplantation in the first 2 years of age remains the lack of availability of size-matched donors and many children die on the transplant list awaiting a suitable organ for transplantation.

The indications and referral for intestinal transplantation were documented in a consensus document published by the Intestinal Transplant Association in 2001 (Box 32.3). However, with the advances in PN, use of fish oil based PN, use of modern techniques for vascular access, and prevention of line infections, the criteria need to be revisited.

A study recently conducted at a single center confirmed the inadequacy of the current criteria and suggested three newly proposed criteria which had high predictive value:

- ≥2 intensive care unit admissions ($P = 0.0001$, OR 23.6, 95% CI 2.7–209.8).
- Persistent bilirubin >75 mmol/L despite lipid strategies ($P = 0.0005$, OR 24.0, 95% CI 3.2–177.4).
- Loss of ≥3 central venous catheter sites ($P = 0.0003$, OR 33.3, 95% CI 18.8–54.0).

There was 98% probability of needing intestinal transplantation when two of these new criteria were present [4]. A larger multicenter collaborative study is needed to validate the criteria for wider applicability and change in referral criteria.

Box 32.3 Indications and contraindications for intestinal transplantation.

Indications for intestinal transplantation

- Irreversible intestinal failure with major complications
  - recurrent or life-threatening sepsis
  - loss of 50% or more central venous access sites
  - recurrent and intractable fluid balance issues
  - liver disease with portal hypertension

Contraindications for intestinal transplantation

- Absolute
  - profound or progressive neurological dysfunction
  - non-correctable disease in organs outside the gastrointestinal tract
  - active systemic sepsis
  - malignancy with a poor prognosis
  - psychosocial problems – severe and irreconcilable
- Relative
  - treatment in the intensive care unit at the time of transplant
  - immunodeficiency
  - drug dependency (opiates, etc.)
  - loss of conventional venous access
  - neoplasia benign or of uncertain prognosis (age per se is not a contraindication)

Difficult indications

In a subgroup of children, early referral or a dialogue with an intestinal rehabilitation/transplant center may prevent the development of complications and also optimize the timing of referral to the intestinal transplant center. These include disorders with poor prognosis as follows:

- Massive resection with duodenocolonic anastomosis.
- Congenital enteropathy.
- Megacystis microcolon.
- Microvillus inclusion disease.
- Trauma, massive resection.
- Multiple fistulae.
- Frozen abdomen.
- Desmoid tumors.

It is important to reassure the family and the referring pediatric gastroenterology/surgery team that the outcome of a referral to a transplant center is not always to list the child for intestinal transplantation, but to decide on alternative treatment options and plan treatment in the best interests of the child and family.

An additional ethical dilemma is when the family or the teenage child with intestinal failure, stable on home PN, demands intestinal transplantation to improve the quality of life. The families should be provided with information regarding the excellent long-term survival of home PN and counseled about the risks, morbidity, and long-term survival following intestinal transplant.

The current consensus within the international transplant community is to consider transplantation only on an individual basis dependent on the ability of the family/

teenager to understand the information and the willingness of the multidisciplinary team to consider this treatment option.

Assessment for intestinal transplantation

Children referred for intestinal transplantation generally have complex medical, surgical, and social histories. A thorough multidisciplinary evaluation is required (Box 32.4) including:

- Confirmation of the cause and extent of intestinal failure.
- Potential for intestinal adaptation and medical or surgical intervention.
- Identification of other organ dysfunction, such as cardiac, pulmonary, or central nervous system.

Key areas in intestinal transplant assessment

Confirmation of diagnosis

It is essential to confirm the diagnosis in patients with motility disorders or mucosal lesions by reviewing the patient's history and radiographic and histological findings. Thus, functional assessment of the entire gastrointestinal tract (including the esophagus) is critical in the selection of the appropriate intestinal graft.

Patients with extensive long-segment Hirschsprung disease require a thorough review of all pathological material prior to transplantation to establish the extent of the disease, with further examination of intraoperative frozen-section samples of residual intestine at the time of transplantation.

The length of bowel in patients with short-bowel syndrome is estimated as far as possible, identifying the length of the ileum and jejunum, the presence or absence of the ileocecal valve, and any evidence of dysmotility.

Intestinal function

The best test of intestinal function is tolerance to enteral feeds, and it is therefore important to obtain a good feeding history in order to establish whether there is any potential for adaptation or medical therapy. It is essential to assess the potential for adaptation in patients with short-gut syndrome by:

- Encouraging either continuous or bolus enteral feeds.
- Treating bacterial overgrowth with antibiotics (amoxicillin and metronidazole).
- Consideration of corrective surgery in children with short gut; re-establishing continuity of bowel or bowel lengthening may improve intestinal function. Reports now exist in the literature of children with "ultra-short-gut syndrome" being successfully weaned from PN using a combination of medical and surgical therapies.

At the end of assessment there should be a decision by the multidisciplinary team to categorize the intestinal function into "reversible" intestinal failure or "irreversible" intestinal failure. Children with reversible intestinal failure should have rehabilitation strategies pursued under the supervision

Box 32.4 Evaluation of pediatric small-bowel transplantation candidates.

Intestinal assessment

- Review of operation notes from the referring hospital to establish the remaining length of bowel
- Upper and lower gastrointestinal barium studies
- Motility studies if available (evaluation of gastric emptying is important)
- Transit time
- Upper gastrointestinal endoscopy and colonoscopy (if indicated) and histology of bowel
- Evaluation of parenteral nutrition prescription
- Nutritional bloods
- Anthropometric measurements (including mid-arm circumference and triceps skinfold thickness)

Hepatic assessment

- Bilirubin (split), alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, bone profile
- Total protein, albumin
- α-Fetoprotein, C-reactive protein
- Prothrombin time, partial thromboplastin time
- Upper gastrointestinal endoscopy to assess the varices
- Endoscopic ultrasound (if available)
- Liver ultrasound for hepatic vessels, presence of ascites, spleen size, and presence of splenic varices
- Liver histology (when indicated)
- CT angiography to look for ectopic varices
- Hepatic venous wedge pressure (if unsure about severity of liver disease)

Vascular access

- Doppler ultrasound for patency of central veins
- CT angiography/MRI venography when in doubt about the patency of the veins

General

- Full blood count
- Blood type (ABO), tissue typing, cross-matching
- Blood urea nitrogen, serum creatinine
- Chest radiograph
- Echocardiogram, electrocardiogram
- Chromium EDTA/cystatin C
- Ventilation/perfusion scan (if indicated)

Investigations for infection

- Blood, urine, throat, feces, ascites culture: bacterial, fungal, viral, hepatitis screen, cytomegalovirus, Epstein–Barr virus
- Meticillin-resistant *Staphylococcus aureus* swabs
- Quantitative stool cultures

of an experienced multidisciplinary team to wean the patient from PN.

Hepatic function

Most children who are referred for intestinal transplantation will have some hepatic dysfunction. The assessment of hepatic function is critical in establishing whether the patient requires an isolated intestinal transplant or a combined liver/intestinal transplant, as well as determining preoperative and postoperative morbidity.

Nutritional and developmental status is established as for liver transplantation (see Chapter 31).

Psychological and social assessment

Small-bowel transplantation remains a complex procedure with an unknown long-term prognosis, and it is important that both child and family are appraised of the risks and complications of this procedure in order to make an informed decision. Psychological preparation of the child is paramount and should include preparation for a stoma and ileostomy.

In addition to the psychological assessment, an assessment of social circumstances is crucial at the time of assessment. A thorough evaluation of family/friend support within the local community is essential to decide about the support that is needed for the child and the family in the post-transplant period. In some extreme circumstances, where neglect of the child is identified, the child may need to be fostered or cared for by grandparents/other family members prior to transplantation and in the post-transplantation period.

On completion of the assessment, the decision will be made whether the child requires a non-liver inclusive graft (isolated bowel transplant, modified multivisceral transplant) or liver inclusive graft (combined liver and small-bowel transplantation, multivisceral transplant) or in rare cases an isolated liver transplant only. Even after listing for transplantation, attempts at weaning from PN should be continued ensuring that nutrition is optimized.

Preoperative management

Management of patients awaiting small-bowel transplantation requires careful attention to the prevention of sepsis, maintenance of nutrition, and prevention and management of vascular and hepatic complications.

Sepsis rates may be reduced by aseptic catheter techniques and by the use of selective bowel contamination (polymyxin B, gentamicin, and amphotericin administered orally). TPN protocols may need modification to ensure adequate nutrition for growth and to overcome hepatic catabolism. Tolerance to oral intake is crucial, since this can affect post-transplantation nutritional management. Children should be stimulated to eat before transplantation, even if no nutritional benefit is gained, as this encourages developmental progress for eating and swallowing in the post-transplant period. Prevention of vascular thrombosis is difficult and the use of anticoagulation is not universally accepted. Hence, the vascular access needs to be managed under guidance by the transplant team.

Hepatic complications should be managed as described in Chapters 21 and 31. Cholestatic patients should be treated with ursodeoxycholic acid (10–20 mg/kg) and supplemented with adequate fat-soluble vitamins parenterally. Bleeding esophageal varices are treated with sclerotherapy or banding, but the majority of these patients will bleed from portal gastropathy, which can be controlled with continuous infusion of somatostatin (octreotide) or oral losartan, an angiotensin II receptor antagonist (2 mg/kg/day; usually 25–50 mg/day), may be effective in reducing portal pressure and bleeding (see Chapters 21 and 27). The use of transjugular intrahepatic portosystemic shunts (TIPS) is rarely indicated, but should be done early in children with refractory bleeding secondary to ectopic varices, which may not be easily amenable to endoscopic therapeutic measures.

Transplant procedure

Selection of donors

Grafts for intestinal transplantation are obtained from ABO-identical brain dead donors varying in age from neonates to 40 years. Donors with a history of prolonged cardiopulmonary arrest or significant inotrope requirement should be avoided, as this may result in significant bowel ischemia. Donors with malignancy are excluded, but those with systemic viral or bacterial infection in the absence of an identifiable thoracic or abdominal source are acceptable. Matching with human leukocyte antigen (HLA) is unnecessary. Cytomegalovirus (CMV)-positive donors are only considered for transplantation into CMV-positive recipients unless the patient is dying from liver failure. CMV-negative patients awaiting isolated intestinal grafts should only receive organs from CMV-negative donors. Graft pretreatment to deplete the lymphoid population of the intestinal allograft with either irradiation or a monoclonal antibody – such as OKT3 (muromonab-CD3) or antithymocyte globulin – as prophylaxis for graft-versus-host disease (GVHD) is performed in some centers, but has not been reported to exclude development of GVHD.

Ideally, donors should be of similar size to the recipient. However, reductions of intestinal and liver/intestine allografts may be necessary because of the prolonged wait for age-matched and size-matched donors.

Donor operation

The procurement of abdominal visceral organs, either en bloc or as separate components, is similar to a large cluster of grapes with a double central stem consisting of the celiac axis

and superior mesenteric arteries (Figure 32.1). The separate grape clusters represent the different abdominal organs, which include the liver, stomach, duodenum, pancreas, small intestine, and colon. These can be removed or retained according to the clinical needs of the recipient, with preservation of the double arterial stem structures in the larger composite grafts, which include the complete multivisceral, modified multivisceral, liver/small-bowel, and modified liver/small-bowel allografts. In the isolated small-bowel allograft, only the superior mesenteric artery stem is retained.

The procurement technique focusses on simple isolation and cooling of the organs to be transplanted, with preservation of the vascular and parenchymal anatomy. This procedure has been simplified using a limited hilar dissection by preserving the duodenal loop and pancreas with the liver/small-bowel allograft, thus avoiding transection of the common bile duct [5]. Procurement is thus limited to simple isolation of the stomach and division at the pylorus, and transection of the ileum at the ileocecal valve with mobilization of the colon. The pancreas is typically preserved. These grafts are ideally suited to very small donors (neonates), thus avoiding manipulation of the hepatic hilum, and larger donors in whom graft reduction is necessary (Figure 32.2). Both situations have allowed for reductions of the liver and intestine component of the graft, thus allowing for increased donor utilization.

The isolated small-bowel graft can be procured as a composite graft with the other organs and then separated as a back-table procedure into separate intestine, pancreas, and liver allografts. The entire multivisceral procurement will take 3–4 h.

These composite grafts are removed with minimal contamination, since the hollow viscera are sealed off by stapling and transection. The succus entericus is left undisturbed and transplanted with the graft.

Graft preservation

After completion of the donor dissection, systemic heparinization is performed and the proximal aorta is cross-clamped. In situ perfusion using cold University of Wisconsin (UW®) solution, with venous bed decompression via a venotomy in the intrapericardial suprahepatic vena cava, is performed. Separation of organs is performed on the back-table, and the graft is stored in ice for transport. Preservation time should be limited to less than 10 h.

Recipient operations

Most children who need an intestinal or multiorgan transplant have had multiple previous abdominal explorations for intestinal resection, lengthening procedures, or treatment of complications. The combination of severe adhesions and portal hypertension presents a significant surgical challenge. The volume contraction of the abdominal cavity resulting

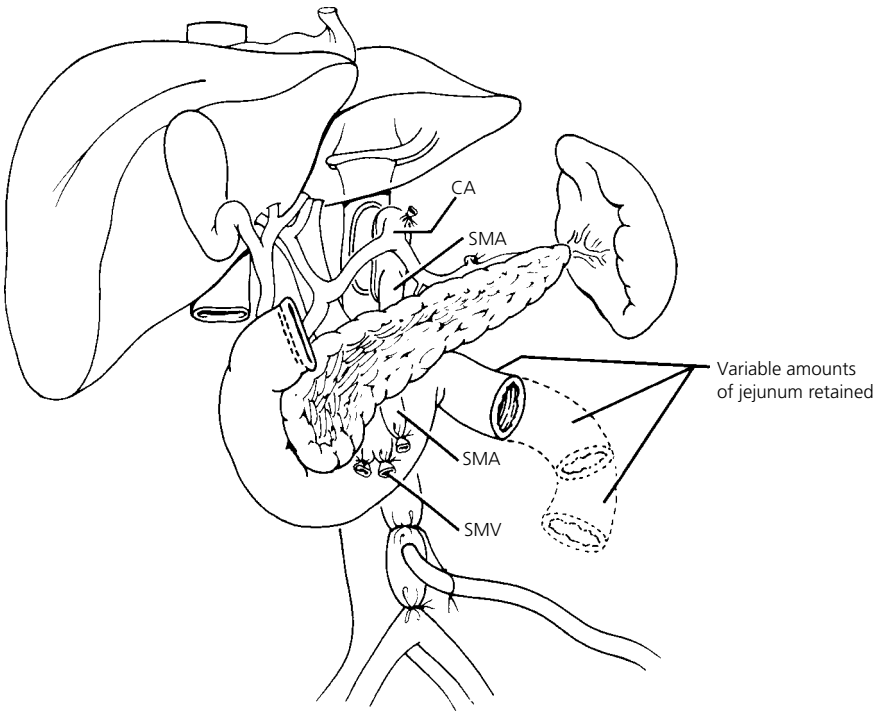


Figure 32.1 A modified "cluster graft" used to replace resected viscera after upper abdominal exenteration. The conceptual flexibility with this technique allows for variable retention or removal of different portions of the gastrointestinal tract. In this illustration of the donor operation, a cannula has been placed in the infrarenal aorta and a clamp has been placed in the supraceliac aorta. The entire cluster graft is removed with the celiac artery and superior mesenteric artery joined in a single aortic patch (Carrel patch). CA, celiac artery; SMA, superior mesenteric artery; SMV, superior mesenteric vein.

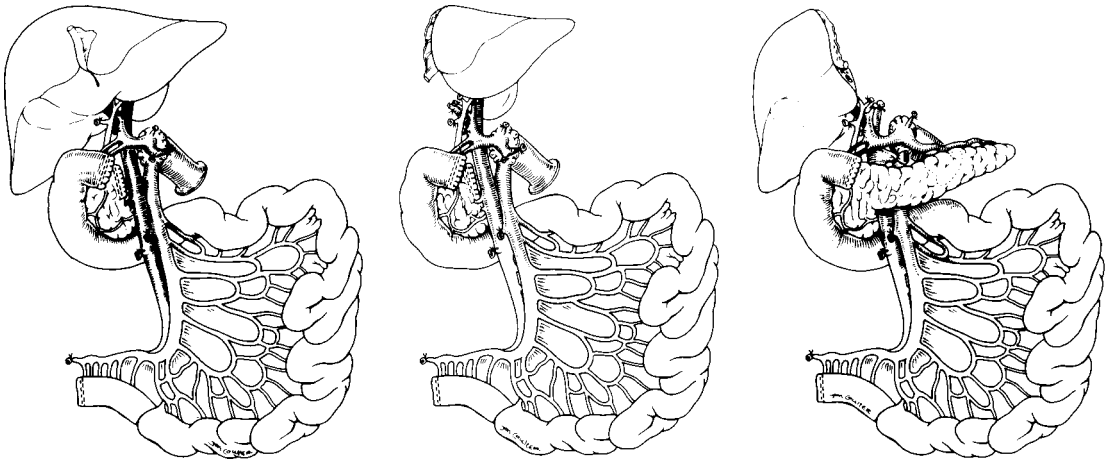


Figure 32.2 The composite liver/small-bowel allograft with preservation of the duodenum in continuity with the graft jejunum and hepatic biliary system is a practical variation of the original cluster graft. The pancreas is transected to the right of the portal vein, but in patients suffering total parenteral nutrition-induced pancreatic disease (with endocrine or exocrine deficiencies), it may be preserved. The aortic patch containing the celiac trunk and superior mesenteric arteries is anastomosed to a conduit of donor thoracic aorta. This technique allows reductions of the liver and/or intestinal components of the allograft, thus permitting the use of larger donor organs for smaller recipients.

from previous resections has required the use of silastic silos (closure using only skin and subcutaneous tissue), and recently, graft reductions of the intestinal and liver allograft by extended right hepatic resection or left lateral segmentectomy (see Figure 32.2). Tissue expanders, applied either before or after transplantation, have been used. The use of abdominal wall from the donor as a composite tissue graft to facilitate abdominal closure after intestinal transplantation has recently been described.

Once the donor organs are found to be satisfactory, the recipient operation begins in order to avoid prolonged cold ischemia times. The recipient operation involves removal of the failed organs, with exposure of the vascular anatomy for arterialization and venous drainage, identification of proximal and distal recipient bowel remnants, and finally allograft implantation.

Composite grafts

Composite grafts include multivisceral grafts and liver/small bowel. For these composite procedures, the native liver is removed, with preservation of the recipient inferior vena cava. It is necessary to ensure that the axial stem of the portal vein between the donor organs remains intact. In recipients of a liver/small-bowel graft, the portal vein of the remaining foregut is attached to the intact portal stem of the donor.

Arterialization of these grafts is accomplished with the double arterial stem of the celiac and superior mesenteric arteries (as a Carrel patch), anastomosed to the infrarenal or supraceliac aorta, with an interposed aortic conduit or iliac artery homograft. The composite graft is connected first to a common conduit of recipient hepatic veins ("piggy-back" to the skeletonized recipient vena cava), and then to the arterioaortic anastomosis. In the multivisceral operation, the recipient's portal vein and gastrointestinal tract, pancreas, and liver are removed with the enterectomy and replaced with the donor portal vein in continuity via the liver, which prevents the need for a portal vein anastomosis. In the modified multivisceral operation, the liver is excluded and only the gastropancreatic–intestinal tract is transplanted. In this operation, the portal venous return is directed into the recipient's portal vein.

Restoration of intestinal continuity requires an anastomosis with native proximal gut (an esophagogastric anastomosis in the multivisceral procedure) and distal native gut, usually a coloenteric anastomosis with the distal ileum allograft. Because the duodenum-preserving composite liver/intestine allograft leaves the hepatic hilum undisturbed, biliary anastomosis is not necessary (see Figure 32.2).

A "chimney" or "loop" allograft ileostomy is performed for routine surveillance of the intestinal allograft. In children with short-bowel syndrome and enteropathies with an intact colon, the ileostomy can be taken down after 6 months to a year when a stable immunosuppressive regimen has been achieved and there has been freedom from rejection without the need for frequent endoscopic surveillance. In children with motility disorders, the decision of a reversal of ileostomy needs to be considered on an individual basis and is also dependent on the experience of the center.

Isolated intestinal grafts

Many patients with functional intestinal failure will not have had abdominal operations or resections. In patients with Crohn disease with multiple abdominal fistulas, enterectomy may be performed before the transplant procedure in order to have a well-healed abdominal cavity. In patients with surgical short gut, the proximal and distal remnants of the intestine are identified. Arterialization of the graft will be from the donor superior mesenteric artery to the infrarenal

aorta. Venous drainage through the superior mesenteric vein may be to the recipient portal vein, superior mesenteric vein, splenic vein, or inferior vena cava. An interposition donor venous graft can be applied to any of the aforementioned native veins in order to avoid a difficult surgical exposure and tension on the vascular anastomosis. Intestinal continuity is provided with anastomoses to previously identified native proximal and distal bowel, with a "chimney" ileostomy for endoscopic surveillance.

The time between procurement and implantation of the allograft (cold ischemia time) ranges from 2 to 17 h, while the warm ischemia time (the time that it takes to sew the graft in) is approximately 30 min. Both of these intervals are important determinants of preservation injury to the intestine.

In order to reduce postoperative fluid loss, early experience with a segment of large intestine was included in 32 patients, but this led to an increased rate of infection and graft failure. With more current infection monitoring and current preservation techniques, colonic transplantation has been revisited with improved results primarily being reduced fluid requirements and improved stool quality [6].

Living donor grafts

A small number of living-donor intestinal transplants have been performed, and in theory living donors may provide a survival advantage due to decreased waiting times, cold ischemia time, and better HLA matching. These living-donor grafts have been performed as an isolated intestine graft using the ileum, and also as a sequential combined (but not composite) liver plus intestine graft, using the ileum and the left lateral segment of the liver. In countries with a well-established cadaveric program, the use of this technique should be restricted especially as outcomes are similar to deceased donor grafts.

Postoperative management

The key to successful postoperative management is effective teamwork between anesthetists, surgeons, pediatricians, and nursing and paramedical staff. Recipients of composite grafts (multivisceral, liver/small bowel, or cluster grafts) suffer from end-stage liver/disease and may require a longer intensive care stay to manage pulmonary, cardiac, and hepatic function. Recipients of isolated small-bowel transplants are less likely to require intensive care, but still present similar infectious risks and the potential to develop hepatic or pancreatic complications. Recipients of isolated liver transplant in the context of intestinal failure may have a prolonged postoperative course because of the need to encourage intestinal adaptation.

The prolonged intensive care stay is related to the potential for graft malfunction, infection, and preoperative liver failure, as well as difficulties with early extubation, which may be exacerbated by the significantly longer operative times for the composite grafts (median time 13 h) in comparison with isolated small-bowel transplants (median time 9 h).

Discrepancies in donor/recipient size may prevent initial closure of the abdominal wall at the time of transplant, requiring the use of silastic or collagen patches until appropriate fluid and electrolyte management can permit definitive closure. The increase in intra-abdominal volume with compression of the thoracic cavity may be an additional factor responsible for respiratory impairment. The measurement of intra-abdominal pressure is essential in children receiving a graft from older donors to recognize abdominal compartment syndrome at an early stage before the manifestation of signs (low urine output, increasing ionotropic requirement, etc.). Staged abdominal closure techniques rather than primary abdominal closure techniques may need to be adopted in the management of the donor: recipient size mismatch grafts [7]. Unusually severe rejection of an isolated small-intestine allograft with systemic venous drainage into the inferior vena cava can produce respiratory insufficiency and an acute respiratory distress syndrome (ARDS) picture.

The main principles of postoperative management are:

- Immunosuppression and prevention of rejection.
- Prevention and treatment of infection.
- Fluid balance and maintenance of nutritional status.
- Assessment of graft function.
- Long-term rehabilitation.

Immunosuppression

The same postoperative immunosuppression is used in both isolated and composite intestinal allograft recipients. This is based on a combination of tacrolimus with or without steroids. Some units begin immunosuppression in the operating room and others immediately afterwards. Induction agents (Thymoglobulin®, Sangstat; alemtuzumab, Campath®, Genzyme Corporation); basiliximab (Simulect®, Novartis Pharmaceuticals) since 2000 have been used to improve clinical outcomes and survival after intestinal transplantation.

As per the Organ Procurement and Transplantation Network/Scientific Registry of Transplant Recipients (OPTN/SRTR) 2013 annual report on intestinal transplantation, for induction therapy in 2013, 54.0% of intestine transplant recipients received T-cell depleting agents, 11.0% received interleukin 2 receptor antagonists, and 38.0% reported no induction [8]. The initial immunosuppression agents most commonly used were tacrolimus (95.0%), steroids (73.0%), mycophenolate (35.0%), and mammalian target of rapamycin (sirolimus) inhibitors (15.0%). Steroids were used in 70.0% of recipients at 1 year post-transplant.

As can be seen from the SRTR/OPTN report, different regimes are used by different international centers. Induction

therapy using humanized immunoglobulin G (IgG)-1 monoclonal antibodies directed at the subunit of the human interleukin 2 receptor (daclizumab or basilimax) has significantly increased rejection-free survival. Indeed, monotherapy with tacrolimus after perioperative lymphoid depletion using rabbit antithymocyte globulin (Thymoglobulin) has improved rejection-free survival, early minimization of tacrolimus exposure, a decreased incidence of opportunistic infections leading to a higher rate of patient and graft survival.

The mainstay of immunosuppression is tacrolimus with steroids at the time of transplantation. In children tacrolimus is usually started at an oral dose of 0.2 mg/kg/day divided every 12 h. Varying preparations of tacrolimus are available commercially. The use of preparations with well-established pharmacokinetic studies is recommended to achieve a steady-state level and prevent the marked fluctuation in levels that can be seen with generic preparations.

The target level of tacrolimus is 12–15 ng/mL within the first few weeks after transplantation, reducing subsequently to 10–12 ng/mL for the next few months until long-term stable range of 5–8 ng/mL is then achieved subsequently. Different centers augment the immunosuppression protocols with mycophenolate mofetil (Cellcept®, Hoffman La Roche); sirolimus (Rapamune®, Wyeth Pharmaceuticals), or azathioprine (Imuran®, Glaxo Smith Kline).

These strategies alter the lymphocyte population of donor and recipient, and have been used principally to avoid GVHD. The paradigm of donor and recipient lymphocyte populations forming "genetic composites" is known as "microchimerism." It is believed that the engagement of these two immunocyte populations leads to mutually canceling immune reactions, with the eventual development of varying disease of non-reactivity. Interestingly, however, the augmentation of microchimerism using the infusion of donor bone marrow cells recovered from donor vertebral bodies at the time of intestinal allograft procurement has not resulted in enhanced intestinal allograft acceptance. The use of donor blood transfusion at the time of transplantation has been used as a minimization strategy and lesser incidence of rejection and other complications have been reported. Although none of the aforementioned protocols is tolerogenic, the lymphocyte-depleting agents have allowed for minimization strategies, thus decreasing the development of complications.

Prevention of infection

Prophylactic antibiotics are given for a varying range of 7–14 days after transplantation. The antibiotic regime will be dependent on each individual center or vancomycin if the patient has been colonized with meticillin-resistant *Staphylococcus aureus* or resistant fecal streptococci; metronidazole 8 mg/kg/dose t.d.s. Co-trimoxazole (240 mg orally if less than 5 years; 480 mg orally over 5 years) is used as prophylaxis against *Pneumocystis carinii*.

Oral non-absorbable selective bacterial and fungal decontamination is given until discharge from the hospital. It is important to note that bacterial translocation can occur with intestinal allograft rejection or other opportunistic infections such as EBV enteritis. It is necessary to treat both the disease causing the immunological damage of the mucosal barrier and the infection.

Antiviral prophylactic strategy is directed towards prevention of infection with CMV and EBV, and includes:

- A 2-week course of intravenous ganciclovir (10 mg/kg/day) with concomitant CMV-specific hyperimmunoglobulin (Cytogam®, CSL Behring).
- Oral aciclovir (200 mg q.d.s. <5 years; 500 mg q.d.s. >5 years) or oral valganciclovir are usually continued for 3 or 6 months, but may be longer depending on the EBV viremia.

Management of fluid balance

In the first 2–3 days after transplantation, fluid shifts between graft, lungs, and peripheral tissues can result in overall fluid retention, but intravascular volume depletion. This is exacerbated by high-dose steroids, tacrolimus, nephrotoxic antibiotics, and antifungals. Early use of renal support, i.e., hemodiafiltration, may be necessary to prevent excessive third space losses and maintain intravascular volume. Ionotropic support may be necessary in the initial few days after transplantation. Fluid balance must take into account fluid losses from abdominal drains, nasogastric and stoma output, and urine output. Fluids should be restricted to two-thirds maintenance and provided as dextrose/saline with colloid as required to maintain central venous pressure (6–10 cmH_2O) and urine output 0.5–1.5 mL/kg/h. Fluids should be liberalized within the first few days after transplantation depending on clinical progress.

Nutrition

TPN is provided with standard TPN formulas and tapered gradually as oral or enteral nutrition is advanced. Enteral feeds by continuous infusion may start as soon as bowel sounds are heard or there is stomal fluid in the stoma bag. In general, it is best to start with simple feeds such as amino acid based formulas with medium-chain triglycerides, glutamine, and glucose polymers. Long-chain triglyceride is added later when lymphatics are established, usually 3 months after intestinal transplantation. Many children will not voluntarily eat after transplantation, as this may be the first time many of them will have experienced normal feeding. Children with motility disorders and primary mucosal disorders who have lagged behind in the development of oromotor skills may take months to years to establish nutritional support. Management requires a multidisciplinary approach involving psychological support. Enteral supplementation may be required on a long-term

basis. Independence from TPN is achieved in the majority of functioning grafts within 4–6 weeks.

Assessment of the graft

Monitoring of liver and pancreatic components of composite intestinal allografts is performed as for isolated transplantation of these solid organs. Regular assessment of liver function tests, amylase, and insulin requirements is routine; abdominal ultrasound and biopsies from transplanted organs are performed as required.

Intestinal grafts

Assessment of the anatomical and functional viability of an intestinal allograft begins in the operating room immediately after graft reperfusion. Venous outflow disturbances may result in congestion and ecchymosis, which must be differentiated from hyperacute rejection and ischemic damage. Similar changes may be seen in the ileal stoma postoperatively.

As intestinal allografts may be composed of varying lengths of the gastrointestinal tract with functionally and anatomically differing segments (stomach, duodenum, small intestine, colon), the assessment of these grafts must be flexible, aggressive, and multidisciplinary. There are no good functional or biochemical markers to assess injury or rejection of intestinal allografts, although a rise in the gentamicin levels in children receiving oral decontamination may be an early indicator. Fecal calprotectin in its initial studies showed promise as a non-invasive marker of rejection. Further studies failed to confirm its validity especially as it can be elevated in severe viral enteritis.

The most effective methods of assessing graft status and diagnosing rejection are:

- Assessment of stomal output for volume, consistency, reducing substances, and bacterial overgrowth.
- Routine surveillance performed twice a week through the allograft ileostomy (either endoscopic procedures or stomal biopsies) provides adequate information in the majority of cases. Occasionally, upper gastrointestinal endoscopy with visualization and biopsy of the proximal allograft is necessary.
- Formal motility testing is occasionally required for long-term functional assessment.

Complications after transplantation

Clinical/surgical factors

Complications related to the surgical procedure or the postoperative clinical management of the recipient are common. The major technical complications are biliary or intestinal anastomotic leaks, intestinal perforation, and thrombosis of the hepatic artery in the larger composite grafts, which may be complicated by severe intra-abdominal infections with polymicrobial and fungal organisms. The most serious clinical management issues are rejection, infection, fluid and electrolyte disturbances, renal dysfunction, and hypertension, which are exacerbated by high levels of tacrolimus and steroids.

Rejection

Historically, early acute rejection was historically seen in 70–90% of transplant recipients in the early 1990s, and it is more frequent and severe in recipients of isolated intestinal grafts in comparison with liver/small bowel or multivisceral allografts. It is now seen in only 30–50% of patients. This has been attributed to the use of newer immunosuppression agents, including monoclonal antibodies (interleukin 2 antagonists, antithymocyte globulin, Campath (anti-CD52)), and sirolimus. Most episodes occur within the first 90 days [9]. The rate of rejection of the liver when it is part of a composite graft is 43%, suggesting that the liver graft may protect the intestinal component. In composite grafts that included other organs, the rejection rates were as follows: colon 34%, stomach 12%, and pancreas 12%.

Symptoms of rejection include fever with abdominal pain and distension, nausea and vomiting, tenderness on palpation, ileus, and increased stomal output. An increase in stomal output suggests either infection or rejection, but does not differentiate between the two. The diagnosis of rejection needs to be made on histopathological examination of graft tissue from endoscopic biopsies or stomal biopsies. The presence of blood in the stool is always an ominous sign, requiring an urgent endoscopy, since this may be due to rejection or infection.

Acute intestinal rejection presents with non-specific symptoms such as the following:

- Sepsis due to bacterial or fungal translocation occurs as a result of disruption of the intestinal mucosal barrier from rejection. Paradoxically, such severe infections can only be treated by stabilizing the allograft mucosal barrier with augmented immunosuppression.
- Endoscopically, the transplanted intestinal mucosa loses its velvety appearance and becomes hyperemic or dusky, as well as hypoperistaltic. Erythema may be focal or diffuse; the mucosa becomes friable, and diffuse ulceration appears (see Figure 32.2).
- Histological criteria for acute rejection of the intestine include edema of the lamina propria with mononuclear cell infiltrates, villous blunting, cryptitis, and crypt cell apoptosis. Varying degrees of epithelial cell necrosis and regeneration may be seen. In severe rejection, there is crypt destruction and complete sloughing of the endothelium, resulting in pseudomembranous enteritis (Figure 32.3). The histological assessment of endoscopic biopsies can be

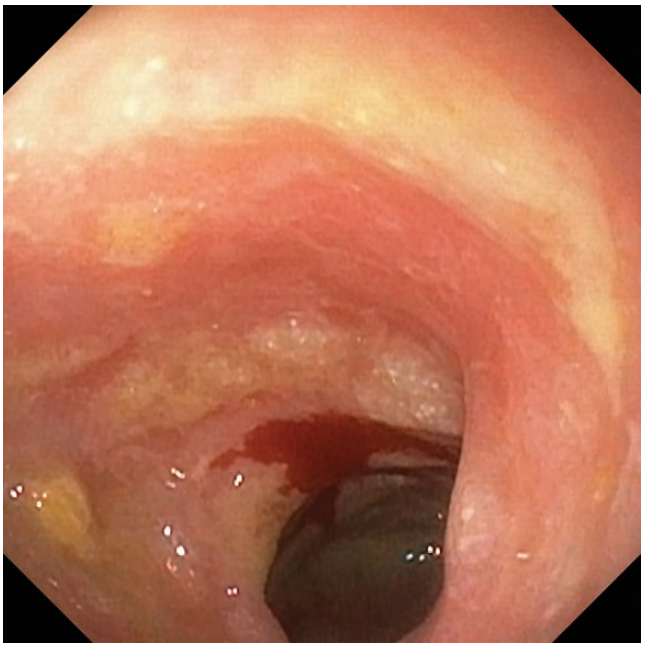


Figure 32.3 Endoscopic appearance of severe intestinal allograft rejection. There has been sloughing of the mucosa and ulceration.

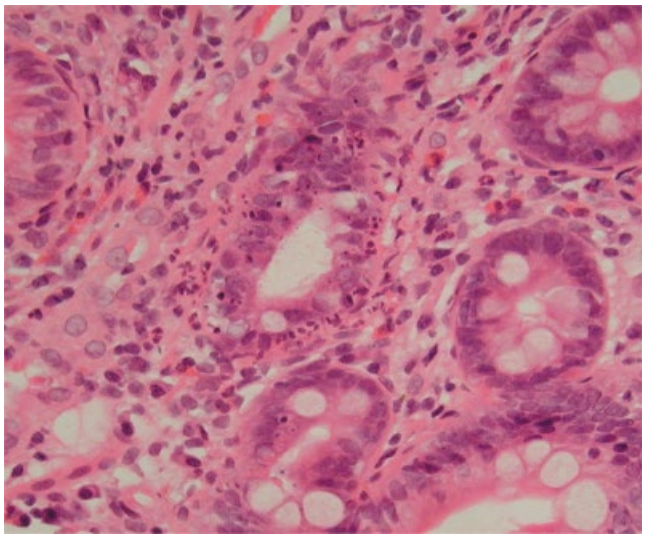
hampered by viral infections and the presence of "patchy" changes; however, a recent grading system has addressed these difficulties.

It is unclear whether, in the most severe form of rejection, the progressively worsening apoptosis or a vascular response to rejection leading to ischemia results in the severe mucosal sloughing and crypt destruction. The mucosal surface becomes replaced by inflammatory pseudomembranes and granulation tissue, which precipitate continuous blood loss, as well as intermittent septic episodes from bacterial and fungal translocation.

Chronic rejection has been observed in patients with persistent intractable rejection episodes. These patients present with:

- Progressive weight loss, chronic diarrhea, intermittent fever, and gastrointestinal bleeding.
- Low albumin in the context of the above symptoms can be a useful marker.
- Endoscopic mucosal biopsies reveal a scant cellular infiltrate, with villous blunting, focal ulceration, and epithelial metaplasia (Figure 32.4B). Full-thickness intestinal biopsies may reveal obliterative arteriopathy, which is uncommon in children.
- Radiographically using barium studies there may be strictures and dilatations, with areas of arteriopathy on angiography.

The diagnosis of chronic rejection is difficult to establish and a high index of suspicion is needed for early recognition of this condition.



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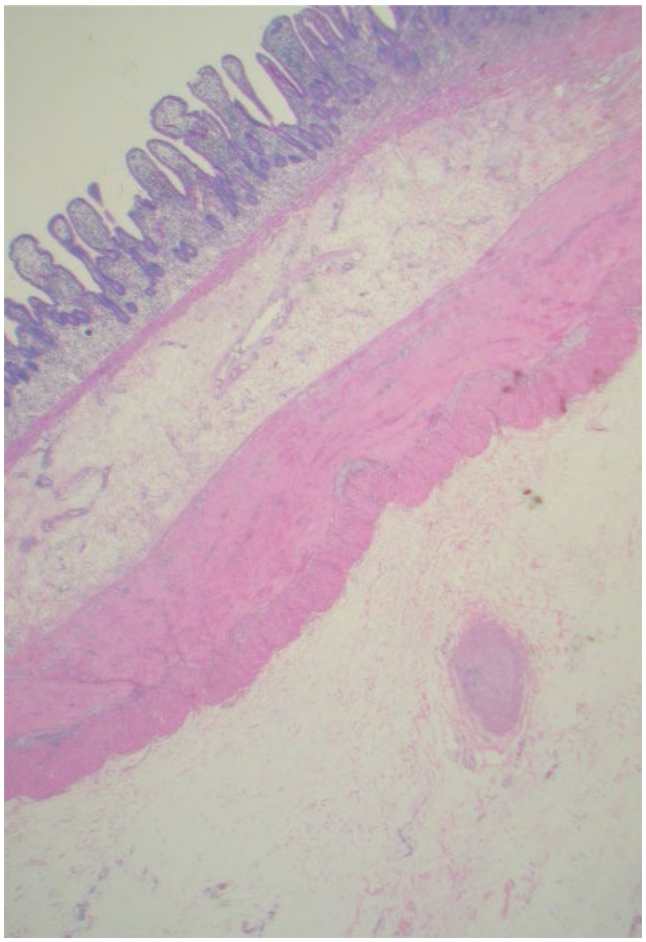


(B)

Figure 32.4 (A) Histological appearance of severe intestinal allograft rejection, with loss of villi and crypts and multiple apoptosis in the crypt. (H&E, original magnification ×400.) (B) Chronic rejection demonstrates thickening and fibrosis of the mucosa, which is best appreciated in the resected bowel. (H&E, original magnification ×40.)

Therapy

Graft rejection is treated with high doses of intravenous methylprednisolone (10 mg/kg) as bolus therapy in cases of mild rejection, and with a taper in cases of moderate to severe rejection. The tacrolimus trough levels in whole blood are maintained at around 15 ng/mL. Alemtuzumab or Thymoglobulin is reserved for steroid-resistant rejection, or in cases of severe mucosal injury and crypt damage. Addition of sirolimus (rapamycin) or mycophenolate mofetil may also be useful in the treatment of resistant rejection.

Infections

Infection remains very common after transplantation according to the intestinal transplant registry records: infection accounts for 60–70% of deaths post-transplant [9]. Infectious complications are frequent due to several predisposing factors including:

- The severity of preoperative liver failure.
- The presence of intra-abdominal, pulmonary, or catheter sepsis prior to transplantation.
- The higher level of immunosuppression required to prevent rejection in intestinal grafts.
- There is a higher incidence of infectious complications in recipients of large composite intestinal allografts, perhaps because of the technically challenging transplant procedures, with increased operative time, transfusion requirements, and intestinal cold ischemia times.

The translocation of bacteria from the gut lumen is the most likely cause of the high incidence of bacterial infections post-transplant. Broad-spectrum antibiotics are routinely administered during the early post-transplant period along with selective decontamination of the gut with oral antibiotics and antifungals.

Bacterial pathogens include staphylococci (intravenous line induced), enterococci, and Gram-negative rods. Recently, the hospital-acquired vancomycin-resistant *Enterococcus* (VRE) has been a problem in the UK. Enteric organisms are usually associated with abdominal wound infections, deep abdominal abscesses, peritonitis, pneumonia, and bacterial translocation from grafts damaged by rejection. Multiple sources of infection can occur simultaneously, or there may be mixed infections from the same source. The multiple antibiotic regimens required to treat these infections may precipitate the development of resistant organisms such as the nascent strain of pan-resistant enterococci, and fungal infections.

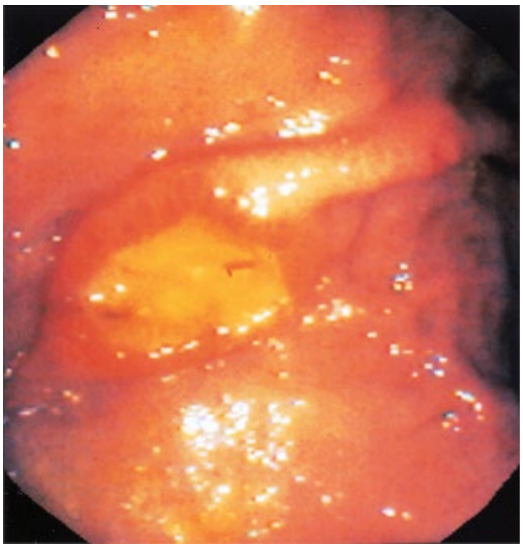
Fungal infections are usually the result of intravenous line contamination, translocation due to rejection, massive antibiotic usage, and intestinal leaks. Aggressive medical and surgical therapy are required in patients with this complication. It is not usually wise to reduce immunosuppression, as the coexistence of cellular rejection warrants maintaining an intact mucosal barrier by appropriate augmentation of immunosuppression. Complete withdrawal of immunosuppression is impossible in this recipient population, due to a high incidence of rebound rejection.

Viral infection such as CMV, adenovirus, EBV, rotavirus and calcivirus, and protozoa (*Giardia lamblia*, *Crytoporidium*) are reported in 40% of recipients [10]. The presentation of these infections can be similar to that of rejection. It is important to distinguish between the two because the treatment is very different. Rejection needs to be treated with increased immunosuppression, in contrast to these infections which are treated with a reduction of immunosuppression.

CMV disease is highest in CMV-negative recipients of CMV-positive grafts; these recipients have more aggressive disease, manifested by a higher incidence of recurrence, persistence of disease, involvement of native intestine, hepatitis, central nervous system disease, and retinal involvement. Diagnosis is usually made after non-specific symptoms have prompted measurement of CMV RNA, CMV antigenemia in the peripheral blood, and/or an endoscopy (Figure 32.5). Endoscopic images usually reveal superficial ulcers against a background of normal mucosa. Histopathology shows typical CMV inclusion bodies, although care must be taken not to associate occasional crypt epithelium apoptosis with rejection. Successful clinical management has been accomplished in over 95% of episodes using intravenous ganciclovir, alone or in combination with CMV-specific hyperimmunoglobulin. It is essential to avoid rejection by maintaining immunosuppression at baseline and reducing it only in the face of deteriorating clinical disease.

EBV is also commonly seen in children post-transplant. Uncontrolled EBV can cause an EBV-driven post-transplant lymphoproliferative disease, resulting in significant mortality and morbidity. Risk factors include recipient age, history of previous splenectomy, and the use of OKT3. Progression of EBV viremia to post-transplantation lymphoproliferative disease (PTLD) can be prevented by reduction in immunosuppression and allowing the body to mount an immune response against EBV infection. Therapy includes the reduction and withdrawal of immunosuppression, antivirals (ganciclovir, aciclovir, hyperimmunoglobulin), rituximab (a monoclonal antibody against the B-lympocyte CD20 receptor), and chemotherapy. More recently, rituximab (a monoclonal antibody) and infusion of HLA-matched T cells have had success in treating this difficult disorder. The introduction of EBV quantitative polymerase chain assay has facilitated the early detection of this virus, and enabled preemptive reduction in immunosuppression to prevent the progression of PTLD (Figure 32.6). In 1992 PTLD was occurring in 20–40% of recipients which decreased to only 5–10% in 2007 [11].

Although viral infections are less common, respiratory syncytial virus, rotavirus, adenovirus, and parainfluenza virus lead to significant morbidity and severe mortality in this population.



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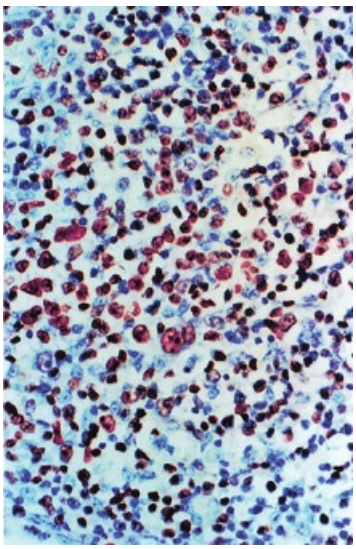


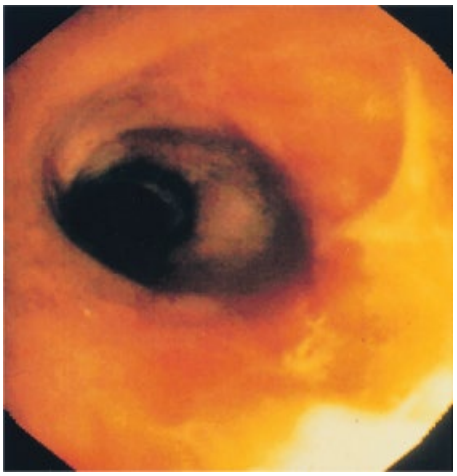
(B)

Figure 32.5 (A) Typical endoscopic appearance of "shallow" ulcer surrounded by normal mucosa. (B) Histopathology shows typical cytomegalovirus inclusion bodies. The number of inclusion bodies has been associated with more severe clinical disease, with gradual disappearance as the disease resolves.

Causes of graft loss

The main cause of intestinal graft loss is infection, which may be associated with rejection or PTLD in 48% of grafts lost. The decision for graft enterectomy should be based on significant intestinal graft dysfunction and the need to withdraw immunosuppression, prior to the development of infectious complications, which may persist after removal of the graft.

Re-transplantation of the intestine is required for acute and chronic rejection, and has a higher failure rate due to the



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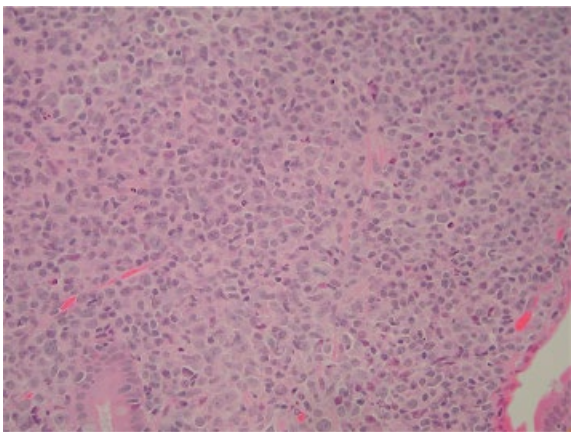

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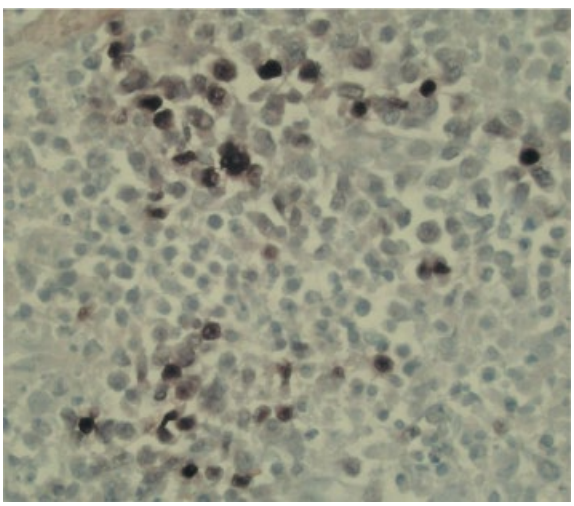

(C)

Figure 32.6 (A) Endoscopic appearance of a post-transplant lymphoproliferative disease (PTLD) lesion, with a raised, "tumorous," ulcerated lesion surrounded by normal-appearing mucosa. Histopathology shows a polymorphic infiltrate (B) (H&E, original magnification ×40), which demonstrates "transformed" PTLD lymphocytes staining positive for Epstein–Barr virus early RNA (EBER). (C) In situ hybridization for Epstein–Barr virus early RNA (original magnification ×600).

precarious clinical status of the patient when the re-transplant is performed. Recent re-transplant experience with 70% survival at 5 years has been reported [12]. Re-transplantation should be restricted to highly selected cases, such as early vascular thrombosis or primary non-function of the graft prior to significant manifestations of other organ failure.

Long-term outcomes and quality of life

Quality of life

The outcome post-intestinal transplantation has progressively improved over the last 10 years, and with that, the emphasis has changed from short survival to long-term survival and quality of life (QoL) assessment. Assessing QoL in intestinal transplant recipients is difficult due to significant patient variability with respect to underlying disease, the postoperative course, long-term complications, and psychosocial factors. Few studies have been performed in this area as there is no standardized measure of QoL currently available. Results may vary depending on how the study is conducted and who is providing the responses; this was highlighted in the Sudan study of 10–16 year olds small-bowel recipients [13]. The recipients rated their QoL as equivalent to healthy children of the same age; however, their parents remained more anxious than parents of health children.

In a QoL study by O'Keefe *et al*. of 46 adult patients 12–36 months after intestinal transplant were evaluated [14]. This group of adults were found to have significant improvements in 13/26 domains assessed after transplant.

QoL assessment in this group of patients is further complicated by the lack of studies in children on long-term home PN, therefore, it is difficult to make comparisons between home PN and life after intestinal transplantation.

Pironi *et al*. compared QoL in adults on home PN and following successful transplantation [15]. The subjective health feeling in adults following intestinal transplantation was better than in adults on home PN, whereas vitality and mental domains were comparable in intestinal transplant and home PN adults.

It is difficult to draw strong conclusions regarding QoL after intestinal transplantation; however these few results are encouraging and suggest QoL is good after transplantation and perhaps similar to that of children with other chronic conditions.

Survival

Survival has improved with the use of tacrolimus, and international experience has confirmed the feasibility and lifesaving potential of this therapeutic modality.

Survival after intestinal transplantation has seen dramatic improvements in the last 5 years, with centers reporting 1-year survival rates varying from 70% to 100%. Data from the international Intestinal Transplant Registry indicate a 1-year graft survival rate of 70%; however, there was a superior survival of 81% in patients who received induction with antilymphocyte globulin and at experienced intestinal transplant centers [16].

Indeed, an analysis of survival statistics after intestinal transplantation shows that the 1-year survival figures reflect surgical experience and appropriate perioperative clinical management, which should result in an approximately 70% rate of patient survival. However, because of the high incidence of rejection and the need for higher long-term immunosuppression, long-term survival continues to decrease, with a 3-year patient survival of 55% [17]. Most of the deaths are from opportunistic infections such as CMV and PTLD-associated EBV infection, which may be prevented by early diagnosis and pre-emptive therapy. Further improvement in survival, however, rests on decreasing the incidence of rejection and providing long-term rehabilitation and graft acceptance without the need for high baseline immunosuppression.

Nutritional status and long-term rehabilitation

Independence from TPN has been accomplished in most children within the first 4–6 weeks. Although all children tolerate a regular oral diet, 87% of these receive their complete nutritional requirements solely from this route. The remainder (17%) require enteral supplementation because of behavioral feeding problems or inadequate calorie intake. Food allergies to lactose and gluten have been common.

Linear growth occurs to a greater degree in older children than in recipients aged 1–5 years, and it progresses after weaning from TPN as calories are provided via oral and/or enteral routes. Deficiencies in red blood cell folate, zinc, and copper levels have been documented postoperatively in some patients.

The intensity of home medical services, however, decreases over a 1–3-year period after transplantation as the patient recovers and is reintegrated into the home and school environment. Children are able to make physical and developmental progress. All school-aged children participate in a school program. Older children and adolescents are often upset about the alterations to their bodies, with protruding lines, tubes, and an ileostomy. Thus, removal of medical appliances may be psychologically and developmentally beneficial, helping the child adapt more easily to life after intestinal transplantation. There is now evidence that although small-bowel transplantation is expensive, the improvement in outcome and the reduction in the costs of home PN make it economically viable.

Recent advances in the field of intestinal transplantation

Donor-specific antibodies and immune monitoring

Increasing evidence has linked the development of donor-specific antibody to graft loss in intestine transplantation [18]. Guidelines to integrate immunological [19, 20] and histological findings must be defined to improve the management of intestinal transplantation grafts more optimally.

Graft-versus-host disease

The phenomenon of donor and recipient cell migration observed in all solid organ recipients has been described as "chimerism." This hypothesis forms the basis of the two-way paradigm of transplantation immunology, in which donor and recipient cell populations interact with mutually canceling effects, producing eventual allograft acceptance. Using bone marrow augmentation with adjuvant donor bone marrow cells, the presence of donor cell chimerism was documented in 100% of the study population and 80% of the control patients.

GVHD is unusual in this population unless there is preexisting immunodeficiency and may be asymptomatic or associated with rejection. Skin changes consistent with GVHD were diagnosed by histopathological criteria in only seven children (8%), and in only two patients who had received adjunct bone marrow, with onset ranging from 6 days to 8 years following transplantation. Histological criteria for GVHD include keratinocyte necrosis, epithelial apoptosis of native gastrointestinal tract, or epithelial cell necrosis of oral mucosa. Spontaneous resolution occurred in all but one patient, who had hereditary IgG and IgM deficiency and died from septicemia. GVHD was diagnosed 4 days after immunosuppression stopped and was confirmed by immunohistochemical studies visualizing donor cell infiltration into the lesions.

Consequently, the impact of GVHD in clinical transplantation is not significant. Also, it is presently unclear whether graft irradiation or donor pretreatment can alter the incidence of clinical GVHD.

Treatment strategies of GVHD following intestinal transplantation differ from the bone marrow transplant with GVHD as the development and progression of disease varies. The use of high-dose steroids should be restricted to children developing an aggressive skin GVHD with areas of widespread erythema and peeling. Although successful use of monoclonal antibody therapies (basiliximab and infliximab), have been reported in the bone marrow transplant population, they have not been proven to be particularly beneficial in our experience. Reduction of immunosuppression with use of extracorporeal photopheresis (ECP) and mesenchymal stem cells should be the treatment options considered.

Future status

Significant advances in medical and surgical therapy have occurred in the field of intestine failure rehabilitation which have decreased the need for intestinal transplantation and have led to the re-evaluation of appropriate criteria for the timing of intestine transplantation evaluation. Long-term outcome improvements will hinge on understanding cellular and humeral components leading to chronic rejection and subsequent therapeutic interventions.

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CHAPTER 33

Combined Liver and Kidney Transplantation

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Key points

- CLKT is a successful transplant option in children.
- CLKT should be undertaken in specialist centers and in carefully selected children.
- Renal graft outcome and function in CLKT may be superior to isolated renal transplantation.

Combined liver and kidney transplantation (CLKT) has become an accepted transplant option for a small number of children because of increasing surgical expertise and better intensive care and medical support. It nonetheless remains a procedure with significant mortality and morbidity in comparison with isolated liver or kidney transplantation, making patient selection and preoperative assessment particularly important. The small number of children requiring this intervention necessitates limiting the number of centers undertaking the procedure to ensure maintenance of expertise and to optimize outcome. While a consensus document for adult patients has been published [1] no similar document is available for children, although some uniformity of practice exists because of the small number of centers undertaking the procedure.

Conditions requiring combined liver and kidney transplant

The main conditions requiring CLKT are primary hyperoxaluria (PH1) and polycystic liver and renal disease (Table 33.1) as reported to the Scientific Registry of Transplant Recipients (SRTR) for 152 children transplanted between October 1987 and February 2011 [2].

In PH1, failure of liver synthetic function is not a feature but the genetic defect in the liver leads to overproduction and accumulation of total body oxalate with systemic deposition and significant renal damage. In some patients with PH1 and relative preservation of renal function, isolated liver transplantation may be possible if the estimated glomerular filtration rate (GFR) is >40 mL/min/1.73 m^2. There is no good evidence upon which to base this recommendation and most centers agree this would be an absolute lower limit, with some units undertaking CLKT at a higher GFR.

There are some conditions with normal liver function that may require CLKT. For example, atypical hemolytic uremic syndrome (aHUS), which is caused by complement deficiency, or methylmalonic academia, in which there is normal liver function but renal dysfunction develops as a result of the metabolic abnormalities. Deficiency of natural anticoagulants (e.g., protein S or C) are other examples of diseases in which the synthetic function of the liver is normal but the hepatic genetic defect leads to distant (including renal) disease.

Fibrocystic liver and kidney disease accounts for a significant proportion of the remaining indications in which CLKT is indicated, usually because of increasing renal failure in association with liver dysfunction (see Chapter 14). Current practice is to recommend CLKT before dialysis becomes necessary.

In children with significant renal dysfunction as a result of the use of nephrotoxic drugs (e.g., hepatoblastoma) CLKT may be required if liver replacement is being considered (see Chapter 28).

Hepatorenal syndrome is not an indication for CLKT because a rapid recovery of renal function is usually seen after successful liver transplantation. The consensus document for adult CLKT [1] specifically excludes hepatorenal syndrome as an indication for combined transplantation, with the exception of patients who have required dialysis for

Diseases of the Liver and Biliary System in Children, Fourth Edition. Edited by Deirdre A. Kelly.

Table 33.1 Diagnoses in 152 children requiring combined liver and kidney transplantation. (From Calinescu *et al*. 2014 [2]. Reproduced with permission of John Wiley & Sons.)

Primary liver disease, n (%)	Primary kidney disease, n (%)
PH1 56 (37%)	Oxalate nephropathy 55 (36%)
Congenital hepatic fibrosis 28 (18%)	Polycystic kidneys 38 (25%)
Metabolic disease (non-PH1) 11 (7%)	HUS 2 (1%)
Polycystic liver disease 7 (5%)	Chronic glomerulonephritis 2 (1%)
Idiopathic cryptogenic cirrhosis 3 (2%)	Renal artery thrombosis 2 (1%)
Hepatoblastoma 3 (2%)	Diabetic nephropathy (type I) 2 (1%)
Alagille syndrome 3 (2%)	Nephritis 1 (1%)
Familial cholestasis 2 (1%)	Focal glomerular sclerosis 1 (1%)
Neonatal hepatitis 2 (1%)	Chronic pyelonephritis/reflux nephropathy 1 (1%)
GSDI 2 (1%)	Progressive systemic sclerosis 1 (1%)
Primary sclerosing cholangitis 2 (1%)	Acquired obstructive nephropathy 1 (1%)
Acute hepatic necrosis 1 (1%)	Hypertensive nephrosclerosis 1 (1%)
Postnecrotic type B HbsAg cirrhosis 1 (1%)	Membranous nephropathy 1 (1%)
Autoimmune liver cirrhosis 1 (1%)	Cancer chemotherapy-induced nephritis 1 (1%)
Cryptogenic liver cirrhosis 1 (1%)	Congenital obstructive uropathy 1 (1%)
Secondary biliary cirrhosis 1 (1%)	Hypoplastic/dysplastic nephropathy 1 (1%)
Other liver cirrhosis 1 (1%)	Malignant hypertensive nephrosclerosis 1 (1%)
Non-syndromic paucity of intrahepatic bile ducts 1 (1%)	Others (not specified in database) 40 (26%)
Cystic fibrosis 1 (1%)	
Alpha-1-antitrypsin deficiency 1 (1%)	
Hepatocellular carcinoma 1 (1%)	
Other primary liver neoplasm 1 (1%)	
Other (not specified in database) 22 (14%)	

CLKT, combined liver and kidney transplantation; GSDI, glycogen storage disease type I; HUS, hemolytic uremic syndrome; PH1, primary hyperoxaluria.
Percentages are rounded to the nearest whole number.

more than 8 weeks as this duration of dialysis dependency suggests irrecoverable renal damage, and single center reports have shown a survival advantage in this group.

Patient selection

Liver failure and chronic kidney disease (not on dialysis)

In some children the primary indication for transplantation is liver disease, but a combined transplant is considered appropriate because of concomitant chronic kidney disease (CKD). The decision to undertake CLKT is rendered difficult in children not on dialysis because there is a spectrum of CKD ranging from mild to severe. In considering the likely need for CLKT it is helpful to classify the severity of CKD using an internationally accepted grading scheme:

CKD 1: GFR ≥90 mL/min/1.73 m^2 with structural renal disease.
CKD 2: GFR 60-89 mL/min/1.73 m^2.
CKD 3a: GFR 45-59 mL/min/1.73 m^2.
CKD 3b: GFR 30-44 mL/min/1.73 m^2.
CKD 4: GFR 15-29 mL/min/1.73 m^2.
CKD 5: GFR <15 mL/min/1.73 m^2.
CKD 5d: GFR <15 mL/min/1.73 m^2 and on dialysis.

In categorizing patients it is necessary to determine the GFR. This can conveniently be undertaken by estimating the GFR using the Schwartz formula [3] in which plasma creatinine and height are substituted into the equation:

$$\frac{\text{Height (cm)}}{\text{Plasma creatinine (}\mu\text{mol/L)}} \times \text{constant} = \text{eGFR}$$

The value for the constant is determined by the methodology for measuring creatinine (30 if using enzymatic methods), and can be further refined for gender. This methodology is convenient and can be undertaken each occasion the plasma creatinine and height are measured, allowing tracking of eGFR to determine a trend and to help predict eGFR change.

While eGFR is useful there is a significant error, especially for values >60 mL/min/1.73 m^2, consequently the formal measurement of GFR is often undertaken to provide an accurate value. Various methods are used but all require the timed clearance of a marker (e.g., ^{51}chromium ethylenediaminetetra-acetic acid, inulin, iohexol) either given as an infusion or as a single bolus.

In children with stable CKD who are not on dialysis, the primary risk of isolated liver transplantation is a reduction in renal function after liver transplantation such that dialysis and renal transplantation become necessary. The causes for such a deterioration include acute on chronic kidney injury from renal insults incurred during and immediately after liver transplantation (Box 33.1), the progression of CKD from

Box 33.1 Causes of acute kidney injury at or immediately after liver transplantation.

- Hypoxia/hypotension
- Severe and prolonged acidosis
- Hepatorenal syndrome
- Multiple transfusion
- Septicemia
- Drugs:
  - direct nephrotoxicity – aminoglycosides, amphotericin, CNI, non-steroidal anti-inflammatory drugs, allergic response (interstitial nephritis), ACEi/ARB
  - indirect toxicity – high-dose inotropes causing intrarenal vasoconstriction

ACEi/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; CNI, calcineurin inhibitor.

calcineurin inhibitor toxicity, the progression of underlying renal disease (e.g., autosomal recessive polycystic kidney disease (ARPKD)), and progressive renal damage from recurrent urinary tract infections complicating immunosuppression. The likelihood of post-transplant progression of CKD also increases with the severity of CKD at the time of liver transplantation. Children who have undergone isolated liver transplantation and who subsequently develop a reduction in renal function may require dialysis. Although, the first choice of dialysis technique in children is usually peritoneal dialysis, it is contraindicated in children following liver transplantation because of the intraoperative damage to the peritoneal cavity. Consequently, such children require hemodialysis.

There is no consensus to base recommendations regarding the choice or timing of CLKT in children with CKD. The practice at this unit is to recommend CLKT for any child requiring liver transplantation with CKD 4 or worse either at the time of listing, or who may deteriorate to this stage within 6 months of listing. It is not clear if this advice is appropriate for children with PH1 and CKD, as some clinicians advocate early or preemptive CLKT [4] because of the deleterious effect of mobilization of systemic oxalate post liver transplant, especially if the immediate post-transplant period is complicated by acute kidney injury (AKI).

Chronic kidney disease requiring renal transplantation and liver disease

Children with CKD may have concomitant liver disease – this is seen most commonly in those with ARPKD or PH1, although Table 33.1 lists a number of other possible diagnoses. In children with mild liver disease who do not require liver transplantation but who have significant renal disease, isolated renal transplantation may be considered. For instance, children with portal hypertension who have adequate synthetic liver function may be considered for a portosystemic shunt and an isolated renal transplantation. However, past experience and that of Tsimaratos *et al.* [5] indicate that it is better to perform a CLKT as hepatic function may deteriorate after the procedure, precipitating encephalopathy and sepsis with a possibly fatal outcome. So any child with deteriorating renal function needs careful evaluation of their hepatic condition [6], and CLKT is the safest treatment for these children (see Chapter 14).

Children on dialysis

Renal transplantation is the treatment of choice for most children on dialysis because while dialysis is able to maintain biochemical stability it does not provide a normal GFR and consequently does not provide optimal biochemical correction of renal failure. Furthermore, long-term dialysis is associated with progressive loss of dialysis access, has an adverse impact on growth, psychosocial development, and school performance, and is also associated with long-term cardiovascular morbidity and mortality from hypertension and coronary artery calcification.

The decision to proceed to CLKT in a child on dialysis who does not have advanced liver failure requires consideration of the risks and benefits of a combined transplant in contrast to an isolated transplant. The main risk of undertaking an isolated renal transplant in ARPKD is the higher incidence of cholangitis in immunosuppressed individuals post-transplant, which may precipitate hepatic decompensation [6, 7]. The use of antiplatelet therapy after renal transplantation, especially in smaller children, increases the risk of gastrointestinal bleeding from peptic ulceration or from esophageal varices.

A major benefit of CLKT is the immunomodulatory effect of the combined transplant which benefits the kidney graft [8, 9]. An analysis of the 1995–2005 United Network for Organ Sharing (UNOS) database included 111 CLKT and 3798 isolated kidney transplants identified from the Organ Procurement and Transplantation Network (OPTN)/UNOS data (Figure 33.1) and shows that 5-year kidney transplant survival after CLKT is better than for isolated kidney transplants. Our own data for 40 CLKT patients shows a similar benefit to graft outcome (Figure 33.2) and function as measured by eGFR (Figure 33.3) and rate of eGFR decline for

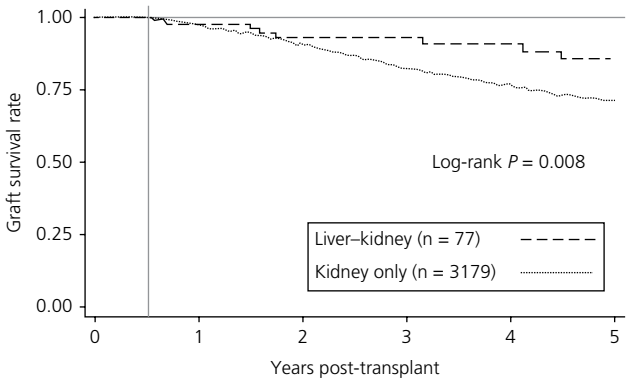


Figure 33.1 Kidney graft survival in combined liver and kidney transplantation compared with isolated kidney transplants. (From de la Creda *et al.* 2010 [12]. Reproduced with permission of John Wiley & Sons.)

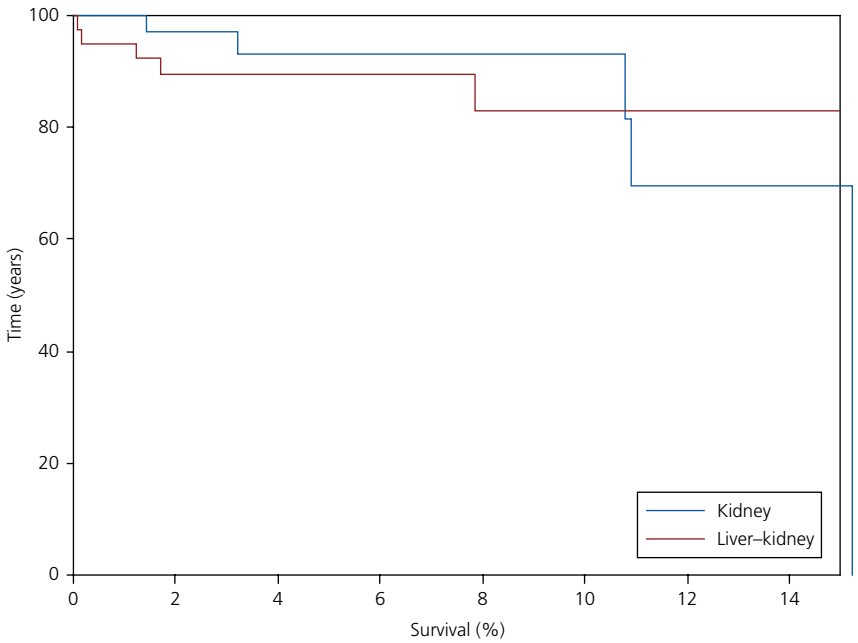
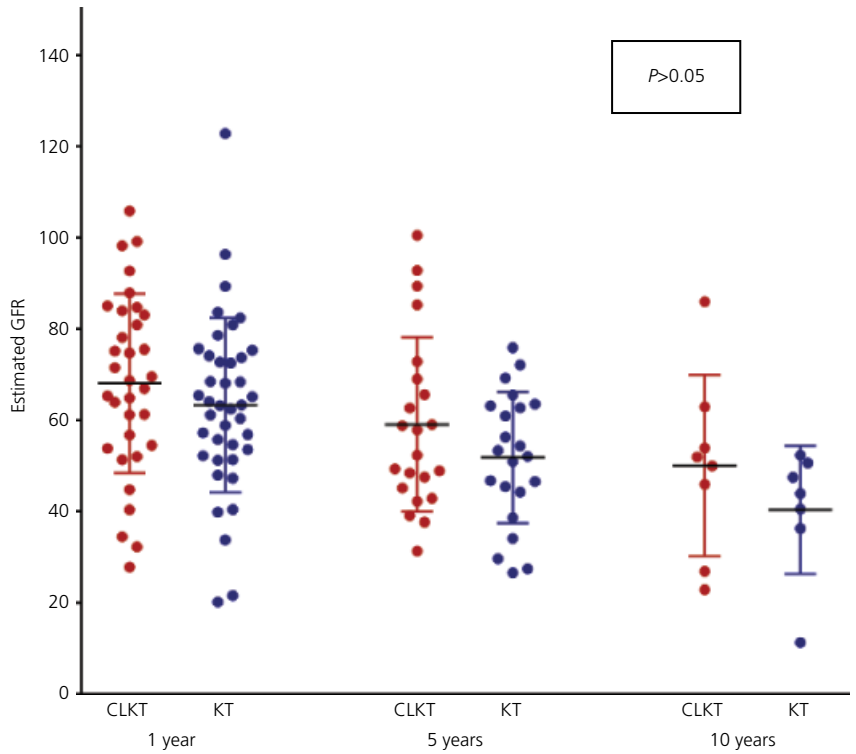


Figure 33.2 Renal outcome after combined liver and kidney transplantation in 40 recipients compared with isolated kidney transplantation. (Data source: Birmingham Children's Hospital NHS Foundation Trust.)



Mean eGFR 1 year: CLKT (33 patients) – 68 mL/min/1.73 m^2 (SD 19), KT (40 patients) – 63 mL/min/1.73 m^2 (SD 19)

Mean eGFR 5 years: CLKT (22 patients) – 59 mL/min/1.73 m^2 (SD 19), KT (22 patients) – 52 mL/min/1.73 m^2 (SD 14)

Mean eGFR 10 years: CLKT (8 patients) – 50 mL/min/1.73 m^2 (SD 20), KT (07 patients) – 40 mL/min/1.73 m^2 (SD 14)

Figure 33.3 Renal graft function in 40 combined liver and kidney transplant (CLKT) recipients compared with matched isolated kidney transplants (KT). (Data source: Birmingham Children's Hospital NHS Foundation Trust.)

isolated kidneys, −3.8 compared with −1.5 mL/min/1.73 m²/year for CLKT between 5 and 10 years after transplantation. This is because of the lower incidence of rejection, in particular a lower incidence of late graft rejection in the kidney.

Many families may be reluctant to consider CLKT when there is an adequately functioning liver because of the increased mortality and morbidity associated with this surgery in comparison to an isolated kidney transplant. However, if the medical team's assessment is that the severity of the liver disease is such that isolated renal transplantation is likely to lead to significant hepatic morbidity, careful explanation of the risks and benefits helps children and families to understand why a CLKT may be the best option.

Children with chronic kidney disease

It is increasingly accepted that pre-emptive renal transplantation (renal transplantation before the need for dialysis) is the ideal management for a child with CKD and deteriorating renal function. Unfortunately, renal function often does not deteriorate in a predictable way, making the identification of the optimum time for transplantation difficult. Furthermore, although live-related transplantation offers the best chance of getting the timing right, cadaveric transplantation is much less predictable. However, it is still possible to plan for pre-emptive CLKT using cadaveric donors, especially in children with ARPKD in whom the rate of renal decline is usually slow and relatively constant. The biggest challenge is predicting the rate of decline in renal function, but by using the approach that children with CKD 4 or worse either at the time of listing or who are likely to deteriorate to this stage within 6 months of listing require transplantation, we have managed 18 pre-emptive transplants out of 41 total transplants. Interestingly, of the 28 children who had fibropolycystic kidney and liver disease, 15 were transplanted pre-emptively, whereas only 2 of 9 children with primary hyperoxaluria had a pre-emptive transplant (Birmingham Children's Hospital NHS Foundation Trust, unpublished data). As in children already on dialysis, the decision to recommend a liver transplant for a child who has CKD but who does not have liver failure is challenging for the multiprofessional team and is often difficult for children and parents to understand, but the arguments are as outlined earlier.

Patient preparation

Education and consent

A combined transplant carries significantly more risk of morbidity and mortality than for isolated organ transplantation. It is consequently essential that parents, and, if possible, the child to be transplanted, fully understand all aspects of the procedure, the risks incurred, and the benefits identified from proceeding.

It is our practice to provide a structured program of assessment and education over a 5-day period following initial referral. During this time the child and family meet with hepatology and nephrology medical, surgical, and specialist nursing staff, as well as members of the multiprofessional team including psychologist, social worker, play therapist, physiotherapist, and dietician. Children and parents are encouraged to visit the pediatric intensive care unit. At the end of the assessment period the decision to recommend CLKT is made by the clinical team and the family is informed of the recommendation.

For the majority of children, an immediate response is not necessary; it is important to allow parents and the child time for deliberation and to provide the opportunity to answer questions so that when consent to surgery is given it is genuinely informed consent. If there is uncertainty in deciding, the child and parents are provided with further opportunities to ask the questions to which they need answers.

Investigations

Routine investigations

Children require routine blood investigations when they are being assessed for CLKT. It is also essential to ensure they have completed all routine and additional vaccinations (Box 33.2) and have mounted a satisfactory immune response by measuring specific antibodies. Up to date ultrasound (or computed tomography (CT) or magnetic resonance imaging (MRI) as required) imaging of the liver and kidneys is required, especially in children with ARPKD, and ultrasound imaging of the neck and pelvic vessels.

Bladder assessment

It is routine practice to undertake a basic bladder assessment in all children being considered for CLKT. This comprises a voiding and intake record over 3 days, bladder flow rates, and a post-void bladder scan to look for evidence of incomplete bladder emptying. In children who have evidence of bladder dysfunction, or have been anuric for more than 12 months, video-urodynamic studies using a suprapubic catheter should be undertaken. Children who demonstrate

Box 33.2 Recommended vaccinations in the UK.

Primary vaccination schedule

- Diphtheria, tetanus, pertussis, inactivated polio
- *Haemophilus influenzae* B
- Meningitis C
- Measles, mumps, rubella

Others

- Varicella zoster
- *Pneumococcus*
- Hepatitis A and B

a small-capacity, high-pressure bladder may require bladder augmentation surgery before CLKT. The decision concerning the timing of this surgery relative to transplantation is beyond the scope of this chapter and varies by urologist.

Cardiovascular assessment

A CLKT gives rise to considerable cardiovascular stress, so a thorough cardiac assessment is required, including an electrocardiogram and echocardiogram. Children with PH1 may have significant cardiac dysfunction as a result of the systemic deposition of oxalate in the myocardium and blood vessels. A dopamine stress test may determine if there is an appropriate increase in cardiac output in these children, and if this is not the case, transplantation could be deferred until the systemic oxalate load has been reduced by dialysis. Children with PH1 who have an unsatisfactory response to a pressor agent such as dopamine are at risk of an inadequate cardiac output postoperatively, which if unresponsive to inotropes because of oxalate deposition in the vasculature and consequent impaired vasoconstriction, will reduce renal perfusion leading to graft dysfunction.

Neurological assessment

An electroencephalogram should be undertaken as part of the assessment to identify any underlying epileptogenic propensity in children with an appropriate clinical history. Any abnormality requires investigation with brain imaging and a neurological assessment and recommendation for specific therapy.

Plasma oxalate

Children with PH1 who have a high systemic load of oxalate may suffer renal graft loss because of the rapid deposition of oxalate if renal graft function is poor in the immediate post-transplant period (Figure 33.4). Assessment of plasma oxalate may be a guide to this.

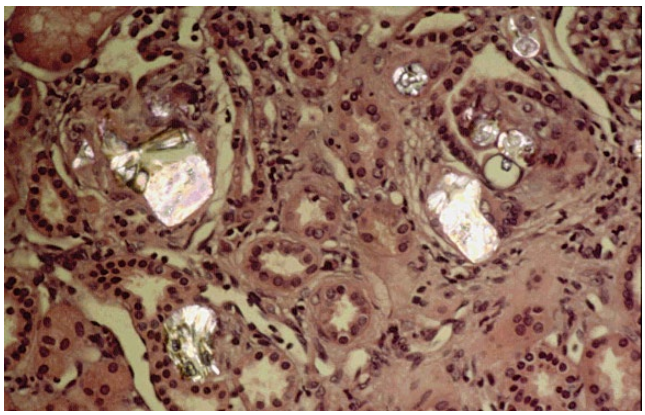


Figure 33.4 Biopsy showing oxalate deposition in the kidney.

Surgical and medical operative management

Surgical challenges for children undergoing combined liver and kidney transplant

There is no lower age or weight limit for children considered for combined organ transplantation, however the surgical challenges involve appropriate graft selection and dealing with the limited abdominal capacity for a dual graft. Most published data from specialist centers report successful CLKT in children >10 kg and these transplants have been carried out from around the age of 1 year. However, CLKT is feasible in children smaller than 10 kg if performed at specialist centers [10].

The lack of an appropriate size-matched pediatric donor population is one of the main limitations for CLKT so children are likely to be on the active transplant list for a considerable time once accepted for transplantation. Although split liver grafts and technical variant liver grafts (reduced liver grafts or "cut down" grafts) and live donation have improved donor availability in the isolated liver transplant setting, the limited capacity of the abdominal compartment is challenging when combined with a renal graft. This is less of an issue in transplantation for fibropolycystic liver and kidney disease as the organomegaly increases the size of the abdominal cavity. Another issue affecting cadaveric organ availability is the pediatric organ allocation system in which combined liver–intestinal graft recipients are prioritized over isolated liver or CLKT, disadvantaging children awaiting CLKT.

Simultaneous CLKT from a cadaveric donor is probably the best surgical option for children as well as providing immunological protection. It is usual to use a split or reduced liver graft, especially as the donor is likely to be an adult. Appropriate size-matched donors are rare so transplantation of a full liver graft is unusual. A donor to recipient weight ratio of up to five times can be accommodated even in small children. Although there are no set rules for a lower age limit for the donor, there is an increased likelihood of technical complications if the organs are obtained from very small donors. Isolated kidney transplantation using organs from donors with anencephaly have been successful, but the small size of the liver vessels precludes using these organs for liver transplantation.

Grafts from donation following brain death (DBD) are more successful because of the increased incidence of organ dysfunction and failure using organs from donation after cardiac death (DCD) in adult liver and renal transplant recipients. The risks taken with "marginal" DCD donors outweigh the benefits, consequently CLKT using DCD donors is not undertaken in either pediatric or adult transplant programs.

An alternative approach for CLKT in children who have waited for a long time on the waiting list is to proceed with live donation, although this will mean that the transplant will be sequential rather than simultaneous. So far no cases of simultaneous live donor CLKT have been reported.

This approach may be a suitable option for children for whom the primary indication for transplantation is liver disease, but could also benefit stable children on renal replacement therapy with advanced liver disease.

A live donor liver transplant is technically similar to its cadaveric counterpart of split or reduced liver grafts. A delay before renal transplantation allows the donor to recover from the first operation before proceeding with kidney donation, improving donor safety.

Surgical aspects of combined liver and kidney transplant

In CLKT, native hepatectomy and implantation of the liver allograft precedes the kidney graft. Technical details of the liver transplantation technique are beyond the scope this chapter and are described elsewhere (see Chapter 31). This allows patient stabilization before embarking on the kidney graft implantation, which is important as the reperfusion syndrome is more pronounced after liver reperfusion. Poor perfusion pressure reduces the rate at which kidney graft function returns and ensuring a good perfusion pressure prior to renal implantation is essential.

It is important to plan the approach to the abdominal cavity and the site of the renal graft placement. Decisions to undertake a native nephrectomy (often right side) should be pre-planned as this gives extra space in the peritoneal cavity. Thus the surgical incision needs to achieve these objectives, if possible, through a single abdominal incision. In very small children this is accomplished by making a bilateral subcostal incision, the apex of which passes midway between the xiphoid process and the umbilicus. This is the usual incision used for an isolated liver transplant but the lower abdomen can also be approached through this incision for kidney graft implantation. In larger children the "reversed L" or J-shaped incision with its horizontal limb extending across to the right flank from a point in the midline just above the umbilicus may serve this purpose.

The native hepatectomy and liver graft implantation is carried out first, although the biliary–enteric anastomosis is deferred until the kidney graft implantation is completed to prevent disruption of the biliary drainage because of manipulation of the bowel during right-sided nephrectomy and kidney graft implantation. It also allows the surgeon a chance to ensure the liver graft is functioning and that hemostasis is complete.

Two approaches are popular for implantation of the kidney graft. In bigger children with a body weight over 20 kg, a traditional extraperitoneal approach is possible even if the organ is from an adult donor. If this approach is agreed prior to the operation, there will be a separate incision for the liver transplant. However, the disadvantage is that the child will have two separate incisions, adding to the surgical complications, which impacts on recovery. Alternatively, an intraperitoneal approach for implantation may be used for children weighing 10–20 kg. It is also possible to place a kidney in the retroperitoneal position in small children by completely mobilizing the right colon (along with the root of the small bowel) towards the midline until the inferior vena cava (IVC) and aorta is exposed, allowing the graft to be placed in a retroperitoneal position. The final position of the renal graft is similar to that of the standard extraperitoneal approach, thus all the advantages of this approach are retained. At the end of the implantation, the right colon is repositioned in front of the kidney graft so that renal transplant biopsy can be safely performed from a posterolateral approach.

Alternatively, the renal graft may be placed within the peritoneal cavity itself, but technical complications from adhesions are more common with intraperitoneal implantation of the kidney graft. In addition, lack of a tamponade effect from surrounding tissues may lead to uncontrolled bleeding. Finally, the graft is more difficult to access for renal allograft biopsy and increases the risk of bleeding after the biopsy. The retroperitoneal approach with bowel mobilization reduces these complications.

If a right nephrectomy is performed at the time of CLKT, the renal bed occupied by the native kidney is an ideal location and provides space for placement of the kidney graft.

The choice for the vascular inflow and outflow anastomoses for the kidney is determined by the size of the graft and the native blood vessels. In smaller children, the lower IVC and aorta are less likely to lead to technical complications than the smaller caliber iliac vessels. The anastomosis of the renal vein and renal artery are standard end-to-side anastomoses. In very small children, complete occlusion of the aorta and/or vena cava with vascular clamps may be required to perform a satisfactory anastomosis. Generally each anastomosis is performed as a "two point" anastomosis in a continuous fashion. In the presence of a very small artery supplying the graft, a half or full circumference anastomosis may be performed with interrupted sutures. An anchor suture placed at the midpoint of the lateral wall of the aortotomy or venotomy maintains a wide opening in these vessels until the anastomoses are completed; this is a safe step practiced by all transplant surgeons. On rare occasions when a double kidney graft is used from a pediatric donor as an en bloc graft, the proximal end of the aorta and the IVC of the graft are oversewn with running sutures and the distal ends of the graft vena cava and aorta are used as the inflow and outflow, respectively. The reversal of the direction of flow could lead to vascular endothelial shear stress and related complications, which can be prevented by removing a distal cuff of aorta and vena cava, re-anstomosing them to the top end of the aorta and vena cava to make them long enough to perform the final vascular inflow and outflow reconstruction. This adds an additional vascular anastomosis to the operation and is a risk factor for graft vessel thrombosis.

The ureteric anastomosis to the bladder may require a separate suprapubic incision, which could be as small as a

few centimeters. Often a ureteric double-J stent is placed at the time of the vesicoureteric anastomosis. Some of the earliest reports of CLKT in children report instances of a ureteroureteric anastomosis, however this leads to an increased risk of ureteric stricture in the follow-up period and almost all transplant centers now perform a vesicoureteric anastomosis.

The success of any solid organ transplant relies on adequate perfusion of the allograft. As discussed earlier, lack of abdominal capacity is the main concern as placing a double graft from a size-mismatched donor in a limited abdominal space could lead to problems with abdominal closure. If the abdomen is too tight after transplantation it may impede blood supply to one or both grafts, leading to ischemia of the graft and high resistance to blood flow culminating in vascular thrombosis. Graft edema after the immediate post-transplant period may occur so the abdominal wall closure is an important surgical step in children undergoing CLKT. Several options are available to the surgeon and these include delayed abdominal closure as well as the use of prosthetic-aided closure. The advantage of delayed and staged abdominal closure is the opportunity to revisit the surgical fields if necessary. It is preferred to perform delayed closure in such situations, combined with a final closure once graft edema has subsided. If the final fascial closure is impossible a modern biological prosthesis is used.

Intraoperative surgical issues

One of the main intraoperative management difficulties is the severe reperfusion syndrome after the liver graft is reperfused, particularly after a cadaveric transplant. It is important to maintain a satisfactory blood pressure using vasopressors. If this cannot be achieved, then proceeding with a renal graft implantation may aggravate the situation and put both grafts at risk. The ideal management in this situation is to defer the kidney transplant until the patient is stabilized. As the kidney grafts can be cold preserved for a longer duration, one option would be to keep the kidney in cold storage; an alternative strategy is to place the graft on a perfusion pump. If these options are not viable the kidney may be offered to another patient. Failure to proceed to kidney transplantation in a planned CLKT is a medically and surgically challenging situation, especially in the postoperative period. The child may require renal support, which should be by hemofiltration rather than hemodialysis due to the significant potential to impact on the hemodynamic stability of the patient with the latter technique.

Medical aspects

Dialysis

Children who are on peritoneal dialysis should not require dialysis immediately preoperatively. Preoperative hemodialysis is required if the last hemodialysis was more than 36 h before the anticipated time of transplantation, if the plasma potassium is greater than 5 mmol/L, or the plasma bicarbonate is less than 20 mmol/L. Children with PH1 should have hemodialysis immediately before CLKT to reduce the oxalate load at the time of reperfusion.

Immunosuppression

The standard regimen in this unit is basiliximab (first dose after implantation, second dose on day 4), tacrolimus, and methylprednisolone (see Chapter 31).

Blood pressure

During implantation of the renal graft the blood pressure is maintained at or above the 75th centile for age by the use of intravenous fluids to maintain the central venous pressure above 5 cmH_2O and, if necessary, by adding inotropes. These strategies are important to ensure prompt perfusion of the renal graft and to provide the optimal hemodynamic circumstances for the graft to function.

Intraoperative renal replacement therapy

There are no published reports of undertaking intraoperative dialysis in children undergoing CLKT, but experience of intraoperative dialysis in adult liver transplant recipients have a favorable outcome if undertaken electively [11]. The perceived advantage of intraoperative dialysis is optimization of fluid balance in anuric children, improved potassium and acid–base control, and, for children undergoing CLKT for PH1 reduction of plasma oxalate, reducing the risk of calcium oxalate deposition in the transplanted kidney.

Intraoperative dialysis for children undergoing transplantation for organic acidemias such as methylmalonic acidemia may be beneficial by eliminating toxic metabolites and facilitating acid–base stability. Although the benefits are clear, there may be logistic problems in providing trained personnel to ensure safe function of the dialysis machine and in maintaining a correct fluid balance, so intraoperative dialysis is not standard intraoperative care.

Postoperative management

Surgical aspects

The surgical management of children undergoing CLKT should be performed with both liver and renal teams. The main surgical issues during the very early post-transplant period are bleeding or graft vessel thrombosis. The risk of postoperative bleeding is substantial if a split or reduced liver graft has been used and a native nephrectomy has been carried out. The renal hilum is a potential site of postoperative hemorrhage from improper preparation of the graft on the bench. Native nephrectomy creates a vast raw area in the retroperitoneum, which may not become readily apparent until significant blood loss has occurred. Unexplained hemodynamic instability should prompt active investigation and early exploration for bleeding.

There is a risk of early thrombosis because of the small size of the blood vessels attached to the graft, and early hepatic artery or renal artery thrombosis causes graft loss. All children undergoing CLKT should have anticoagulation with titration of heparin according to the activated partial thromboplastin time (APTT) or PTT ratio. Routine Doppler scans of both the liver and kidney allografts on the first 5 days should detect thrombosis and any concern about the patency of graft blood vessels requires a formal assessment with a contrast-enhanced CT scan.

Medical aspects

Postoperative management for CLKT is different from the management of an isolated liver transplant. There is greater emphasis on the need to maintain a positive fluid balance by adequately replacing insensible fluid losses (300–400 mL/m^2/day) and fully replacing drain and urinary losses, especially in the first 72 h after surgery. Maintenance of a satisfactory blood pressure (>75th centile for age) is important to encourage urine production and also to avoid renal vein thrombosis, and can be achieved by adequate fluid replacement. There is significant redistribution of water across body compartments after major surgery leading to intravascular contraction and hypotension – this must be aggressively managed with boluses of 4.5% human albumin solution (or another suitable colloid) to maintain a central pressure above 5 cmH_2O.

Dialysis is sometimes required for delayed renal graft function, which is more likely if the cold ischemia time is prolonged. If required, dialysis must be undertaken with care to avoid excessive fluid loss resulting in hypovolemia and hypotension; anticoagulation must be kept to a minimum to avoid precipitating hemorrhage. Dialysis is recommended in the immediate post-transplant period in children with PH1 to avoid the deposition of oxalate in the transplanted kidney as this can lead to rapid renal transplant dysfunction. It is generally accepted that regular dialysis should be undertaken even if there is urine production, and should be continued until the estimated GFR is greater than 40 mL/min/1.73 m^2.

Immunosuppression is maintained by introducing tacrolimus the day after surgery, and giving a second dose of basiliximab on postoperative day 4. A "minimal steroid" regimen is used in which prednisolone is weaned down to zero by day 5. Mycophenolate mofetil therapy commences on day 15 provided the platelet and white cell count is satisfactory.

It is important to ensure appropriate play and psychology support for the child as they recover from CLKT. The parents also require support from the clinical staff caring for their child. However, social worker contact may be helpful, especially if the transplant center is far from the family home leaving the parents isolated from their local network of friends and family.

Follow-up

Frequency of review

The focus on follow-up is the renal graft and is more frequent than for isolated liver transplant, with regular visits every 3–4 days for the first month and a gradual reduction in frequency once stable. Ideally, both renal and liver transplant teams should assess the patient in the same clinc. In contrast to follow-up for liver transplantation, routine renal transplant ultrasound scans are not required provided the graft function is satisfactory.

What is monitored

Renal transplant function is monitored by plasma creatinine measurement. Electrolyte abnormalities are frequently encountered as a result of the tubulopathy caused by tacrolimus, necessitating monitoring of the acid–base balance, potassium, phosphate, and magnesium. Electrolyte supplements are frequently needed but the dose required usually falls with time and in response to gradually lower permissible levels of tacrolimus. Dip testing of the urine is important to exclude urine infection and to screen for the development of new-onset diabetes mellitus after transplantation. The development of proteinuria is a worrying feature and may indicate chronic rejection.

A detailed account of monitoring of the liver graft and long-term management is discussed in Chapter 31.

Long-term complications

The long-term renal outcome for children who have had CLKT remains uncertain because of limited long-term outcome data. In a review of experience in one center, and a review of outcome in multiple centers contributing to the UNOS database, renal transplant outcome to 5 years was better in children who had a CLKT in comparison with those who had an isolated renal transplant [12], suggesting the liver confers immunological protection to the kidney (see Figure 33.1). However, despite this immunological protection there are several non-immunological factors that may lead to a decline in function, including calcineurin inhibitor (CNI) toxicity, the primary disease, recurrent urinary tract infections, BK nephropathy, and non-concordance.

Significant CNI toxicity is seen much less commonly than previously because of the change from ciclosporin to tacrolimus in the primary immunosuppression regimen. In addition, most centers use lower tacrolimus trough levels than previously because of adding mycophenolate mofetil. Unlike in isolated liver transplantation, there is no evidence that the CNI can be stopped in CLKT because renal transplants require more immunosuppression than isolated liver transplants, although a switch to sirolimus may be successful.

Metabolic diseases such as primary hyperoxaluria and the organic acidemias may lead to renal graft damage.

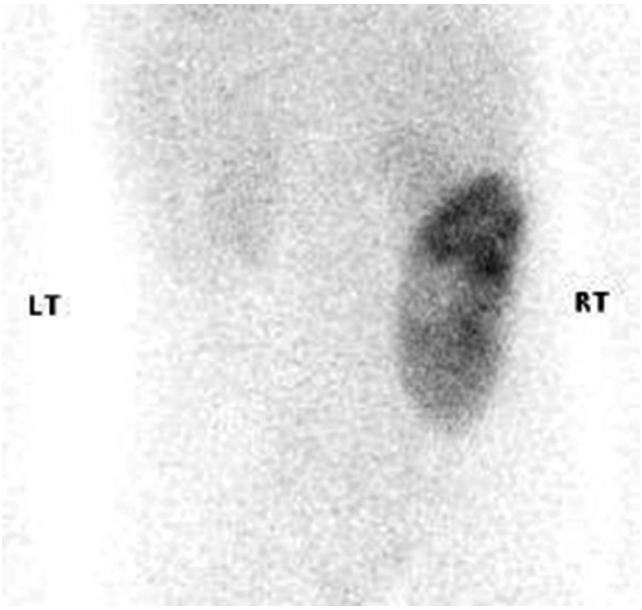


Figure 33.5 DMSA scan of a transplanted kidney showing patchy isotopic uptake indicative of scarring.

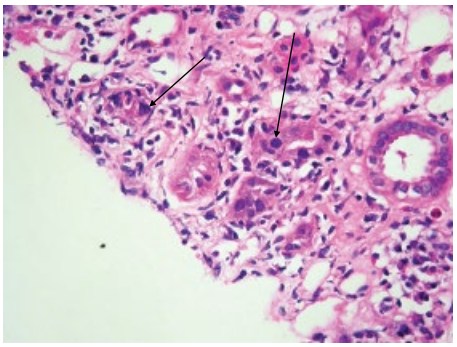


Figure 33.6 Renal transplant biopsy: the arrows show polyomavirus inclusions

In primary hyperoxaluria, the substantial oxalate load in bones and other tissues slowly dissolves and can become trapped in renal tissues (see Figure 33.4) leading to an inflammatory response with progressive fibrosis and poor graft outcome [2]. Excessive oxalate deposition can be reduced by postoperative dialysis until the eGFR is above the level at which isolated renal transplantation is undertaken (40 mL/min/1.73 m^2).

The organic acidemias are partially resolved by liver transplantation but a well-functioning kidney transplant clears toxic metabolites and facilitates metabolic stability. Dialysis may be required in the immediate postoperative period when the GFR is low.

Recurrent urinary tract infections are common after renal transplantation, in part because of immunosuppression. Inattention to hygiene when catheterizing predisposes to urinary tract infection. Recurrent urinary infections cause renal transplant scarring (Figure 33.5) and reduce renal transplant function. Prophylactic antibiotics with or without a daily live yoghurt preparation may be beneficial. Attention to taking adequate fluids throughout the day and regular voiding is also important.

Children who have been anuric from an early age (e.g., with PH1, after nephrectomy for ARPKD) may have small-capacity, high-pressure bladders and require bladder augmentation as well as a drainage channel to allow catheterization of the bladder (Mitrofanoff procedure). The commonest finding is incomplete bladder emptying with a significant residual urine volume, providing a suitable environment for urinary pathogens to proliferate and progress to symptomatic infection. Treatment of constipation, attention to bladder emptying by undertaking "double voiding" (attempt a second void 1 min after completing the initial void), and pelvic floor relaxation strategies together with prophylactic antibiotics and live yogurt preparations may prove beneficial, although young children may find it difficult to co-operate.

Polyoma (BK) virus infection is becoming increasingly common and causes a nephropathy that necessitates a reduction in immunosuppression. Failure to diagnose the infection can lead to increased immunosuppression on the assumption that the renal transplant dysfunction is caused by rejection, resulting in viral proliferation and progressive graft damage. The diagnosis is made by identifying viral inclusions in a cellular urinary sediment, on renal biopsy (Figure 33.6), or by polymerase chain reaction (PCR) in the blood or urine. The correct management requires discontinuation of mycophenolate mofetil and cautious reduction of other immunosuppressive therapy if there is a poor response to initial management. A variety of antiviral therapies have been advocated but there are no therapeutic trials to provide an evidence base upon which to base recommendations.

Malignancy is a complication of long-term immunosuppression with a risk that correlates with the duration and degree of immunosuppression rather than with transplantation of specific organs. There are no long-term data for the incidence of malignancy in CLKT, but it is likely to be similar to malignancy rates in recipients of isolated liver or kidney transplants. Post-transplant lymphoproliferative disease is the commonest malignancy in the early post-transplant period and the risk is greatest when an organ from an Epstein–Barr virus (EBV) positive donor is transplanted into an EBV-negative recipient who subsequently develops EBV infection.

Skin malignancies are the most common malignancies in medium- to long-term follow-up of adult transplant recipients. In children both benign and atypical nevus counts are higher in children with frequent episodes of sunburn [13]. As both nevi and sunburn are risk factors for melanoma,

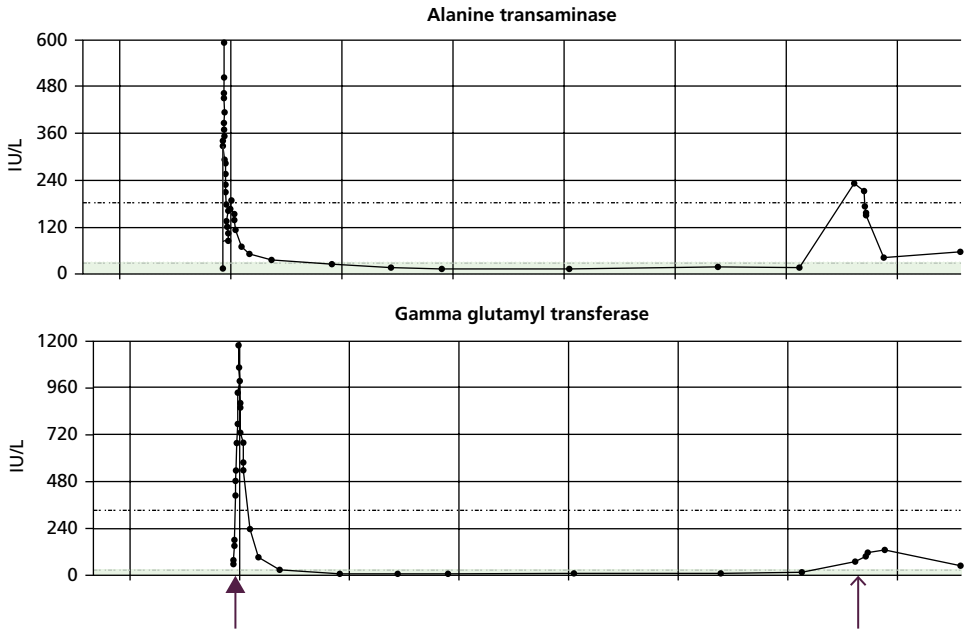


Figure 33.7 Non-compliance after combined liver and kidney transplantation: the thick arrow shows liver function tests at the time of transplant, the thin arrow is at 3 years from transplantation when there was non-compliance.

fair-skinned transplant recipients with nevi should have intensive sun avoidance strategies.

As with all transplanted organs, adolescence and young adulthood are high risk times for non-adherence with medication. There are no specific data for CLKT, but there is no reason to consider this group of patients is any different from other transplant recipients (see Chapters 35 and 36). It is necessary to have a high level of suspicion if there are unexplained variations in CNI levels, plasma creatinine, or liver function tests (Figure 33.7). Adolescents often demonstrate other patterns of risk-taking behaviour such as smoking, use of non-prescribed drugs, sexual activity, and clinic non-attendance, so the problem of non-adherence should be addressed in the context of these other behaviors. Referral to a youth worker, counselor, or psychologist at an early stage may be helpful in either reversing or reducing this pattern of behavior. In some instances this behavior might be a consequence of disturbed family dynamics, necessitating family therapy.

Transfer to adult services

There is no uniformity in the age of transfer of adolescents to adult services. For many families the move from a pediatric environment to an adult unit is traumatic and unsettling and it is important to ensure an adequate transition plan (see Chapters 35 and 36) to prevent clinic non-attendance and non-concordance with medication, ultimately leading to graft loss. There are a limited number of centers undertaking CLKT, consequently it may be necessary to transfer renal transplant care to an adult renal unit with liver transplant follow-up undertaken at the adult liver transplant unit. While this arrangement is not ideal it may be necessary for geographical reasons; there is a UK initiative to develop rare disease centers that may be appropriate for CLKT follow-up by providing the opportunity for occasional review by adult and pediatric clinicians familiar with CLKT. This development will provide much needed follow-up data to inform future discussions concerning transplant outcome and identify specific needs of adolescents and young people with CLKT.

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SECTION 13

Liver Disease Around the World

CHAPTER 34

Liver Disease in the Developing World

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Key points

- Diseases involving the liver are a common problem in the developing countries.
- Infections including viral hepatitis, tuberculosis, dengue fever, typhoid, and malaria are a common cause of liver disease.
- With the changing pattern of the incidence of infectious disease, the impact of genetic and metabolic disease is increasingly recognized in these countries.
- Neonatal cholestasis constitutes about a third of hepatobiliary cases seen at centers in the developing world.
- One-third of cases of cholestasis are idiopathic owing to lack of specialized diagnostic facilities, although this is changing.
- Lack of awareness of neonatal cholestasis and its consequences leads to a delayed referral to specialized centers in many countries.
- Liver transplantation is well established in many centers in developing countries, mostly living-related liver transplantation.

Liver disease in children is a common problem in the developing world. The most important etiology is infection, which includes viral hepatitis, dengue fever, typhoid, malaria, and tuberculosis. As a result of improved environmental sanitation and socioeconomic conditions, there has been a change in the pattern of infectious disease in the developing world. In addition, international programs such as the universal hepatitis B vaccination program in hyperendemic areas have changed the natural history of hepatitis B and have implications for the control of infectious liver diseases in other parts of the world.

As modern medicine and sanitation control reduce the incidence of infectious diseases, it has become apparent that both genetic and metabolic liver diseases are as important a cause of liver disease in children in the developing world as in developed countries. Unfortunately, lack of resources, training, and specialized laboratory support makes the diagnosis and management of these rare diseases a problem in many areas.

The success of liver transplantation internationally has led to its development in many countries. As the organization of an effective cadaveric donor program is difficult for many reasons, the most effective form of transplantation for treatment of children with end-stage liver disease is a living-related donation program.

Much needs to be done to improve child health in many developing countries. It is important to continue to control the remaining infectious causes of liver diseases and to develop better ways to diagnose and treat genetic and metabolic liver diseases.

Neonatal liver disease

The spectrum of neonatal liver disease and cholestasis in developing countries differs significantly from that in developed nations with regard to both etiology and management (Table 34.1). As in the developed world, biliary atresia and idiopathic neonatal hepatitis are the most common causes of neonatal cholestasis.

Diseases of the Liver and Biliary System in Children, Fourth Edition. Edited by Deirdre A. Kelly.

Table 34.1 Etiological factors of neonatal cholestasis in India.

Etiologic factors	Consensus statement of Indian Academy of Pediatrics, 2000* (n = 1008)	Data from All India Institute of Medical Sciences, New Delhi† (n = 420)
Obstructive causes	38%	34.4%
Biliary atresia	34%	29.8%
Choledochal cysts	4%	4.6%
Hepatocellular causes	53%	63.8%
Infections	17%	18%
Metabolic causes	4.2%	12%
Miscellaneous	2.2%	2.9%
Unknown etiology	29.6%	30.7%
Ductal paucity	3%	1%
Undifferentiated	6%	1.2%

* Based on cumulative data from eight tertiary care centers [2].
† Data from the largest single series [3].

Etiology

Neonatal cholestasis constitutes up to one-third of hepatobiliary disorders in children reporting to tertiary care hospitals in India [1]. The relative incidence of conditions leading to neonatal cholestasis, as reported from a consensus statement reported from India, is shown in Table 34.1. While 20–66% of cases of neonatal cholestasis were idiopathic in earlier studies, recent reports documented this proportion to now be lower owing to better diagnostic facilities. In a series of 50 consecutive cases of obstructive jaundice seen at a tertiary center in Singapore, 66% were due to idiopathic hepatitis, 28% due to biliary atresia, 4% to choledochal cyst, and 2% to galactosemia. In Taiwan, about 49% of the neonatal hepatitis was due to cytomegalovirus (CMV) infection. In contrast to Europe and the USA, α1-antitrypsin deficiency is rare in certain countries like India, Singapore, or Taiwan.

Diagnosis

The average age at which the child presents to a tertiary care center is delayed owing to a variety of reasons. Infants with biliary atresia appear well and have normal growth and development in spite of their jaundice, and this leads to parents and physicians underestimating the seriousness of the problem. As a result, infants are referred too late to benefit from palliative surgery for biliary atresia. There is also a misconception amongst many health-care professionals that all well babies with icterus have physiological jaundice (which is unconjugated and associated with normal urine color). The diagnosis is described in detail in Chapter 8, but many of the specialized investigations are now available in developing countries and accurate diagnosis of rare conditions is now possible.

Treatment and outcome

The details of treatment of specific disorders are given in Chapter 8. Some of the unique problems faced in developing countries are discussed here.

Delayed referral

The final outcome in a significant proportion of cases is dependent on the age at diagnosis and the availability of appropriate management. The delay in diagnosis and referral is reflected in the reported prevalence of cirrhosis: 75–100% of patients undergoing laparotomy for neonatal cholestasis. Of 44 children who underwent the Kasai procedure in India, only 20% became jaundice free, which was attributable to their advanced age at presentation (>90 days) in 60% of cases. This is in contrast with western counties (60% clearance of jaundice) and with Singapore, where 37% of patients with biliary atresia have a successful portoenterostomy. However, in children presenting early in India, a 15-year survival rate of 87% has been reported in those with successful biliary drainage, which is comparable with European or Japanese results. Early identification of biliary atresia is of great importance and essential if a hepatic portal enterostomy is to be performed early enough to establish bile flow. Although there are centers of excellence in the developing world with pediatric surgeons able to perform a hepatic portal enterostomy, there are also many countries where this cannot be performed. Infants with biliary atresia and without a hepatic portal enterostomy will not survive beyond 2 years of age.

Limited availability of specialized formulae and medication

Specialized formulae for the treatment of malnutrition or specific disorders is often not available (e.g., for tyrosinemia or maple syrup urine disease). In addition, the cost of treatment might be too high for the families (e.g., nitisinone for tyrosinemia). In tyrosinemia, it is often easier for the family to raise funds for a one-time liver transplant then to incur the cost of monthly nitisinone therapy.

Limited awareness

A greater awareness regarding the clinical presentation, diagnosis, and need for referral of patients, a uniform approach to investigation and treatment, and strengthening/improvement of laboratory and surgical facilities at referral centers should improve the outcome for these infants in developing countries in the future.

There are recent positive improvement in India, as the mean age of patients with biliary atresia at presentation has decreased from 132 days to 97 days and delays in referral has fallen from 121 days to 78 days after measures to raise awareness. Recently, a consensus statement has been published from India that highlights the management approach from a developing country perspective [1].

Chronic liver disease

Most chronic liver disease in the developing world is due to chronic viral hepatitis or autoimmune disease. The differential diagnosis depends on the age of the child (see

Table 34.2 Common causes of chronic liver failure in children as reported from studies done in developing countries.

Cholestatic liver disease	Metabolic liver disease	Chronic hepatitis
Biliary atresia	Alpha-1-antitrypsin deficiency	Autoimune hepatitis ± sclerosing cholangitis
Choledochal cyst	Galactosemia	Non-alcoholic fatty liver disease
Idiopathic neonatal hepatitis	Tyrosinemia type 1	Hepatitis B and C
Alagille syndrome	Wilson disease	Fibrocystic liver disease
Familial intrahepatic cholestasis	Cystic fibrosis	
	Glycogen storage disease type IV	

Chapters 11, 13, and 21). Cirrhosis and portal hypertension is the end point for all forms of progressive chronic liver disease although the rate of progression to cirrhosis is variable. The commonest causes of chronic liver failure in the developing world are biliary atresia and metabolic liver diseases in infants, and post viral hepatitis in older children (Table 34.2). The accuracy of the diagnosis depends on local medical expertise and the level of laboratory support. A detailed history and thorough medical examination is important to determine appropriate investigations.

There are wide socioeconomic disparities in the developing world, with differences in the level of health-care provided by the state and medically insured patients as well as the level of care available in rural and urban areas.

Treatment of these conditions is similar to the developed world, but is limited by the lack of availability and the high cost of medications for standard therapy. This highlights the importance of recognizing and diagnosing treatable diseases, such as autoimmune hepatitis, early on to prevent progression of the disease.

Children with chronic liver disease have a significant degree of malnutrition and nutritional support is important. Nutritional assessments are difficult to interpret because the common anthropometric measurements are affected by organomegaly and ascites. Mid upper arm circumference is an anthropometric measurement widely used by health-care workers in developing areas with a high prevalence of malnutrition and is a good indicator of nutritional status. Growth failure and malnutrition occurs because of malabsorption of fat and fat-soluble vitamins, ongoing catabolism, and increased metabolic demand. An increased caloric intake of 125% of the recommended daily allowance, as well as an adequate protein diet of 2–3 g/kg/day, should be provided.

Infection with hepatotropic viruses can cause acute deterioration in children with chronic liver disease. Children in the developing world have a high exposure rate to hepatitis A, B, and E viruses. It is recommended that children with chronic liver disease be vaccinated against hepatitis A and B if not already immune to them [4].

Successful liver transplant programs have been established in the developing world and transplantation should be considered for children with chronic liver diseases.

Autoimmune hepatitis

Autoimmune hepatitis (AIH) is a progressive inflammatory disease of the liver characterized by high aminotransferase levels, elevated γ-globulins, the presence of non-organ-specific autoantibodies, and histologically by interface hepatitis on liver biopsy in the absence of a known etiology, such as chronic viral infection (see Chapter 11).

The clinical presentation of AIH is variable and been broadly grouped into three clinical patterns:

- Acute hepatitis (40% of patients).
- Insidious onset with relapsing jaundice, headache, and malaise (40%).
- Complications of portal hypertension (10%).

In an audit (unpublished) in Cape Town, South Africa, 20 children were diagnosed with AIH over a 5-year period. The age at diagnosis (2.6–12.0 years) and female preponderance was similar to that found in the developed world. Acute hepatitis was the most common presentation (65%), followed by insidious onset (20%), and complications of portal hypertension (10%). The acute presentation was higher in this setting than in the developed world, probably as the acute presentation is more noticeable. The majority (60%) of children were type 1 AIH (antinuclear antibody/smooth muscle antibody positive), 40% were seronegative, and none were type 2 (liver, kidney muscle antibody 1 (LKM-1) positive).

Diagnosis

This variable presentation of a disease that may fluctuate with spontaneous remissions and relapses, results in a delay of diagnosis and appropriate referral. Thus the diagnosis needs to be considered and excluded in all children presenting with unexplained signs and symptoms of acute or chronic hepatitis and/or cirrhosis, particularly as the disease responds well to treatment with prednisone and azathioprine.

The diagnosis is as in western countries with polyclonal hypergammaglobulinemia with selective elevation of immunoglobulin G (IgG) in most cases. Hypergammaglobulinemia is also found in children with chronic infections, especially human immunodeficiency virus (HIV) and tuberculosis, so these must be actively excluded, particularly in areas with a high prevalence of these infections.

Immunological diagnosis and disease classification is difficult in patients with liver disease similar to AIH but who do not express the typical autoantibodies. The diagnosis of autoantibody-negative AIH may be due to a lack of standardization of the assays or to the unavailability of these tests in areas of the developing world. Autoantibody-negative AIH may respond to immunosuppressive treatment, so a -month trial

with prednisone should be considered in all patients suspected of having AIH regardless of the serological findings.

Liver biopsy is recommended for all patients suspected of having AIH unless there is a contraindication to the procedure. The typical histological findings of piecemeal necrosis are characteristic but not specific for AIH (see Chapters 2 and 11).

Treatment

Treatment should be initiated as soon as the diagnosis is made to avoid severe liver damage. Standard therapy is a combination of prednisone and azathioprine (see Chapter 11 for treatment details). The aim is to obtain clinical and biochemical remission of the liver disease and avoid the need for liver transplantation. The majority of patients respond within 4–8 weeks but complete normalization may take many months. Relapses can occur on treatment and are managed by temporarily increasing the steroid dose. Nonadherence is the most common cause of relapses.

Compliance is of utmost importance as treatment in the developing world is usually life-long and liver transplantation is not an option for the majority of children.

Autoimmune sclerosing cholangitis

The diagnosis of autoimmune sclerosing cholangitis (ASC) is made on a combination of histology, endoscopic retrograde cholangiography (ERCP), and magnetic resonance cholangiopancreatography (MRCP), but neither of these latter techniques are widely available in the developing world so it is likely that this disease is underestimated.

ASC responds to the same immunosuppressive treatment as AIH. Ursodeoxycholic acid, which is often not available, is usually added to the steroid and azathioprine treatment but its long-term benefit has not been established.

Portal hypertension

Portal hypertension (PHT) in childhood can give rise to severe and life-threatening complications. The effective prevention and management of these complications are necessary to improve outcomes for patients (see Chapters 21 and 27). Whilst treatment is no different to that in the developed world, the high cost of some drugs and the lack of expertise in pediatric endoscopy in some areas are serious management constraints.

Extrahepatic portal vein obstruction

Extrahepatic portal vein obstruction (EHPVO) is a commoner cause of PHT in children from lower socioeconomic classes in the developing countries when compared with the western world. It is the most common cause of PHT in Indian children [5]. Patients with EHPVO have normal liver function and liver parenchyma, unlike patients with PHT secondary to liver disease.

In many cases there is no obvious etiology for the portal vein obstruction although a history of umbilical vein catheterization, abdominal sepsis, trauma, or pancreatitis is suggestive. There is a strong association with levels of hygiene and poverty as the disease affects the socially disadvantaged. It may also occur following liver transplantation. The most common presentation is upper gastrointestinal bleeding. Mortality of between 5% and 30% has been reported for a single bleeding episode [6]. Transient ascites is seen in about 20% of children following a bleeding episode.

Growth retardation has been a great concern and occurs in up to 50% of children with EHPVO [7]; growth may improve after shunt surgery. Mild cognitive dysfunction is also seen in these children. Ectopic varices at sites like the duodenum, gallbladder, or rectum are more common than in patients with cirrhotic PHT.

The diagnosis of EHPVO is based on the clinical signs and symptoms of PHT-related complications such as a variceal bleed, splenomegaly, anemia, and thrombocytopenia in the absence of any chronic liver disease. An ultrasound is the investigation of choice, showing portal vein cavernous transformation, usually associated with splenomegaly and collateral blood vessels.

Treatment

There is controversy in the optimal management of these patients. The initial outcome depends on the control of the variceal bleeding and whether appropriate resuscitation facilities are available. The treatment options are medical therapy with β-blockers, endoscopy, and surgery (see Chapters 21 and 27). Esophageal variceal band ligation is easier to perform, and is safer and more effective, in eradicating varices than injection sclerotherapy. However, it is technically difficult to perform in very young children, in whom injection sclerotherapy has a role. Children with EHPVO with poorly controlled variceal bleeds or without easy access to specialized medical centers are good candidates for bypass surgery. The meso-Rex shunt is a curative procedure as it bypasses the extrahepatic obstruction and restores physiological blood flow to the liver, but mesocaval or splenorenal shunts may be technically easier to perform (see Chapter 27). Long-term outcome depends on shunt patency. Growth usually returns to normal.

Budd–Chiari syndrome

Obstruction to outflow at any level from the small hepatic veins to the junction of the inferior vena cava (IVC) with the right atrium constitutes Budd–Chiari syndrome (BCS). BCS most often presents as chronic obstruction with hepatomegaly, ascites, abdominal distension, and abdominal pain. Abdominal and chest wall collaterals are often prominent and liver enzymes and bilirubin levels may be minimally to moderately elevated. BCS in the developing world differs from the west in a number of ways. The disease usually involves both the IVC and hepatic veins, whereas the hepatic veins are predominantly involved in cases from the west. Presentation is mostly

chronic and usually there are no underlying predisposing factors in cases from the developing world. Management is similar to that reported from the west and involves interventional therapeutic radiological procedures like angioplasty, stenting, and transjugular intrahepatic portosystemic shunt (TIPSS) [8]. Liver transplantation may be required.

Veno-occlusive disease (sinusoidal obstruction syndrome)

Hepatic veno-occlusive disease (VOD) is a clinical syndrome characterized by hepatomegaly, ascites, weight gain, and jaundice due to sinusoidal congestion that can be caused by alkaloid ingestion.

The epidemiology, pathophysiology, treatment, and prognosis of VOD are distinct from other forms of hepatic venous outflow obstruction. It is for this reason, therefore, that that this entity is often described separately. In the developing world, VOD is most commonly caused by plant alkaloid poisoning (herbal teas, tribal remedies), whereas in the developed world it is most commonly seen after stem cell or bone marrow transplantation.

Herbal medicines are widely used both in the developed and developing world. In many parts of the developing world, herbal remedies are integral parts of traditional healing with a large proportion of the population in Africa using such remedies. The hepatotoxicity of herbal remedies is well documented, especially for pyrrolizidine alkaloids. *Senecio* is a common plant species in Africa containing these alkaloids and they are commonly used in foods and remedies.

The diagnosis of VOD is made from the distinctive clinical features and retrograde flow in the portal vein on ultrasound, and is confirmed on liver histology which shows narrowing or occlusion of the terminal hepatic venules, sinusoidal congestion, and necrosis of hepatocytes, with a mild inflammatory infiltrate in the centrilobular zone. Treatment in western counties is with thrombolysis with defibrotide, combined with antithrombin III, but these agents are not usually available elsewhere and therapy focuses on supportive management and treatment of ascites.

Metabolic liver disease

The liver is a highly metabolic active organ where many pathways of intermediary metabolism are located. Dysfunction in any step of these pathways may lead to deleterious hepatic or extrahepatic manifestations known as metabolic liver disease (MLD). These diseases are genetically determined and inherited mainly as autosomal recessive, and associated with significant morbidity and mortality. They are relatively rare but when all grouped together they affect an important portion of the world population, estimated by the World Health Organization (WHO) as 10 out of 1000 births.

MLD represents a heterogeneous group of disorders with different manifestations (Table 34.3). Some conditions are characterized by hepatic parenchymal injury with subsequent fulminant liver failure, cholestasis, cirrhosis, and tumors. Others are liver-based genetic disorders associated with normal liver structure and function where extrahepatic manifestations are the main cause of morbidity and mortality. In a third group, liver disease is part of a systemic involvement with both hepatic and extrahepatic expression. Therefore, the clinical presentation of MLD is variable, based on the specific defect involved (see Chapters 9 and 19).

Table 34.3 Metabolic liver disease (MLD) categories.

MLD with primary hepatic expression and parenchymal damage	MLD with both hepatic and extrahepatic expression	MLD with primary hepatic expression and without parenchymal damage
Galactosemia	Wilson disease	Crigler–Najjar syndrome
Tyrosinemia type 1	Cystic fibrosis	Urea cycle disorders (except ASL)
Fructosemia	Progressive familial intrahepatic cholestasis type 1	Primary hyperoxaluria type 1
Hereditary hemochromatosis	Organic aciduria	Maple syrup urine disease
Alpha-1-antitrypsin deficiency	Glycogen storage type 1b, 4, 9	Homozygous familial hypercholesterolemia
Progressive familial intrahepatic cholestasis (types 2, 3)	Erythropoietic protoporphyria	Familial amyloid polyneuropathy
Bile acid synthesis defects	Citrin deficiency	
Glycogen storage disease types 1, 3, 6	Lysosomal storage diseases	
North American Indian cholestasis	Perixosomal disorders	
Indian childhood cirrhosis	Mitochondrial cytopathy	
Cholesterol ester storage disease		
Gaucher disease type 1		
Fatty acid oxidation defects		
Urea cycle (ASL)		

ASL, argininosuccinate lyase deficiency.

Frequency and prevalence

Metabolic liver disease was reported in many countries from the developing world; however, the exact epidemiological figures are not well established. Recently, there has been a noticeable focus on the diagnosis of genetic disorders within the developing world. Controlling environmental problems by achieving better immunization programs, improving primary health care, and lowering perinatal and infant mortality rates, has led to the emergence of genetic conditions in those countries. The prevalence of these disorders has been increasing due to the large population, consanguineous marriages, and increased birth rates. Consanguineous marriages are practiced in many developing populations depending on the religion, culture, and geography – increasing the frequency of genetic disorders [9].

In some developing countries, MLD makes up to 40% of infant liver problems. Most of the published MLD reports are from India, the Far East, Middle East, and North Africa. Reports from the rest of Africa are scarce. The frequency of MLD types is variable between different countries as well as between different regions within the same country. Of 948 children with chronic liver disease in India, MLD accounted for 194 (19.7%) patients. Wilson disease was the most common metabolic liver disease in this study, although the exact frequency was not estimated. Other frequently diagnosed MLDs were α1-antitrypsin deficiency, galactosemia, hereditary fructose intolerance, glycogen storage diseases, tyrosinemia type l, Niemann–Pick disease, and Gaucher disease. Most metabolic defects were diagnosed on the basis of clinical features and liver histology, as specific enzyme assays were rarely available. Isolated metabolic diseases such as tyrosinemia type 1 have been reported from Singapore.

Presentation

The presentation in patients in the developing world is similar to that in developed nations. Most genetic diseases present in the first 5 years of life. MLD is a major etiology of neonatal cholestasis and acute liver failure (ALF) in both neonates and older children. It accounts for 33% of ALF in children younger than 3 years in India. Also, it was reported as the most common cause of liver cirrhosis in children in countries such as Iran, Oman, and Saudi Arabia.

Genotype–phenotype correlation

The majority of MLD is inherited with known gene defects; however, different mutations are prevalent in certain groups and places. In some developing countries where molecular studies are available, several novel mutations have been reported peculiar to an ethnic group and different to those found in the developed world. Wilson disease is a good example of the heterogeneity of mutations in the *ATP7B* gene; cystic fibrosis has different *CFTR* gene mutations in different groups, with the p.F508 del being common in Caucasians. Nevertheless, the correlation between genotypes and phenotypes in many disorders was not found to be significantly different to that found in the west.

Examples of common metabolic liver diseases in the developing world

Wilson disease

Wilson disease is considered to be the most frequently reported MLD in many regions within the developing world, probably due to the availability of appropriate diagnostic tests. It is considered to be a common cause of chronic liver disease in variable percentages among different populations. In contrast to the reported prevalence of between 12 and 29 per 100,000 for Wilson disease in European populations, the prevalence in Asian countries varied between 33 and 68 per 100,000.

Multiple novel gene mutations, different to those reported from Europe, are found across the developing countries. Studies from India demonstrate that a total of 51 mutations of *ATP7B* have been documented including 34 novel mutations. Of the mutations documented in India, *C813A* is the commonest mutation. There is no single dominant mutation seen in the Indian population, unlike the findings from studies in other countries [10]. There are no specific clinical features that differ from those in the developed world.

In Egyptian children, Wilson disease was found to be associated with an early onset of liver disease and neurological manifestations, with early Kayser–Fleisher rings. These children also have a high rate of homozygous mutations reflecting the high rate of consanguinity. In addition, Wilson disease carries a high risk of mortality – up to 50% if presenting with ALF in certain developing areas.

Indian childhood cirrhosis

Indian childhood cirrhosis (ICC) was once endemic and unique to India, although it has been described to a lesser extent in other countries. It presents in children less than 3 years old with serious liver disease progressing to cirrhosis. The liver is characterized by a marked degree of excess copper deposition and Mallory hyaline in injured hepatocytes identical to alcohol-induced hepatotoxicity. The exact etiology of ICC has not been identified. It was assumed that excessive copper exposure in early infancy triggered the liver damage; however, epidemiological data from large well-controlled studies in India failed to prove an exogenous copper toxicity. Other environmental agents, such as zinc, were also implemented in the pathophysiology as it was found in excess in ICC livers, supporting the theory of an exogenous toxin trigger. ICC was recorded in siblings and other family members of known patients supporting the idea of inheritance susceptibility; nevertheless, the exact pattern of inheritance is not yet defined.

There has been a sharp decrease in ICC cases across India in the past three decades, even in centers where many cases were initially reported, due to a change in the use of copper cooking vessels. Recent reports have highlighted the

development of this disease in older children aged up to 14 years. Therefore, ICC should be considered in children of any age diagnosed with cryptogenic cirrhosis, especially those from the Indian subcontinent, despite the declining incidence [11]. Treatment is with penicillamine.

Cystic fibrosis

Cystic fibrosis (CF) was earlier believed to exist only in Caucasians, but although uncommon in the Asian population, is now confirmed in multiple ethnic groups within the developing world. CF has been increasingly diagnosed in Japan, Latin America, the Middle East, and populations derived from the Indian subcontinent that have emigrated to western Europe, thus implying the presence of CF in significant amounts in India and Pakistan. Reports from South Africa also show the presence of CF in persons of pure African descent, thus demonstrating that the earlier observation of the presence of *CFTR* mutations in African Americans was not simply due to a mixture of European genes.

The clinical presentation is similar to that in the western world, but the overall distribution of *CFTR* mutations is considerably different. The frequency of p.F508 del, found in more than 50% in Caucasians, is much less in CF populations in other countries, and *CFTR* mutations differ considerably between neighboring developing countries.

Alpha-1-antitrypsin deficiency

Alpha-1-antitrypsin (AAT) deficiency is the commonest genetic cause of liver disease in Caucasians, especially from northern and western European descendants. The main presentation of AAT deficiency is with neonatal cholestasis; chronic liver disease, although infrequent, can also be a problem. It is uncommon in non-Caucasians and scarcely reported in the developing world. Investigators from India tested 1250 children with liver disease – only 7.8% were suspected of having AAT deficiency based on screening tests. None of the patients who were subjected to genotype sequencing had the PIZ or PIS alleles described in the west. Based on what is currently known about AAT deficiency, the disease does not constitute a major problem in the developing world.

Progressive familial intrahepatic cholestasis

Progressive familial intrahepatic cholestasis (PFIC) is a group of autosomal recessive liver disorders of childhood that present with cholestasis and progress to end-stage liver disease. It has been reported in many developing countries with novel mutations in several ethnic groups and variable frequencies between different populations. The disorder is common in the Middle East, especially among Arabs, mostly because of high consanguinity rate. In certain Arab countries like Oman and Saudi Arabia, PFIC is the commonest etiology of chronic liver disease in children. Unfortunately, the published data on PFIC from India and the Far East is in the form of case reports and small case series. In Africa, the prevalence is unknown.

The disorder is characterized by significant morbidity, high mortality, and the need for liver transplantation in most cases. PFIC is the most common indication for pediatric liver transplantation in Arab populations. It is difficult to confirm the diagnosis of PFIC and its subtypes in the developing countries due to a lack of genetic testing.

Inborn errors of metabolism

Inborn errors of metabolism (IEM) are common in the developing world, especially in places where consanguinity is widely accepted, where levels can reach up to 40–50% of marriages. In Saudi Arabia, the incidence of organic acidemias and lysosomal storage disorders is considered the highest in the world. These diseases carry a great burden on the local health authorities since they have significant morbidity and a high mortality rate.

Diagnostic problems

Diagnosing MLD requires a high index of suspicion with a careful history and clinical examination. A thorough family history is crucial, looking for evidence of consanguinity, affected sibling/s, neurological/developmental delay, or unexplained sibling deaths. Manifestations of liver disease with or without encephalopathy are common either alone or as part of other organ involvement. It is difficult to differentiate MLD from other etiologies of liver problems when liver disease is the sole presentation.

To confirm the diagnosis, a panel of tests is considered which requires a specialized metabolic laboratory equipped for performing and the interpretation of such sophisticated tests. Unfortunately, most developing countries lack such laboratories and trained metabolic specialists who can interpret these tests. In addition, molecular genetic testing, which is an important diagnostic tool for many disorders, is also unavailable in several countries. Therefore, due to limited financial resources, the identification and diagnosis of MLD remains a challenge in the developing world leading to underestimation, failure of diagnosis, and early death.

Treatment

Early intervention can improve the outcome and prevent irreversible damage in most MLD disorders (see Chapters 9 and 19). Treatment includes dietary modification, which is the cornerstone in managing several metabolic diseases, using specialized formulae restricted in certain constituents based on the defective metabolic pathway.

In glycogen storage disorders, frequent feeding or nasogastric feeding is reqiured due to limited fasting tolerance. The commercial metabolic formulae are quite expensive and often unaffordable. Modification of the local diets is utilized but carries the risk of inadequate metabolic control if improperly prepared. Besides, restricted local diets may

Table 34.4 Liver disease presentations of common infections in the developing world.

Jaundice	ALF	Hepatomegaly	Hepatosplenomegaly	Abscess	Cirrhosis
Viral hepatitis A, B, C, D, E Malaria Dengue fever Yellow fever Typhoid fever Hepatobiliary ascariasis Leptospirosis Visceral leishmaniasis Liver flukes Hydatid disease	Viral hepatitis A, B, E Malaria Dengue fever Yellow fever Typhoid fever Tuberculosis Leptospirosis Visceral leishmaniasis	Viral hepatitis A, E Tuberculosis Typhoid fever Brucellosis Leptospirosis Dengue fever Yellow fever Amoebic abscess Liver flukes Hydatid disease	Viral hepatitis B, C, D Malaria Brucellosis Visceral leishmaniasis Schistosomiasis	Amoeba Tuberculosis Brucellosis Hydatid disease	Viral hepatitis B, C, D Schistosomiasis

ALF, acute liver failure.

deprive infants and young children of nutrients required for growth, especially in a region where malnutrition and infection are common.

Other modalities of treatment include oral replacement of enzymatic cofactors, chelating agents to promote excretion of toxic metabolites, and enzyme replacement therapy in specific conditions such as Gaucher disease. Most of these treatment modalities are not readily available or are costly and difficult to maintain in many developing countries.

Liver transplantation may be an alternative treatment of MLD using living-related heterozygous donors (see the section on orthotopic liver transplantation below and Chapter 31).

Recommended strategies to improve outcome

A high index of suspicion for the detection of MLD is recommended, especially in areas where consanguinity is high. Training of health-care professionals in the diagnosis and management of such disorders is essential. It is also important to facilitate the development of specific investigations for early diagnosis and confirmation of MLD. Collaborating with metabolic specialists, hepatologists, and liver transplant centers in the same or neighboring countries may resolve resources issues.

Educating and supporting families and encouraging the correct dietary restriction may reduce severe consequences. Encouraging local companies within the developing world to produce special dietary formulae and replacing commercially expensive ones needs to be explored. The role of genetic counseling for families to understand the inheritance risk may reduce further affected infants. Finally, investment into neonatal screening programs for MLD will help in estimating the exact frequency of the disorders and in implementing early therapy.

Infectious diseases

The liver is frequently involved in infections that are prevalent in the developing world. Different organisms are peculiar to certain regions and can lead to deleterious consequences including death. Presentations of different infections can be variable (Table 34.4). History and geographic region usually limit the likely diagnoses of hepatobiliary dysfunction. Certain organisms, such as those causing viral hepatitis, are widely spread in the developing world, whereas others, such as those causing yellow and dengue fevers, are confined to specific geographic regions.

The following are common infections causing hepatobiliary dysfunction in the developing world other than viral hepatitis A–E:

Bacterial infections

Tuberculosis

Tuberculosis (TB) is one of the most common and endemic infections in the developing world, constituting a major public health problem. Liver involvement was reported in 25–50% of patients dying from active TB. The liver is affected in two ways, either through hepatic infection or through the effect of antituberculous drugs. Hepatobiliary TB manifests in several ways, as shown in Table 34.5. The tubercle bacilli reach the liver via hematogenous spread and the liver responds by granuloma formation, caseating or non-caseating, mainly located in the periportal areas [12]. Multiple granulomas can coalesce, producing a focal tuberculoma. A liver abscess is formed if caseation is extensive into the tuberculoma.

Hepatic TB is divided into two main types, miliary and local. *Miliary TB* occurs when the mycobacterium spreads to the liver via the hepatic arteries resulting in diffuse granulomatous hepatitis. Involvement of other organs might also be seen in this type, including pulmonary TB. Granulomatous hepatitis was reported following BCG vaccination in immunocompromised patients. Presentation is non-specific with fever, anorexia, weight loss, hepatomegaly, and deranged transaminases. Ultrasound shows hepatomegaly and diffuse small metastatic lesions with calcification. Cheesy irregular nodules on the liver can be diagnostic on laparoscopy.

Local TB occurs when the mycobacterium spreads to the liver via the portal vein with resultant formation of

Table 34.5 Presentations of hepatobiliary tuberculosis (TB).

Hepatic TB	Biliary TB	Mixed presentation
Diffuse hepatitis associated with pulmonary or miliary TB Diffuse hepatitis (granulomatous hepatitis) without pulmonary involvement Focal tuberculoma or abscess Calcified hepatic granuloma	Biliary stricture Gallbladder involvement Biliary obstruction by lymph node masses	Granulomatous hepatitis and biliary stricture Calcified hepatic granuloma and lymph node masses

tuberculoma or tuberculous abscess without evidence of pulmonary TB. This lesion will be clearly visualized on ultrasound imaging. ALF is uncommon in TB unless within the context of multiorgan failure in miliary TB or secondary to isoniazid.

Biliary TB is rare, occurring either due to primary infection of the biliary ducts or due to compression of the bile ducts by a hepatic granuloma or affected lymph node. Patients usually present with signs of obstructive jaundice. Stricture formation, indistinguishable from sclerosing cholangitis or malignancy, can be a major complication. Diagnosis is by ultrasound, computed tomography (CT), or MRCP imaging. ERCP and surgery might be required for decompression of an obstructed biliary system.

Diagnosis and outcome

An elevation of hepatic aminotransferases is usual. The diagnosis is based on finding caseating granulomas on liver biopsy with evidence of TB elsewhere in the body. In children with a liver abscess, liver function tests may be normal. A diagnostic tap of the abscess is rarely helpful, but a biopsy from the wall of the abscess may show caseating granulomas or acid-fast bacilli. Alternatively, the diagnosis should be suspected if the diagnostic tap reveals a sterile yellowish fluid, the immunodiagnostic tests for amoebiasis are negative, and there is no response to combined therapy with antibiotics and antiprotozoal agents.

Anti-TB therapy

Liver disease may be induced by anti-TB medications (Table 34.6) since most are hepatotoxic, with a higher risk with combination therapy, mainly within 2–3 months of initiating treatment (see Chapter 12). Hepatotoxicity manifests with abdominal pain, nausea, vomiting, and jaundice or can be asymptomatic with raised transaminases. Patients with chronic liver disease are not only at a higher risk of developing TB due to their immunocompromised status, but their underlying disease may also be aggravated by anti-TB medications. Treatment is as for pulmonary disease if liver involvement is part of miliary TB.

For granulomatous hepatitis, tuberculoma, or liver abscess, therapy follows extrapulmonary TB. No modification of drug therapy is required because liver involvement does not increase hepatotoxicity risk. Nevertheless, in patients with chronic liver disease, the number of anti-TB drugs should be reduced with increasing treatment duration (Table 34.7). Close monitoring of liver function is essential in patients treated with anti-TB medications.

Table 34.6 Recommended doses and regimens of antitubercular drugs in children.

Drug	Dose (mg/kg/day)
Isoniazid	5
Rifampicin	10
Pyrazinamide	25
Ethambutol	20

Table 34.7 Treatment categories and regimens for childhood tuberculosis as recommended by the Indian Academy of Pediatrics [13].

Category	Intensive phase	Continuation phase
New cases	2H 3R 3Z $3E^3$*	4H $3R^3$
Previously treated cases	2S 3H 3R 3Z $3E^3$ + 1H 3R 3Z $3E^3$	5H 3R $3E^3$

E, ethambutol; H, isoniazid; R, rifampicin; S, streptomycin; Z, pyrazinamide.
* The number before the letters refers to the number of months of treatment. The subscript after the letters refers to the number of doses per week.

Typhoid fever (enteric fever)

This is a serious multisystem infection endemic in multiple regions within the developing world, mainly tropical countries. It results from ingesting contaminated food or water with *Salmonella* species, *S. typhi* and *S. paratyphi*. The disease manifests with fever, rigors, abdominal pain, and constipation. Multiple complications involving various organs can develop in the course of the illness such as heart failure, gastrointestinal bleeding, bowel perforation, delirium, and disseminated intravascular coagulation (DIC).

Hepatomegaly and mild derangement of liver enzymes are common, but significant liver disease is not [14]. Hepatic dysfunction usually manifests in the second week of illness with jaundice, cholecystitis, or tender hepatomegaly. Clinically, typhoid hepatitis is similar to viral, malarial, or amoebic hepatitis; hence, proper and timely diagnosis is essential to initiate treatment. In viral hepatitis fever subsides after the appearance of jaundice, while in typhoid hepatitis

fever persists. Hepatic encephalopathy has been reported although it can be mistaken with neuropsychiatric manifestations of typhoid fever. Children younger than 4 years carry a significantly high mortality.

The pathogenesis of typhoid hepatitis can either be due to liver invasion by bacteria or due to endotoxemia with immune-mediated liver damage. Histopathology may show non-specific inflammation, cloudy swelling, moderate fatty change, or the appearance of typhoid (Mallory) nodules, a typical feature of typhoid hepatitis due to hyperplasia and aggregates of Kupffer cells. Fortunately, the changes are reversible with treatment.

Typhoid fever should be considered in any febrile patient who develops jaundice in the developing world with or without hepatomegaly. Blood culture is usually positive and detection of *S. typhi* by polymerase C reaction (PCR) is helpful. Serological tests have limited sensitivity and specificity. Fluroquinolones, azithromycin, and cephalosporins are the antibiotics of choice. Prognosis is favorable with early therapy; however, the chronic carrier state occurs in 2–5% of patients when the organism colonizes the gallbladder and biliary system. Immunization should be offered for travelers to endemic areas.

Brucellosis

Brucellosis is caused by *Brucella* spp., intracellular bacteria of the reticuloendothelial system. It has a worldwide distribution with high endemicity in the Mediterranean region, Middle East, and Latin America. *B. melitensis* is the most prevalent and virulent type responsible for most cases. Childhood brucellosis accounts for 10–30% of all infected cases and the main source of infection is ingestion of unpasteurized dairy products [15]. Older boys are more often affected, probably because they are more in contact with animals. Fever, constitutional symptoms, and peripheral arthritis are common presenting features but the infection can be complicated by multisystem involvement. The bacteria reach the liver via blood, inducing epitheloid granuloma formation with necrotizing appearance. Hepatomegaly or hepatosplenomegaly with mild elevation of liver enzymes is common but jaundice is rare. A liver abscess, known as brucelloma, is another complication of acute brucellosis that is extremely rare in children, while it represents a chronic form of disease in adults. A single lesion is more likely although more than one is possible.

Diagnosis is based on:

- Serology and PCR since positive culture of blood or drained pus from the hepatic abscess has a low yield.
- Radiological evaluation with ultrasound and CT of the abdomen in hepatic brucelloma. The presence of an abscess with central calcification in a febrile patient is suggestive of brucelloma.

Treatment of brucellosis requires combination therapy with doxycycline for 45 days and streptomycin for 14 days. Gentamicin or netilmicin may be substituted for streptomycin for the first 7 days. Second-choice regimens consist of combinations of doxycycline and rifampicin (rifampin) for 45 days, or monotherapy with doxycycline for 45 days.

Aspiration or percutaneous/surgical drainage should be considered for patients with hepatic or splenic abscess or brucelloma.

Leptospirosis

Leptospira, a spirochete bacterium, infects animals and humans causing leptospirosis. Transmission to humans can either be via direct contact with infected animal urine or indirectly through contact with contaminated water, especially during rainy seasons. In endemic areas it is commoner and more severe in adults than children. The infection commonly presents with mild non-specific febrile illness but in some cases it progresses to severe hepatorenal dysfunction, pulmonary hemorrhage, myocarditis, and vascular collapse (Weil disease). The organism damages blood vessels, leading to hepatic centrilobular necrosis and acute renal tubular dysfunction [14]. Liver involvement presents with jaundice and tender hepatomegaly associated with very high serum bilirubin but normal or mildly deranged transaminases. This picture is highly suggestive of leptospirosis rather than viral hepatitis. Jaundice can also be due to hemolysis. Meningitis or encephalitis mimic hepatic encephalopathy. Clinically, patients develop characteristic conjunctival suffusion.

Diagnosis is made by serology, and treatment should be initiated early with intravenous penicillin and cephalosporins. Supportive therapy and careful management of the liver, renal and central nervous system, and of any hematological complications, are mandatory.

Viral infections (non-hepatitis)

Dengue fever

Dengue infection (dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS)), caused by a mosquito-borne flavivirus, is endemic in many areas of the tropics and subtropics including South East Asia, India, the Pacific Islands, and the Caribbean. Up to 50% of infected children may be asymptomatic or have a mild febrile illness. Liver injury in dengue fever is multifactorial. It may be caused by direct viral effect on the liver, host immune responses, or the effect of hypotension and shock. Dengue fever is usually characterized by:

- Biphasic fever (saddle-backed).
- Myalgia/arthralgia.
- Severe headache and retro-orbital pain.
- Rash, which may be petechial.
- Leukopenia and lymphadenopathy.
- Hepatomegaly in almost all patients (79–100%) and elevated transaminases. Mild hepatitis is found in up to 75% of cases with a higher aspartate aminotransferase (AST)

than alanine aminotransferase (ALT) probably due to muscle injury, but transaminases can be 10 times the normal value. They usually peak in the second week of illness with gradual normalization by the third to fourth week of illness.
- Jaundice; this is a bad prognostic sign and is usually associated with ALF with a mortality of up to 50%.

If the disease progresses to DHF, additional features include:
- Abdominal pain and diarrhea.
- Mucosal bleeds, petechiae, and gastrointestinal bleeding.
- Renal failure.
- Fulminant hepatitis with encephalopathy and Reye syndrome.
- Myocarditis with a congested liver and ascites.
- Pleural effusion.

According to WHO, DHF is characterized by:
- High-grade fever.
- Thrombocytopenia.
- Hemorrhagic phenomena.
- Increased vascular leakage.

DSS additionally shows:
- Protein-losing shock syndrome with a rapid pulse.
- Weak and narrow pulse pressure (less than 20 mmHg).
- Hypotension for age.
- Cold, clammy skin and restlessness.

Diagnosis is based on serology and viral detection by PCR on liver tissue. Liver histopathology shows centrilobular necrosis at zone 2, fatty changes, Kupffer cell hyperplasia, monocyte infiltration of the portal tract, and Councilman bodies.

There is no approved vaccine for dengue fever and management is based on multiorgan supportive care.

Yellow fever

Yellow fever is endemic in tropical regions of Africa and South America. It is caused by the yellow fever virus, which is transmitted to humans via mosquitos. Clinical features are similar to those of dengue fever, and vary from mild febrile illness with muscle and joint aches to severe multiorgan failure and shock with fatality up to 50%. Hepatomegaly is common and liver histopathology shows hepatocyte swelling, necrosis, apoptosis, and steatosis, mainly in the mid-zonal region sparing the central vein area [16]. Apoptosis is more prominent than necrosis, hence the appearance of Councilman bodies, the hallmark of yellow fever (Figure 34.1). They represent hepatocytes with focal eosinophilic condensation in the cytoplasm. Yellow fever hepatopathy seems to be the effect of direct liver injury by the virus.

Yellow fever should be suspected in residents of or travelers to endemic areas who present with fever and jaundice. Elevated bilirubin and transaminases occur acutely and may persist for several months. As in dengue fever, AST tends to be higher than ALT and ALF carries a high mortality rate.

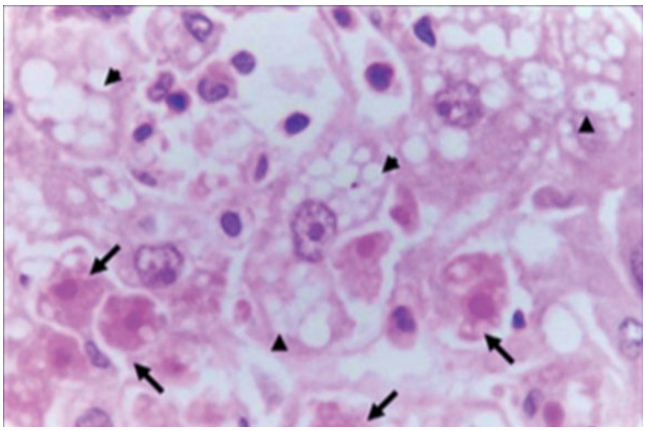


Figure 34.1 Liver histopathology in yellow fever showing Councilman bodies (arrows) and steatosis (arrowheads). (From Monath and Vasconcelos 2015 [17]. Reproduced with permission of Elsevier.)

Thrombocytopenia and coagulopathy are common, which explains the hemorrhagic diathesis in yellow fever.

The diagnosis is confirmed by serology and PCR detection of the yellow fever virus in the blood or other tissues including the liver. Unfortunately, there is no specific treatment and supportive therapy is critical. Prevention is achieved by the YF-17D vaccine that is included in the vaccination programs of children above 6 months in many endemic countries. It can also be given to travelers to endemic areas.

Parasitic infections

Malarial hepatopathy

Jaundice is a common manifestation in malaria, an endemic tropical disease, due to intravascular hemolysis. In addition, jaundice may be secondary to hepatobiliary dysfunction and malarial hepatopathy, reported mainly in *Plasmodium falciparum* compared to *P. vivax* malaria [14]. Jaundice and hepatomegaly are more common in pediatric malarial infections (68%) compared with adult infections (6%). A mild elevation of hepatic aminotransferases is common. Severe infection may occur in neonates who present with clinical features suggestive of cholestasis.

Clinical presentation is with fever, jaundice, and hepatomegaly with often significant splenomegaly due to hemolysis. Liver dysfunction ranges from conjugated hyperbilirubinemia with a mild elevation in transaminases to fulminant hepatic failure. The international normalized ratio is usually normal even with a marked elevation of liver enzymes unless there is an associated DIC. Occasionally, coexisting cerebral malaria can be confused with ALF encephalopathy.

The exact pathogenesis of malarial hepatopathy is not well understood but liver damage is believed to be due to cytoadherence of parasitized red blood cells (RBCs) to small blood vessels obstructing blood flow with resultant ischemia. DIC occurring in severe malaria and coinfection with viral hepatitis A and E may complicate hepatocellular damage.

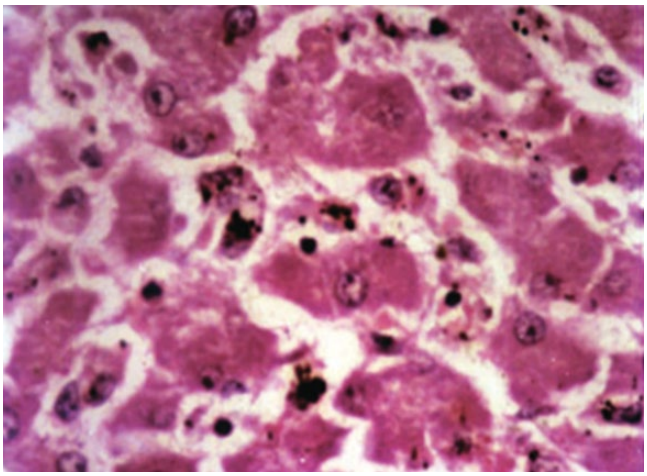


Figure 34.2 Liver histopathology in malaria showing Kupffer cell hyperplasia and malaria pigment deposits. (From Anand and Puri 2005 [18]. Reproduced with permission of John Wiley & Sons.)

Malarial hepatopathy should be suspected in any patient presenting with acute fever and jaundice or ALF in a tropical area.

The diagnosis is made by demonstrating the parasite on peripheral blood smears or the detection of malaria antigen by an immunochromatographic method. Liver histopathology includes centrizonal necrosis, Kupffer cell hyperplasia with loaded brown malarial pigment, steatosis, and parasitized RBCs (Figure 34.2). Cholestasis and parenchymal inflammation of the liver are uncommon.

Management is by standard antimalarial therapy and supportive care. Hepatic dysfunction is reversible with no residual damage if the infection is properly managed. Liver dysfunction can also be induced by antimalarial medications used in treatment or prophylaxis, especially with combination therapy. This varies from asymptomatic minimal elevation of transaminases to fatal acute hepatitis.

Amoebiasis

Amoebiasis is an aggressive protozoal disease caused by *Entamoeba histolytica* that is endemic in tropical regions. The parasite usually infects the human bowel leading to severe dysentery and fulminant colitis [19]. The trophozoites reach the liver by invading the bowel wall into portal circulation causing hepatic amoebiasis. Hepatocyte death occurs with small areas of focal necrosis that coalesce to form a larger hepatic amoebic abscess. The lesion is usually solitary and mainly located within the right lobe of the liver; however, multiple lesions can develop in advanced cases of amoebiasis.

Clinical presentation is with fever and abdominal pain with or without intestinal dysentery. Jaundice is uncommon and, if present, suggests a large or multiple hepatic lesions. Tender hepatomegaly is the most common physical sign in hepatic amoebiasis.

Diagnosis is by ultrasound imaging or abdominal CT scan. In addition, serology is useful for detecting circulating antibodies against *E. histolytica* thus differentiating amoebic from pyogenic abscesses. *E. histolytica* can be detected in aspirated pus or necrotic material from the hepatic lesion.

Complications include:

- Spread to adjacent structures such as the peritoneum, lung, and pericardium.
- Pulmonary involvement, which is suggested by a development of cough and dyspnea and is diagnosed with a CT scan of the chest.
- Rupture into the pericardium, which produces signs of pericarditis that can be lethal.
- Acute abdominal pain and signs of peritonitis; these are suggestive of abscess rupture into the peritoneum.
- Subphrenic abscess with secondary bacterial infection, which is not uncommon.

Medical therapy is the treatment of choice for hepatic amoebiasis using imidazole derivatives, mainly metronidazole. It is important to eradicate the amoebic cysts and trophozoites from the intestine by luminal amoebicides to prevent relapses even in the absence of dysentery.

Ultrasound-guided percutaneous aspiration or drainage is required for large abscesses with imminent rupture or failure of clinical improvement despite medical therapy.

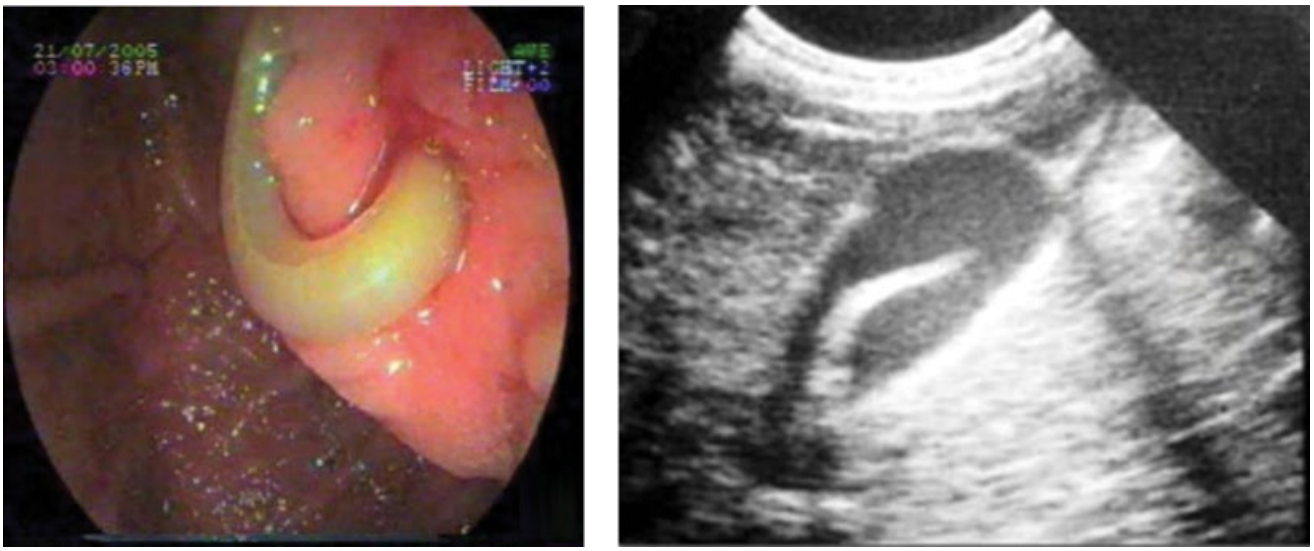
The prognosis of hepatic amoebiasis is favorable if diagnosed early and properly treated. Prevention of amoebiasis can be achieved by improving public health measures and education in endemic areas.

Hepatobiliary ascariasis

Ascariasis is the most common helminthic infection worldwide, caused by a parasitic worm *Ascaris lumbricoides*. It is endemic in tropical and subtropical countries, mainly in children living in unhygienic conditions. The patient becomes infected from ingesting contaminated vegetables and fruits with *Ascaris* eggs that mature in the intestinal tract [20]. Usually, the infection is asymptomatic, but heavy infestations cause abdominal pain, poor growth, or bowel obstruction.

In 10% of patients the worms migrate and invade the biliary system and pancreatic duct causing hepatobiliary ascariasis, which is the second most common cause of morbidity and mortality in children with this infection after intestinal obstruction. The worms may reside in the biliary ducts, or gallbladder or even colonize in the liver parenchyma. Clinical symptoms include obstructive jaundice with associated cholangitis, cholecystitis, or pancreatitis. Liver abscess and rarely liver perforation may occur. Clinical examination reveals a tender right upper abdominal quadrant, a palpable gallbladder, hepatomegaly, and jaundice.

In endemic countries, ascariasis should be suspected in patients presenting with hepatobiliary disease. Diagnosis is usually confirmed by ultrasound imaging showing worms in the bile ducts and gallbladder (Figure 34.3). Ultrasound is helpful in monitoring worm movement and activity in



(A) (B)

Figure 34.3 Biliary ascariasis. (A) *Ascaris* worm projecting from the ampulla of Vater on duodenoscopy. (From Ibrarullah *et al.* 2011 [21]. Reproduced with permission of Elsevier.) (B) Ultrasonography showing a linear echogenic filling defect in the gallbladder. (From Anand *et al.* 1999 [22]. Reproduced with permission of Wolters Kluwer Health.)

the biliary ducts. MRCP is another helpful imaging tool, but of limited use due to the cost.

Management of hepatobiliary ascariasis is by conservative methods. Antihelminthic medications are needed to eradicate intestinal worms but are ineffective for those in the biliary system. Approximately 60–80% of biliary ascariasis responds to conservative management and worms will spontaneously return to the intestine. Extraction of biliary worms can be performed during ERCP for critically ill patients who need biliary drainage or those who have failed conservative management. Surgery is required in those with failure of ERCP, intrahepatic bile duct worms, gallbladder worms, and pancreatitis.

Teaching children to wash their hands and raw vegetables and fruits before eating is an important measure to prevent ascariasis infestation.

Schistosomiasis (bilharzia)

Schistosomiasis is a tropical disease caused by worms of the genus *Schistosoma*. It is the third leading endemic parasitic disease in the world after malaria and amoebiasis. High rates of infection occurs in areas close to fresh water and children carry the heaviest burden of infection [23]. The parasite penetrates the human host skin during contact with infected water and matures in the intestinal venules. The excreted eggs reach the liver via the portal system, inducing an inflammatory reaction with subsequent granulomatous formation or cellular infiltrate of eosinophils and neutrophils surrounding the eggs, mainly in the periportal areas. Hepatic schistosomiasis is usually caused by *S. mansoni*, *S. japonicum*, and *S. mekongi*. It consists of two distinct syndromes: early inflammatory and late fibrotic hepatic disease.

Inflammatory hepatic schistosomiasis is most commonly seen in children, and is an early reaction to the ova inside the liver. The clinical features are hepatomegaly and variable splenomegaly ranging from mild to severe. Fibrotic hepatic schistosomiasis is a chronic liver disease developing several years later and so is more likely in adults. It is characterized by severe and dense fibrosis in the periportal area causing portal hypertension and esophageal varices with risk of significant bleeding.

The diagnosis of hepatic schistosomiasis is done by the following:

- The detection of schistosomal eggs on stool or urine microscopy in a patient with hepatomegaly residing in an endemic area.
- Serological tests and PCR.

Praziquantel is used for treating all schistosomal species. It is safe and effective in children, but has no effect on the eggs, which continue to be excreted several weeks after treatment, exposing the patient to reinfection. Therefore, a repeat dose after 6–12 weeks may be needed. Patients with portal hypertension should have surveillance endoscopy and treatment as required for esophageal variceal bleeds. There is no vaccine yet and prevention depends on improving sanitation and water supply, socioeconomic conditions, and education in endemic areas.

Mass antihelminthic drug administration to school-age children has been implemented in some endemic countries.

Visceral leishmaniasis

Visceral leishmaniasis, known as kala-azar, is a systemic parasitic infection caused by protozoa of the genus *Leishmania*, mainly, *L. donovani* and *L. infantum*. The infection is endemic

in tropical and subtropical areas. Transmission to humans is via the bite of sand flies and the parasite primarily infects the reticuloendothelial system including the liver [24].

Clinical presentation occurs months or years after infection with fever, anorexia, and weight loss. Hepatosplenomegaly, with a more prominent spleen than liver, and lymphadenopathy are detected on physical examination. Jaundice can be a presenting feature, and causes a diagnostic issue in a febrile patient in an endemic area in which viral hepatitis and dengue fever are common, potentially delaying the diagnosis of visceral leishmaniasis.

Mild derangement of biochemical liver function is common, but acute hepatitis and ALF are rare presentations. Pancytopenia due to bone marrow suppression is suggestive of visceral leishmaniasis. The diagnosis is based on visualization of the amastigotes in bone marrow aspirate, and splenic, liver, and lymph node tissue, and serology to detect antibodies against *Leishmania*. Liver histopathology shows Kupffer cell hyperplasia, ballooning degeneration of hepatocytes, fatty changes, and fibrosis that can be focal or diffuse. Epitheloid granulomas are not usually seen in visceral leishmaniasis.

Visceral leishmaniasis is progressive and fatal if not properly treated. Supportive care is crucial to control intercurrent infection, anemia, and bleeding. Secondary bacterial infection and hemorrhage are common complications. Systemic therapy with sodium stibogluconate (Pentostam®), liposomal amphotericin B, or miltefosine may be indicated. Hepatic dysfunction is reversible with early treatment; however, severe jaundice and profound derangement of liver function carry a bad prognosis. The diagnosis is commonly missed, and should always be considered in a patient presenting with jaundice, fever, hepatosplenomegaly, and pancytopenia in an endemic area.

There is no vaccine to prevent visceral leishmaniasis. Therefore, the preventive method is protection against sand flies by covering exposed skin, using insecticides, bed nets, and repellents.

Liver flukes

Liver flukes are food-borne trematodes. The main diseases caused by liver flukes are clonorchiasis, opisthorchiasis, and fascioliasis [25].

Clonorchiasis and opisthorchiasis

Those diseases are caused by the fish-borne liver flukes *Clonorchis sinensis*, *Opisthorchis viverrini*, and *O. felineus*. They are endemic in South East Asia and eastern Europe. Humans become infected after eating raw or undercooked fish containing the metacercariae. The adult worm matures in the bile ducts and gallbladder. Infected patients may have acute pain in the right upper quadrant and hepatomegaly with hepatitis-like symptoms. Severe infection and chronic symptoms may present with obstructive jaundice, cholangitis, cholecystitis, or cirrhosis. Cholangiocarcinoma is a serious complication and carries a poor prognosis.

Fascioliasis

Fasciola hepatica and *F. gigantica* are the causative agents of fascioliasis in humans. The highest prevalence was reported in northern Bolivia, Egypt, and Iran; however, it has also been reported in Europe. Transmission occurs following ingestion of contaminated freshwater plants, drinking untreated water, or washing utensils with infected water. Like other liver flukes, the parasite matures in the bile ducts. Two clinical phases are seen in this infection. There is an acute phase, which corresponds to the migratory stage of the parasite when the patient develops fever, abdominal pain, nausea, and vomiting. The chronic phase corresponds to the presence of the adult worm in the bile ducts and the patient may either be asymptomatic or present with cholecystitis, cholangitis, and obstructive jaundice. The disease may be fatal without treatment.

Diagnosis is by detecting eggs on stool microscopy, or in bile and duodenal fluid. Fecal antigen detection by enzyme-linked immunosorbent assay (ELISA) or PCR and serology for *F. hepatica* is now possible. Praziquantel is the drug of choice for fish-borne liver flukes, while triclabendazole is used for the treatment of fascioliasis.

There is no available vaccine and prevention depends on avoidance of contaminated water, raw water plants, and raw fish.

Hydatid disease

Hydatid disease is caused by larval forms of *Echinococcus* tapeworms found in the small intestine of carnivores. Human echinococcosis is endemic in South America, the Middle East, East Africa, Central Asia, and China. Two species are important – *E. granulosus* causes cystic echinococcosis (CE) and *E. multilocularis* causes alveolar echinococcosis (AE) [26].

In cystic echinococcosis, the cysts are mainly localized in the liver but may develop in the lung and other intra-abdominal sites. Patients are usually asymptomatic but may have chronic abdominal pain due to cyst expansion within liver tissue. Cholangitis and obstructive jaundice develop if the cyst ruptures into the biliary system.

Diagnosis is based on clinical findings and ultrasound imaging in which there are multiple daughter cysts within a well-defined circular cyst (Figure 34.4). Degenerative cysts are calcified and visible on plain X-ray. An abdominal CT scan or magnetic resonance imaging (MRI) will demonstrate disseminated disease. Serological tests have limited specificity due to cross-reactions with other helminthic diseases. Needle aspiration of a suspected hydatid cyst should be avoided to prevent leakage of cyst contents causing anaphylaxis or abdominal dissemination.

Treatment option for hydatid cysts depends on the cyst characteristics and the availability of medical and surgical

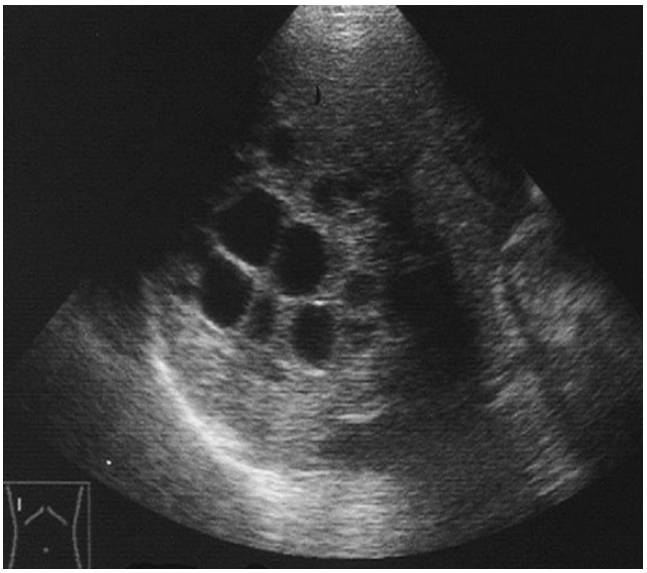


Figure 34.4 Liver hydatid cyst appearance on ultrasound; multiple daughter cysts are visible near the surface and posterior calcified pericyst membrane. (From Beeching and Dassanayake 2016 [27]. Reproduced with permission of Elsevier.)

expertise. Some cysts require no treatment and should be followed up with ultrasound imaging. Medical therapy using albendazole for several weeks to months may be effective except in large cysts. Surgical resection of large cysts eradicates the infection and prevents rupture and spillage of the contents. In inoperable patients, percutaneous aspiration of cyst contents, instillation of hypertonic saline or alcohol, and reaspiration (PAIR) is an alternative.

Alveolar hydatid disease, caused by *E. multilocularis*, has a worse prognosis than cystic hydatid disease. It spreads within the liver like a malignant tumor and invades the lung and other tissues by metastasis formation. Patients present with fever, weight loss, and tender hepatomegaly. Ultrasound shows pseudotumors and irregular hypoechogenic areas with scattered calcifications. CT scanning and MRI are more useful for delineating the morphology of the lesions. On histopathology, epitheloid granulomas are visualized lining the parasitic vesicles and *Echinococcus* can be detected by PCR on liver tissue.

Treatment is by albendazole, which should be given for years. This should be associated with resection of the entire parasitic lesion in the liver. Liver transplantation has been performed to treat patients with inoperable disease; however, larva regrowth with immunosuppression is possible.

Viral hepatitis

Viral hepatitis is a worldwide health problem and is most prevalent in children in the developing world. Hepatitis A to E viruses are the main causes of viral hepatitis. Hepatitis A and E viruses are enterally transmitted and do not lead to chronic liver disease, while hepatitis B, C, and D viruses are parenterally transmitted and lead to chronic viral hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) (see Chapter 13).

Hepatitis A virus infection

Hepatitis A virus (HAV) is a non-enveloped, single-stranded RNA virus. It has four genotypes, with a single serotype. It belongs to a new genus, *Hepatovirus*, within the family *Picornaviridae*. The main route of transmission is through the fecal–oral route by contaminated food, water, or household contact. Uncooked or partially cooked shellfish is an important source of HAV infection, while transmission from food handlers and infected children to medical personnel has been reported.

Epidemiology

Hepatitis A virus infection is prevalent in Asia, southern Europe, South America, and many other parts of the world. The epidemiological status of hepatitis A is divided into high, intermediate, and low endemicity. The prevalence has declined gradually in the past 20 years as a result of improved sanitation and socioeconomic conditions With these changes in epidemiology, there has been a decrease in immunity against hepatitis A, increasing the number of children and adolescents who are now susceptible to HAV. This is well demonstrated among the children in Pacific Island Nations [28] and in Singapore [29]. Outbreaks of hepatitis A still occur regularly among the developing countries due to contaminated water supply and overcrowding [30].

Clinical features

Hepatitis A remains an important cause of morbidity in the developing world. The incubation period ranges from 15 to 50 days. The prodrome is abrupt, with general malaise, fever, anorexia, nausea, vomiting, and abdominal pain or discomfort. Jaundice appears within 1 week and lasts for <2 weeks in the majority of cases. Hepatomegaly and elevation of aminotransferases are usual during the prodromal period. Serum bilirubin and aminotransferases return to normal within 2–3 months. HAV infection is often asymptomatic in young children, in contrast to infection in adults. It is more severe in those with chronic liver diseases, such as chronic hepatitis B or C.

Extrahepatic manifestations are rare and may range from neurological, renal, and hematological manifestations to acute pancreatitis. Both the relapsing and cholestatic forms of hepatitis A are seen in children, with an increased incidence in older age. Prolonged cholestasis is seen as a protracted period of jaundice for more than 3 months, which resolves without intervention. The cholestatic course may present with pruritus, fever, diarrhea, weight loss, and serum bilirubin of more than 10 mg/dL. Relapsing acute viral hepatitis can be seen in 3–20% of patients with hepatitis A, with the patient initially showing partial or complete resolution of clinical and biochemical manifes-

tations, but relapsing within a period of 3 weeks with a clinically milder presentation. Multiple relapses are uncommon and a tendency to increased cholestasis in patients may be noticed.

The severity of the hepatitis and the numbers of symptomatic cases depend on age. The case fatality rate has been reported to be 0.1% in children <14 years old, 0.4% in adolescents and young adults (15–39 years), and 1.1% in patients 40 years of age or over. Although HAV is mostly a self-limited acute disease, it is the commonest cause of ALF in developing countries.

Prevention

Improvements in sanitation and water supply and the introduction of HAV vaccine are important factors contributing to the control of HAV. Before the introduction of hepatitis A vaccine, immunoglobulin was used to control local epidemics or to prevent infection in travelers, but only provided immunity for 3–6 months. The development of effective hepatitis A vaccine has produced long-term immunity. The vaccine is administered in two doses on a 0- and 6-month schedule starting at 11–18 months of age.

Education is an important tool in the prevention of hepatitis A infection. Personal and food hygiene are of utmost importance. All drinking water should be thoroughly boiled before consumption. Shellfish must be properly cooked before eating.

Hepatitis B virus infection

Epidemiology

Hepatitis B virus (HBV) infection is a worldwide health problem. It is prevalent in Asia, Africa, southern Europe, and Latin America, where the hepatitis B surface antigen (HBsAg) seropositive rate ranges from 2% to 20%.

In highly prevalent areas, primary HBV infections occur mainly during infancy and early childhood. The age of primary infection is an important factor affecting the outcome. Infection during infancy and early childhood leads to a high rate of chronicity. After the introduction of universal vaccination, the incidence of hepatitis B in many developing countries has markedly decreased.

Perinatal transmission. Perinatal transmission from HBsAg carrier mothers to their infants is a very important route of transmission leading to chronicity in hyperendemic areas. It accounts for the transmission route in 40–50% of HBsAg carriers in Asia.

Horizontal and parenteral transmission. Horizontal transmission from highly infectious family members such as elder siblings is common, particularly in Africa. Parenteral transmission from improperly sterilized syringes or other contaminated instruments remains a problem in the developing world. Other risk factors include institutionalized children and multiple or large amounts of blood transfusions.

Clinical features and natural history

Acute or fulminant hepatitis B. HBV infects all ages. The normal incubation period is 2–6 months, and symptoms may occur as early as 2 months of age in infants of HBsAg carrier mothers. Acute hepatitis B usually runs a self-limited course, and recovery follows anti-HBs seroconversion. Acute or fulminant hepatitis B occurs mainly in infants of hepatitis B e antibody-seropositive mothers in areas of high prevalence. There is a high mortality rate in children with fulminant hepatitis B, but those who survive do not develop chronic liver disease.

Chronic hepatitis B. Children with chronic HBV infection are usually asymptomatic. The liver histology findings are mild initially, but may progress to severe liver damage in later life during the process of acute exacerbation and hepatitis B e antigen (HBeAg) seroconversion.

HBeAg is an important marker reflecting active viral replication and infectivity, and its clearance is therefore used as a marker for seroconversion or the success of antiviral therapy. HBeAg clearance is usually preceded by an acute exacerbation, with elevated aminotransferase levels and active inflammation of the liver. The peak levels of aminotransferases range from ALT <300 IU/L to >800 IU/L in adolescents and young adults. It is occasionally accompanied by bridging necrosis on liver histology. Seroconversion takes place in 40% of children within 1 year of an acute exacerbation.

After HBeAg clearance, aminotransferase levels gradually return to normal, and anti-HBe develops spontaneously. Unfortunately, it is likely that permanent liver damage and integration of the genome of HBV may have already taken place, despite the disappearance of HBeAg. Viral genetic factors and titers and host factors may affect the clinical course and outcome of HBV infection.

Complications

The development of liver cirrhosis or HCC is rare but occasionally observed during childhood, particularly in areas hyperendemic for HBV infection. It is estimated that the lifetime risk of HCC in HBsAg carriers is around 25% and that it is higher in those with persistent HBeAg positivity.

Prevention of hepatitis B and related liver diseases

Immunoprophylaxis is the most cost-effective way of achieving global control of HBV infection and its related complications. Passive immunization using hepatitis B immunoglobulin (HBIg) provides temporary immunity, but the cost of antenatal screening and HBIg is beyond most developing countries. The most important strategy has been the universal immunization program to prevent both perinatal and horizontal transmission of HBV infection.

The world's first universal hepatitis B immunization program was launched in Taiwan in July 1984. In this program, a combination of passive and active immunization effectively prevents HBV transmission from highly infectious

mothers (HBeAg-positive mothers). HBIg is given within 24 h after birth for infants of high-risk mothers with positive HBeAg and HBsAg. To all infants, the first dose of hepatitis B vaccine is given within the first week after birth, and the second and third doses 1 and 6 months later. The efficacy of prevention for infants of high-risk mothers is approximately 85%.

In areas in which the prevalence of HBV infection is low or financial resources are limited, immunization with three doses of HBV vaccine on a schedule of 0, 1, and 6 months without antenatal screening of the mothers or administration of HBIg is a reasonable strategy to save costs.

Effect of universal hepatitis B immunization on the control of liver diseases in children

Universal hepatitis B vaccination has effectively reduced both perinatal and horizontal transmission of HBV and thus the rate of chronic HBV infection worldwide.

The hepatitis B carrier rate has fallen from 5–10% to <1%, demonstrating that universal vaccination is more effective than selective immunization for high-risk groups The reduction in HBV infection after the launch of the universal hepatitis B vaccination program has had a dramatic effect on the incidence of HCC in children. The annual incidence of HCC in children aged 6–14 years was reduced to one-fourth, from 0.52–0.54 per 100,000 children born before July 1984 to 0.13–0.20 per 100,000 children born after July 1984. This trend has continued, and it is expected that there will be a subsequent decline in the incidence of HCC in adults in the future.

Long-term immunity after HBV immunization and booster doses

The levels of antibody against HBsAg (anti-HBs) wane gradually with time after primary HBV immunization in infancy. Hyporesponders who have lower initial antibody titers after vaccination lose detectable antibodies within a shorter period than the responders. The determination of serum levels of anti-HBs after hepatitis B vaccination is currently the only simple test available to predict the decay of protection and to plan the administration of booster doses. Long-term protection against HBV infection is high, and booster vaccination at age 7 did not significantly increase this protection against HBV infection at age 16. Even in the absence of detectable anti-HBs, the maintenance of HBsAg-specific cellular immune memory after primary HBV immunization confers protection against clinical breakthrough infection. For a discussion about therapy and monitoring see Chapter 13.

Hepatitis C virus infection

Epidemiology

The seroprevalence of hepatitis C virus (HCV) infection is around 0.8–3.0% in the adult population and <0.2% in children in most parts of the world, except for some hyperendemic areas. In a retrospective review of 3932 children with liver disease in China, hepatitis C accounted for some 20% of the cases [31]. HCV infection occurs mainly in high-risk children, such as those who have been exposed to blood products (children with hemophilia, thalassemia, blood transfusions, hemodialysis, malignancy, and organ transplantation) or in children of HCV-infected mothers. In hyperendemic areas, such as the Nile delta in Egypt, anti-HCV prevalence increases sharply with age, from 9.3% in those <20 years of age to >50% in those over 35. A history of antischistosomal injection therapy (reported by 19% of anti-HCV-positive patients) was found to be a risk for anti-HCV.

Perinatal transmission. Perinatal infection with HCV is much lower than that in HBV.

Parenteral exposure to HCV. Transfusion and injection using unsterile needles were the most important causes of HCV transmission in the developing world. They continue to be important routes of HCV transmission in children in those areas of the developing world with limited resources, where mass blood screening for anti-HCV is not universally carried out even nowadays.

Clinical features

After primary infection with HCV, 60–80% of children have a chronic course, as in the west. HCV RNA is detectable in serum 2 weeks after exposure, and anti-HCV is detectable in serum by 4–8 weeks. Most chronically infected children remain asymptomatic, with normal liver function profiles. However, transient or persistent elevation of aminotransferase levels is not uncommon, particularly in perinatally infected children.

Prevention

In contrast to hepatitis B, there is currently no approved effective vaccine for the prophylaxis of HCV infection. Screening of blood and blood products and the use of disposable syringes and needles have reduced post-transfusion HCV infection and hepatitis effectively in those countries in the developing world with sufficient resources and organization.

Treatment

Treatment for hepatitis C with sustained viral clearance is more effective than that for hepatitis B, particularly since the development of combination therapy and oral direct acting antivirals (see Chapter 13). Sustained viral response was more frequent in children with HCV genotypes 2 and 3 (84%) than in those with HCV genotype 1 (36%) with combination therapy with peginterferon and ribavirin, but it is likely that this therapy will be replaced by the highly effective oral antivirals, subject to cost.

Other causes of viral hepatitis

Hepatitis D, hepatitis E, and other types of non-A–E hepatitis are uncommon forms of viral hepatitis in children. Hepatitis D is particularly rare in children in the developing world. Hepatitis G, transfusion-transmitted (TT) virus, and SEN

virus (acronym from the original patient's initials) can be detected frequently in the serum of children with or without hepatitis and do not cause viral hepatitis in children.

Hepatitis E virus infection

Hepatitis E virus (HEV) infection is endemic in south and central Asia and is transmitted through the enteric route. It is a major cause of epidemic water-borne hepatitis in tropical and subtropical countries in areas with poor sanitation. Seroprevalence in children can be as high as 75% in some countries. Signs and symptoms of hepatitis E do not differ significantly from those in other viral hepatitides. After an incubation period ranging from 15 to 60 days, the illness presents with a prodromal pre-icteric phase with symptoms of fever, anorexia, vomiting, abdominal pain, diarrhea, or constipation lasting 2–3 days. This is followed by an icteric phase lasting 10–14 days during which fever subsides and appetite returns to normal. Mild hepatomegaly is often present, as well as elevation of liver enzymes that persist for up to 6 weeks. Cholestatic symptoms with pale stools and itching can occur in up to 20% of cases. Non-hepatic manifestations include acute pancreatitis, immune hemolysis, thrombocytopenia and Henoch–Schönlein purpura (see Chapter 13). Diagnosis is by serology or PCR and a vaccine has been licensed in China, but is not available globally.

Orthotopic liver transplantation

Orthotopic liver transplantation (OLT) is now an accepted treatment for children with end-stage liver disease worldwide (see Chapter 31). As the 5-year actuarial survival rate is well over 90% for children in many international centers, many developing countries have successfully set up liver transplantation programs. This section discusses the indications, pre-transplant issues (including issues related to donors), post-transplant outcomes, and the challenges faced by OLT programs in the developing world and will highlight the differences from developed countries.

Indications for transplantation

Chronic liver disease

The main indication for transplantation in the developing world is chronic liver disease secondary to biliary atresia. The numbers of children reaching referral centers for Kasai portoenterostomy represent one-third of the estimated cases. In a recent paper looking at 100 solid organ transplants from New Delhi, out of 50 cases of liver transplantation, biliary atresia was the most common indication for such a transplantation (38%) (Figure 34.5) [32].

Acute liver failure

In children in the developing world, ALF is mainly due to infection or to drugs. Studies in India have reported acute viral hepatitis as the etiology in up to 75% of patients with ALF. Hepatitis A has been identified in more than 50% of cases of ALF, followed by multiple infections with hepatitis A and E. Experience in a center in New Delhi showed that out of 81 children with fulminant hepatic failure, 41 were identified as having hepatitis A, of whom almost 44% had glucose-6-phosphate dehydrogenase deficiency. Thirty-five children satisfied the criteria for liver transplantation, of whom 31 children died without a transplant, mainly due to multiorgan failure and sepsis. In the above-mentioned

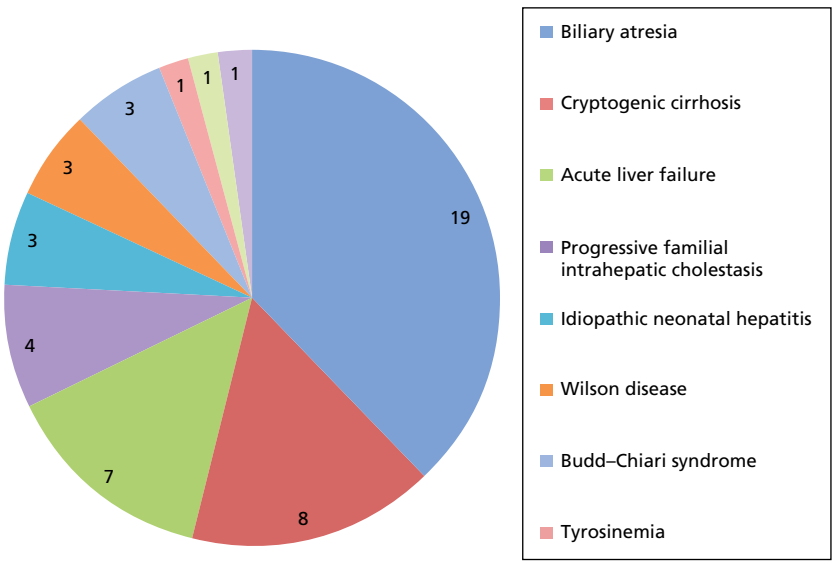


Figure 34.5 Indications for liver transplantation at Indraprastha Apollo Hospitals, Delhi. Biliary atresia is the commonest indication. The high proportion of cryptogenic liver disease, thought to represent metabolic liver disease, should be noted. (From Sibal *et al.* 2016 [32]. Reproduced with permission of John Wiley & Sons.)

study from New Delhi, ALF accounted for 14% of cases undergoing LT [32].

Others

The other important indications of OLT are cryptogenic causes, metabolic conditions, and PFIC. Wilson disease is the most common metabolic indication for a transplant (6%) in older children, followed by a recent increase in PFIC due to improved diagnosis. Drug-induced liver failure including accidental or non-accidental paracetamol overdose and the ingestion of native herbal medicines is not an important indication for OLT in the developing world.

Pre-transplant issues

Certain pre-transplant issues are unique to the developing world:

Infections

Hepatitis B infection is endemic in eastern and South East Asia, and although hepatitis B vaccination has been incorporated into many national vaccination programs, it is still not universally implemented. In countries such as Taiwan, Malaysia, Hong Kong, and Singapore, where hepatitis B vaccination has been implemented since the 1980s, chronic hepatitis B carriers are less common in the pediatric age group. However, even with hepatitis B vaccination in infancy, 5% of infants born to HBeAg-positive mothers fail vaccination and become chronic carriers. Thus, a small number of pediatric patients with end-stage liver disease are chronic hepatitis B carriers and may require a transplant in adulthood.

Pre-transplant management includes oral lamivudine, HBIg during the perioperative period, and then long-term oral lamivudine with reduced levels of immunosuppression. An alternative strategy is high-dose hepatitis B revaccination after transplantation, which has shown conflicting results.

TB is endemic in many developing countries, and childhood TB is not uncommon. Children with end-stage liver disease who are both malnourished and immunodeficient are more likely to be infected. It is well known that BCG vaccination during early childhood does not fully protect against the infection. Thus, it is essential to exclude TB by chest radiography, sputum culture, and the Mantoux test during the pre-transplant assessment. Family members should be screened as the child will be at high risk of infection during the immediate postoperative period, and potentially experience a fulminant course. Prophylactic isoniazid should be considered if there has been any such contact, including transmission via the donor graft.

Nutritional status

Malnutrition in liver disease is a common problem and improved strategies for supporting and improving nutritional status prior to transplantation have significantly improved post-transplantation outcomes. Severe malnutrition is a particular problem in the developing world and is compounded by late referral. The implications of liver disease for nutritional status and the focus on nutritional management are often underestimated. Thus, many pediatric patients in the developing world present with protein malnutrition, gross nutritional rickets, iron-deficiency anemia, and coagulopathy. In addition, many primary health-care pediatricians are not fully aware of the benefits and possibilities of liver transplantation in their own country and refer patients late.

Parental attitude

Many parents in developing countries are unwilling to accept liver transplantation as a viable treatment option. Poor education, fear of long-term medications, concerns about infections, and cultural factors contribute to rejection of this treatment option, particularly, in India, if the child requiring the transplant is a girl.

Donor issues

Survival rates with liver transplantation have improved with experience and medical advances, thus creating more demand for such procedures. The increasing demand has caused a shortage of cadaveric organs in the west and poses an even greater challenge in the developing world. Cadaveric organ donation is not widely accepted in many developing countries, due to lack of awareness and for religious and cultural reasons, and thus few national donor programs have been established. Lack of political agreement, poor communication, and variable standards of health care are major factors preventing the sharing of organs within different parts of the same country and so there are few networks for sharing donor organs.

In India, the Human Organ Transplantation Act was passed in 1994, and regulations were made in 1995 that recognized the concept of brain death and allowed cadaver organs to be removed for transplantation. In spite of this, there is a lack of knowledge and reluctance on the part of the public to donate the organs of their brain-dead relatives. There may also be lack of awareness among doctors, who do not approach the relatives of brain-dead patients with a request for donation. In addition, the lack of infrastructure and organizational difficulties compound the problem. However, recently, efforts to increase cadaveric donation have been successful in South India leading to increased donation.

The organ donation rates in general are lower in the developing world than in the west. In Singapore, where there is a well-established organ donation program, cadaveric liver donation is about 10 per million population, which is still lower than in western Europe and North America.

Living-related organ donation

In view of the difficulties with cadaveric donation, living-related donor liver transplantation is an essential option in pediatric transplantation in countries such as Japan, Korea,

Taiwan, Hong Kong, and India. Studies show comparable patient and graft survival rates in both living-related donor and cadaveric donor groups, with a trend toward less initial graft malfunction in living-related donor liver transplantation. The advantages and disadvantages are detailed in Chapter 31.

Ethical debates on living-related donor liver transplantation continue, with concerns about emotional pressure on a potential living-related donor. In addition, in countries like India, most organ donors are women, partly because of social and family pressure, or because the husband is the wage earner.

A strict pre-assessment protocol should be followed to ensure the donor is fit and not being coerced into donation. These include diagnostic tests of liver and other organ function, psychological support and counseling of the whole family. The donor must be aware of potential risks, which range from death to complications such as bile leakage, hemorrhage, infection, and postoperative pain. In recent years, attitudes towards donation are changing and now more fathers are coming forward to donate livers for their children [33].

In many endemic areas in the developing world, there is a high incidence of donors positive for antibody to the hepatitis B core antigen (anti-HBc). The exact incidence varies from country to country. In Singapore, anti-HBc antibody is detected in about 30–40% of healthy adults and in 53% of organs donors, while in Taiwan anti-HBc antibody is found in up to 80% of adults. Due to the shortage of donor organs, it is not possible to reject these anti-HBc antibody-positive donor organs, despite the risk of reactivating hepatitis B and infecting the immunosuppressed recipients, who have a high risk of fulminant hepatic failure. In Singapore, of the anti-HBc-positive grafts that have been transplanted, 18% of the recipients subsequently developed hepatitis B de novo and are receiving long-term antiviral treatment.

Pre-transplant preparation includes HBV vaccination if the recipient is not immune, post-transplant hepatitis B immunoglobulin, and/lifelong treatment with lamivudine.

In South East Asia, many donor grafts are rejected because of severe fatty infiltration. In Singapore, out of 98 consecutive cadaveric liver donors, 16 (16%) were rejected because of severe fatty change. Among 61 healthy living donors who volunteered for liver donation, nine organs (15%) were rejected for the same reasons. This is because of underlying obesity, the dietary pattern of Asians, and/or ingestion of native herbal medicine. Further research is necessary to solve this problem, but in the meantime it is a major limitation for obtaining grafts.

Organization of liver transplant program

In contrast to the rest of the world, many transplant programs in developing countries have been initiated by the medical professionals with or without institutional or government support.

It is essential to obtain governmental approval before establishing a transplant program, particularly when a living-related donor program is being considered. Before considering a transplant program, time and effort should be spent in training the multidisciplinary team, supporting staff and the team to manage all aspects of liver transplantation. Pre-transplantation care and assessment, immediate post-transplantation care, and long-term care are as important as the surgical technique. Liver transplantation is very much a team effort, and all the members of the team must be fully committed to the care of the patient.

Intensivists play a key role in the successful management of a transplant, particularly in the immediate postoperative period, when hemodynamic instability can adversely affect graft function. In developing countries, there are few well-trained intensivists, and many hepatologists may have to function as intensivists as well or rely on anesthetists.

It is also essential to have appropriate support services, especially good laboratory and radiology support. The laboratory must be equipped with up-to-date facilities and able to perform the essential tests over 24 h. These tests include routine liver and kidney function tests, coagulation tests blood counts such as drug monitoring (ciclosporin and tacrolimus), viral serology such as hepatitis B DNA, hepatitis C RNA, Epstein–Barr virus, and CMV polymerase chain reaction or antigen detection.

Adequate radiological imaging of the vascular and hepatobiliary system is crucial for both pre-transplantation and post-transplantation assessment. Interventional radiologists play an important role postoperatively in the management and treatment of many complications. Such expertise is now available in the developing world. Doppler ultrasonography, MRI, and CT angiography, although expensive, are now available in many centers in the developing world.

Post-transplantation outcomes

The outcome of OLT in developing countries varies. Few patients survive long enough on the cadaveric waiting list to receive a donor, and thus only countries with living-related donor programs are able to offer a viable procedure. In countries with relatively well-established programs, such as South Africa, Hong Kong, India, Singapore, and Taiwan, the graft and patient survival rates are acceptable, with actuarial survival ranging from 75% to 90%.

As in the west, ciclosporin and tacrolimus are the two main immunosuppressants used, but other agents such as mycophenolate mofetil, interleukin-2 receptor antagonists, and sirolimus have become available. According to one study from South India, the cost of immunosuppression varied from Rs. 28,705 (US$ 450) in the first month to Rs. 8820 (US$ 138) per month at the end of first year, amounting to an average monthly cost of Rs. 17,250 (US$ 270) [34]. According to their calculations, one-fourth and half of the cost was for mycophenolate and for drug level measurement of tacrolimus, respectively. This cost of immunosuppression after living donor liver transplantation in India is much lower than that reported elsewhere. This could be due to the use of lower drug doses and the availability of cheaper generic drugs.

Long-term survival depends on the availability of immunosuppression and the rate of intercurrent infections. With 5-year survival rates of 90%, India has now become a major center for liver transplantation for international patients as well because of the high-quality, low-cost value proposition. The average cost of a pediatric transplant in India is US$ 30,000. This is only about one-fifth to one-tenth the cost in the west. Age and size are no longer barriers as babies as young as 6 months have been transplanted. In fact now children from different parts of India and more than 20 countries have been transplanted.

To conclude, liver transplantation is now a well-established therapy for children with acute liver, end-stage liver disease, and a variety of metabolic disorders. The first successful pediatric liver transplantation in India was performed in 1998 and the child remains well 15 years post transplantation. With increasing acceptance of liver transplantation amongst the medical community and the public at large, there is now potential for the number of liver transplants to increase significantly so as to offer hope to the thousands of children who suffer from liver failure.

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SECTION 14

Pediatric Liver Disease in Adult Life

CHAPTER 35

Transition to Adult Care

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Key points

- Increasing numbers of young people with childhood or adolescent onset of liver disease, some of whom have significant morbidities, are surviving into adulthood and are requiring health care in adult services.
- Poor transition from pediatric to adult services can be a risk factor for poor clinical and psychosocial outcomes and increased health-care costs; conversely effective transitional care can maximize outcomes.
- Transitional programs should include delivery of knowledge and skills to young people and be individually tailored to meet the needs of each young person.
- Knowledge and skills training in adolescent health should be provided to both pediatric and adult health professionals.
- Pediatric and adult health teams should provide coordinated care to young people.
- Parents/carers should be involved in the transition process such that a balance is found between increased autonomy for the young person and appropriate parental involvement.
- Transition services should be routinely monitored and evaluated to ensure that they are effective and meet the needs of young people, families, and health professionals.

The previous edition of this book included the addition of a chapter about adolescence and transition to adult care, reflecting both the increasing numbers of young people with childhood-onset liver disease surviving into adulthood and the increasing need to recognize the specific needs of young people, their development into adulthood, and what this means for health-care services. Since the previous edition there has been further recognition of this important area with a focus on management and intervention with young people in transition and a consideration of the role of both parents and health-care professionals. However, despite progress in the field, transition from pediatric to adult care is still a risk factor for poor clinical and psychosocial outcomes and increased health-care costs. There are already some excellent reviews of transition [1, 2] so the aim of this chapter is to identify practical considerations and provide an outline of what a transition program should comprise and some ideas about how this might be achieved.

The incidence and prevalence of chronic conditions are increasing, with estimates that 10–20% of adolescents worldwide have a chronic health condition or complex disability [3]. Advances in medical therapies and technologies have resulted in growing numbers of young people with childhood or adolescent onset of illness surviving into adulthood, often with significant morbidities, resulting in the need for effective transitional care for young people as they move from pediatric to adult services. For both pediatric and adult health-care providers a key challenge is how to effectively collaborate so that the move from one service to the other is as seamless as possible for the young person, their families, and health professionals. Furthermore, as survival rates continue to improve, the importance of addressing not only physical outcomes but also psychosocial, educational, and vocational outcomes is being recognized, with further emphasis on the impact of the transition process itself on such outcomes. However, describing the process of transition into adulthood as one stage can be limiting as it tends to oversimplify this complex and diversely experienced developmental stage of life. This can be particularly problematic if it leads to generalizations about the adolescent population as this can then have a significant impact upon how services, including

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those related to physical and mental health, are designed, structured, and implemented. It is therefore important to recognize the complexities associated with the stage of adolescence to better understand the patient population.

Understanding adolescence

Adolescents can be challenging for both pediatric and adult health-care professionals and a lack of clarity surrounding concepts of adolescence and the changes that take place during that period, together with frequently held misperceptions and erroneous beliefs, further add to the reluctance that many professionals demonstrate when confronted with an adolescent patient. Contrary to commonly held beliefs, adolescents are neither older children nor younger adults but are developmentally, psychologically, and medically distinct from both children and adults. The World Health Organization defines adolescence as the period of growth and development occurring between childhood and adulthood, typically starting at around 10 years of age and continuing up to around 20 years old, with the focus being on the physical, psychological, and social stages of development which occur during this period rather than on strict age criteria [4]. The challenges for any adolescent, whether or not they have a chronic illness, include achieving the desired independence and autonomy whilst also maintaining close and supportive links with the nuclear family, creating a sense of identity and self-understanding through social relationships with peers, self-reflection, and future expectations. Further support for a definition of adolescence which is not based on chronological age comes from the evidence that key milestones of adolescence can be delayed in the context of chronic illness.

The adolescent brain

Understanding the development of the adolescent brain may help to explain some of the ambiguity of adolescence. Until relatively recently it was believed that the majority of human brain development occurred during the first few years of life and that changes plateaued after mid-childhood. However, advances in brain imaging technologies have highlighted that significant and substantial changes take place in the brain during adolescence and into the mid-20s, providing new and important insights into the development and behavior of adolescents.

Adolescence is a time of egocentrism, a change from concrete to more abstract thinking, and a time of experimentation and risk taking, whether a young person is healthy or has a chronic illness. Whilst brain development continues during adolescence it does not do so evenly, with areas controlling physical coordination, emotion, and motivation developing first. The limbic system is one of the main regions of the brain to develop in adolescence and is responsible for emotional responses, social interactions, memory consolidation, spatial orientation, and reward and pleasure. The development of the nucleus accumbens – that part of the limbic system involved in reward and pleasure – is particularly pertinent during this time. Adolescence is frequently identified as a period of particular vulnerability, with increased rates of risk-seeking behaviors compared to other age groups, and the continual development of the limbic system during adolescence helps to explain this pattern of behavior. The limbic system, which controls the feeling of reward experienced as a result of taking risks, develops at a quicker rate than the frontal lobes, which are involved in behavioral inhibition, the ability to control impulses and emotions, and the processing of cause and effect. Decisions therefore tend to be made on impulse as a risky choice has a strong emotional incentive and the immature prefrontal control system is unable to compete and influence the decision-making process behind the risk. The dissonance in the developmental trajectory of the different systems helps to explain why young people undertake certain behaviors which adults would consider risky or hazardous, which for the adolescent with health issues can also include behaviors such as non-adherence to medications, clinic attendance, or advice about health.

The development of the prefrontal cortex during adolescence and the subsequent impact on behavior is also an important consideration for understanding particular aspects of health care related to transition. The prefrontal cortex, which is involved in a range of higher level cognitive functions including decision making, planning, self-awareness, and social interaction, undergoes substantial growth and change during adolescence, particularly with respect to the processes of myelination and synaptic pruning. However, full maturation of the prefrontal cortex is not achieved until around the age of 25 years or older, which explains why some expectations of adolescents, such as planning future careers or vocations, planning when to fill prescriptions, take medications, or attend clinic or indeed planning the move from childhood to adult services, can be so challenging for them.

Impact of liver disease in adolescence

As with many other chronic health conditions, the demands of managing a liver disease in adolescence can have an impact on development. Continuous medical supervision and the daily use of medications may have implications for social and cognitive development, educational attainment, and quality of life. Quality of life is, for example, reported to be lower in adolescent liver transplant recipients compared to the general population and this is associated with psychosocial factors and the perceived burden of immunosuppression [5]. For the young person with liver disease there may be effects on their appearance of either the disease itself and/or the medication. Puberty may be delayed, growth may be stunted, and there may be other effects such as ascites or jaundice.

The disease and treatment may be unpredictable – particularly if liver transplantation has been or will be necessary – making it difficult for young people and their families to plan realistically for the future.

Illness and management of a chronic condition exaggerate the challenges of adolescence and may result in increased social isolation and delayed development of peer support networks, as well as delays in the achievement of biological and sexual maturation. Furthermore, the demands of managing a chronic condition, particularly one which necessitates regular medical follow-up and adherence to medication or treatment regimens, can make achieving the desired adolescent goal of autonomy difficult as the young person may have to depend on their parents to facilitate elements of their management. One developmental challenge for any adolescent is the need to establish a sense of self but the adolescent with a chronic condition has to cope with the additional challenge of integrating and accepting their identity as an ill person with other dimensions of their identity. If an adolescent's identity as an ill person overshadows other elements of their identity, self-management difficulties may arise.

Transition

General transitions for young people

Life for any one of us can be thought of as a series of transitions, some of which go well, others less so. Some transitions we choose and control, others are imposed on us, but adolescence is characterized by a number of important personal, social, family, and educational transitions happening in tandem – not only the transition from childhood to adulthood but also the move from family relationships to peer relationships; from platonic to intimate relationships; from school to further education and work; and from dependent to interdependent and independent living. Young people make these transitions at different times and speeds – some will move quickly, such as teenage parents living independently, whilst others may be on a slower pathway living with, and financially dependent on, their parents into their late 20s or beyond. Patterns of transition are clearly individual, irrespective of health status, but what is often overlooked is that the life transition goals for the young person with a chronic illness do not differ from those of a healthy young person.

Health-care transition

Against the backdrop of multiple life transitions in adolescence, a further layer of complexity is introduced for the young person with a chronic health condition who, in addition to the "usual" transitions of adolescence, has to embark on the often challenging journey from pediatric to adult health-care provision. Furthermore, whilst there is a widely held acceptance of the individuality of life transitions at this time, health-care systems and policy makers frequently impose rigid, age-determined criteria about transitions in health care, resulting in the transfer to adult care occurring in mid-adolescence when physical growth and puberty are incomplete and other transitions are yet to be started.

Within the specific domain of health, transition has been defined as, "a multi-faceted, active process that attends to the medical, psychosocial and educational/vocational needs of adolescents as they move from the child-focused to the adult-focused health care system" [6]. It represents a time of change, not only for the young person and their family but also for those providing their health care, although it is apparent that many young people have a rather different experience of transition which falls far short of the aspirations alluded to in the definition above [7].

One stumbling point is the confusion surrounding transition terminology, with professionals often misinterpreting transition as a single event of transfer to adult services. However, transition is a dynamic process, starting in the pediatric center and continuing on into adult-centered care, and within that the transfer to the adult center is but one constituent element. The beginning of this process involves the decision to start the preparation for transition and ideally should begin by early adolescence. During the middle phase the focus is on the readiness for transition of young people, their families, and health-care providers. The end of the process is when the young person has not only transferred to the adult center but is also actively participating in adult care activities such as decision making and self-management. The goal of transition is to provide young people with an individually tailored, developmentally appropriate, and efficiently coordinated program, with the ultimate aim of young people being able to function competently in the adult service. Key principles of transition are outlined in Box 35.1.

There is now general agreement that in transitional care there are more commonalities and similarities amongst young people with chronic conditions than differences

Box 35.1 Key principles of transition.

- Active, dynamic process
- Future focussed
- Young person-centered
- Inclusive of parents/carers
- Begins in early adolescence
- Focussed on a resilience framework
- Multidisciplinary, interagency
- Involves pediatric and adult services (health, social services, youth) and primary care
- Coordinated, uninterrupted health care
  - age appropriate and developmentally appropriate
  - culturally appropriate
  - comprehensive, flexible, responsive
  - holistic: medical, psychosocial, educational/vocational
- Skills training for the young person in communication, decision making, assertiveness, self-management

related to their specific disease or condition. Furthermore, there is little published evidence about transition in liver disease although it is acknowledged that some version of transitional care is likely to exist in the majority of liver disease services. A non-categorical approach will therefore be used in the remainder of this chapter, recognizing that young people with liver disease will face many of the same issues during transition as other young people with a chronic illness, albeit that some of the disease-specific consequences may be different. Clinicians and families also face common challenges, making a non-categorical approach to transitional care more efficient and effective than a disease-specific approach.

Equipping young people for transition

Risk factors for poor transition

Barriers to effective transition have been described in terms of five main concerns:

1. Vulnerability to risk-taking behaviors (including non-adherence).
2. Psychosocial barriers to transfer (attachments to parents/clinicians/services; parent/carer anxiety; reluctance of pediatric team to transfer care to adult service; unrealistic expectations of adult providers).
3. Lower rates of clinic attendance.
4. The cultural difference between pediatric and adult services (family centered and developmentally focussed versus patient-centered and focussed on independence and self-management).
5. The challenges from organizational continuity (such as lack of formal systems to support handover and poor communication between centers) [8].

Other risk factors may include aspects such as a change or lack of insurance coverage and other financial pressures as parental support reduces.

Some of the psychological, cultural, and organizational barriers to transition were highlighted in a recent qualitative study involving liver transplant recipients who were either within a year of transfer to adult services or who had already transferred. They identifed that young people found it difficult to end relationships with pediatric clinicians and to form new relationships with professionals in adult care. They also expressed frustration about a perceived lack of continuity of care after transfer and a fear of the unknown nature of adult services [9].

Knowledge and skills in a resilience framework

In order to provide optimal transitional care for young people it is important to understand the role of resilience in young people's capacity to cope with adversity. Resilience is acquired through experience when young people are encouraged to positively appraise their competence and belief in their ability to cope. Resilience has been defined as a "dynamic process involving an interaction of intrinsic and extrinsic processes of both risk and protection that act to modify the effects of an adverse life event such as illness" [10]. The key concept of a resilience framework underpins all aspects of adolescent health, including transitional care. In the context of chronic illness, and transition in particular, the attainment of relevant knowledge and skills plays a pivotal role in the development of resilience, as outlined below, and it is widely acknowledged that knowledge and skills training forms an integral part of any successful transition program [11].

Knowledge

Age-appropriate and developmentally appropriate information that is relevant and salient is an integral component of transitional care for young people. A wide range of issues related to specific aspects of their condition and treatment, the role of key health professionals involved or likely to be involved in their care, health behaviors, and factors related to living as an adult with their condition should be addressed with the young person through the period of transition. A structured education program is important to ensure that all topics are covered appropriately. For a number of topics information will need to be delivered over several sessions. Issues which should be addressed in an education program are outlined in Box 35.2.

As important as the content of the education program is the way in which the information is delivered. Methods of delivery need to be developmentally appropriate, a fact which can be overlooked, particularly in those situations where a young person has some degree of mild cognitive impairment. This latter fact may be particularly pertinent when providing care for some young people with liver disease. Education programs need to be tailored to the individual young person, in terms of what, when, and how information is provided. Information needs to be accessible to young people when they need it, in a format that they want, and it has to be perceived by them as relevant to their own particular generic and disease-specific health needs. Checking understanding is important and simple strategies such as asking a young person to explain to their parents what has been discussed or decided about their future management when they return after a one-to-one consultation can be useful for assessing what they have understood and retained. Use of self-completion checklists whilst a young person is waiting can be useful but these should be used in conjunction with a more active and interactive assessment of understanding and knowledge. Simply asking a young person to complete a checklist is insufficient in isolation. Over recent years there have been a number of innovative developments to help facilitate successful transition, one of which is the "My Health Passport". As part of their

Box 35.2 Key areas to be covered in an education program.

Condition and treatment, including effects on body, medical history, prognosis

- Diagnosis, previous surgery and treatments, co-morbidities and their significance – particularly for those diagnosed in early childhood
- Medication regimen including names, doses, side effects, rationale for use, risks of non-adherence, important drug interactions
- Purposes of tests and procedures
- Relevant medical terminology
- Specific issues – e.g., immunizations, antibiotic prophylaxis
- Future treatments/surgery – possible and probable

Role of individual health-care providers, what they do, and how to access their services

- Differences between pediatric and adult care
- Meaning of transition
- Health insurance issues, prescription charges at transfer, other changes in the mechanisms for providing care

Healthy lifestyle in terms of nutrition, exercise, emotional wellbeing, general health, dental health

- Impact of drugs, alcohol, and smoking on condition and therapy
- Impact of condition and therapy on sexual and reproductive health
- Impact of condition and therapy on education/vocation/employment opportunities
- Advice on tattoos, body piercing
- Advice on travel, sun exposure

Confidentiality, consent, and rights

- Understanding of rights to confidentiality
- Consent process for medical procedures as an adult
- Social support groups and community organizations
- Reliable sources of information on condition, therapy, general health, vocation, etc. (and unreliable sources to avoid)

Box 35.3 Skills for transition.

Health

- Feeling confident about seeing health professionals independently of parents
- Health information-seeking skills – ability to find reliable, accurate, relevant, and up-to-date information and developing the skills to distinguish from inaccurate information; using widely accessible resources appropriately (supported by professionals providing relevant resources)
- Accessing health care independently, including booking own appointments, contacting medical teams for advice (in both emergency and non-urgent situations), refilling prescriptions
- Awareness of own health – e.g., taking temperature, recognizing signs of intercurrent infection, measuring blood glucose
- Self-management of condition – specific tasks related to underlying condition – e.g., related to immunosuppression for liver transplant patients
- Adherence to medical regimen, advice about health behaviors, clinic appointments
- Pain management skills – including chronic pain associated with condition and procedural pain management (e.g., having some procedures without sedation or anesthetic)
- Fatigue management skills (including approaches for dealing with peer pressure to engage in activities that are too tiring and strategies for managing academic/employment demands)
- Emergency strategies
- Practical skills – e.g., urine and blood testing

Psychosocial

- Independent living skills – e.g., meal preparation, household chores, budget management, self-care, mobility (including independent travel away from home), driving, hobbies, and leisure activities
- Peer support, including independent social life
- Social competencies

Educational/vocational

- Communication skills
- Work experience
- Part-time job
- Disclosure to potential partners, employers

Good 2 Go Transition Programme, SickKids in Toronto, Canada, host a freely accessible web-based program for My Health Passport (https://www.sickkids.ca/myhealthpassport/ last accessed July 2016) which enables young people to complete a brief online form and print out a customized, wallet-size card which provides them (and others if necessary) with instant access to the key aspects of their medical information. This includes details of the underlying diagnosis and its presentation, medications, immunizations, allergies, hospitalizations, previous surgery, frequency of tests and investigations, and details of health professionals involved in their care. Health passports have been developed to be relevant to the condition, so there is one to complete for young people with liver disease which differs to the one for liver transplantation or cystic fibrosis. Completion of the information can be done by the young person independently but can also be undertaken interactively with a health professional, thus providing an opportunity for assessing a young person's understanding and knowledge and providing clarification as necessary.

Skills

Appropriate skills training in self-advocacy alongside the acquisition of relevant knowledge is key to the development of resilience in young people. Within the domain of health such skills training can be associated with improved medical and quality of life outcomes. Box 35.3 outlines important areas where skills for transition need to be developed.

Generic adolescent health issues

There are a number of recognized long-term morbidities of liver disease and transplantation, such as hyperlipidemia, osteoporosis, and premature arteriosclerosis, which mean that generic health issues such as nutrition, exercise, substance abuse, and alcohol take on more salience than for the adolescent who is not at risk of developing these disease- and/or treatment-related morbidities. Furthermore, at a time when obesity, decreased exercise, and increased alcohol consumption are issues for young people generally, it is important that those with liver disease are encouraged to adhere to advice about health behaviors. Mental health is another key component of transitional care, particularly in situations where there may be side effects of medication or of the disease itself on appearance.

Drugs and alcohol

Risk taking is one of the normal and adaptive behaviors used by adolescents to achieve the developmental tasks of identity formation, establishing relationships outside the nuclear family, and separation–individuation. Risk-taking behaviors are on a spectrum, ranging from age-appropriate relatively benign activities to those which are high risk, such as substance abuse. Adolescents with liver disease may not be any different to their healthy peers in terms of engaging in risk-taking behaviors, and indeed behaviors which put their health at risk are reported to be as common in adolescents with chronic illness as in the general population, but the consequences can be very different and potentially far more damaging for them. All recreational drugs affect the liver and should therefore be strongly discouraged, as should smoking. For patients with severe liver disease, alcohol should also be discouraged whilst for those with less severe disease, moderate alcohol intake is acceptable (within national guidelines). However, as well as providing advice about health behaviors it is also important to help young people develop appropriate coping strategies to deal with peer pressure concerning drug and alcohol intake, which should be included as part of a young person's individualized transitional care program.

Sexual and reproductive health

Sexual health is rated as a key issue of transitional care but is least effectively dealt with by health professionals [12], despite the evidence that young people with a chronic illness are as sexually active as their healthy peers. Whilst they have all of the same concerns as their healthy peers the young person with liver disease additionally has to deal with the impact of their underlying health condition and treatment on the development of their sexual identity. Evidence indicates that they want counseling and advice about contraception, fertility, pregnancy, and relationships [13]. However, it is also important that they are not only given appropriate knowledge (e.g., about how their condition or treatment may affect fertility, interactions between the contraceptive pill and any medications they are taking, immunosuppression during pregnancy, increased risks of sexually transmitted infections for patients on immunosuppression) but also the skills to deal with these issues in the context of their liver disease. As with all elements of skills training, it is important that health professionals assess young people's understanding of what they have been told, particularly in view of the sensitivity of the issues and the difficulties that some young people may have in asking questions or seeking clarification.

Adherence

Non-adherence to the treatment regimen has been conceptualized as part of the spectrum of risk-taking behavior, with estimates that 30–50% of adolescents with a chronic illness are non-adherent to some element of their treatment protocol [14–16]. It is also reported that non-adherence is associated with other risk-taking behaviors such as substance use, highlighting the importance of taking a thorough psychosocial history as part of transitional care. Regular use of screening tools such as the home, education/employment, eating, activities, drugs, sexuality, suicide/depression, and safety (HEEADSSS) tool [17] can be useful for determining risk as well as providing important information for formulating interventions to improve adherence and management. The importance of including mental health screening routinely was emphasized in a recent study of liver transplant recipients who had been followed up for a year after transferring to adult services [18]. In this study, psychological distress and medication non-adherence were significantly correlated and there was a significant interaction between adherence status and changes in mental health from pre- to post-transfer. Whilst it is easy to take a judgemental approach to non-adherence it is important for health professionals to gain an understanding of the challenges faced by the individual adolescent in terms of their prescribed medications (number, frequency), requirement for follow-up and monitoring, and impact of the disease and treatment on their daily lives. Treatment protocols can interfere with many aspects of life, such as peer and social interactions, personal freedom, spontaneity and leisure time, and remaining adherent can be particularly challenging when the young person feels well. Becoming non-adherent may be one way in which a young person can exert some control and may signal their wish to have a voice in the decision-making processes regarding their health and their future. Whilst self-medication is a key element of becoming an independent young adult, it has to be seen in the context of shared decision-making, self-care, and self-management. In the field of liver disease, non-adherence has been assessed in liver transplant recipients, with one study identifying that three-quarters of adolescents were non-adherent on at least one measure of adherence [19]. Non-adherence is a major cause of graft loss or

rejection in adolescent liver transplant recipients and accounts for 17% of liver graft dysfunction [20]. Within this context, transition to adult services has been highlighted as a particular risk factor for non-adherence, and there is evidence to suggest that adherence to medication, clinic visits, and medical advice significantly decreases after transfer to adult services [21], with an increased incidence of graft loss [15]. Such evidence highlights the importance of a good transfer process and skilled adult health providers who understand, and are sympathetic to, the range and complexity of generic and health-related issues faced by young people with liver disease.

Transition readiness

It is widely accepted that the process of transitioning to adult care should start early in adolescence and involve individualized knowledge and skills development (see earlier) [22] and that the timing of transfer to adult services should be based on a young person's readiness and ability to negotiate the adult health-care system. However, evaluation of an individual's readiness and acquisition of the required skills has, until recently, been impeded by the lack of validated tools to assess these parameters. Whilst there are a number of checklists in use in various transition programs, many of these have a yes/no response to questions and do not enable an assessment of the different stages of readiness that can exist prior to skill acquisition and mastery. To address this gap, the Transition Readiness Assessment Questionnaire (TRAQ) has been developed [23], a generic, practical tool with a strong theoretical underpinning which measures skills needed to successfully transition from pediatric to adult health care in the domains of self-management and self-advocacy. Other disease- and condition-specific tools have also been developed, including one for liver transplant recipients [14] which measures perceived and demonstrated self-management skills, allocation of responsibility for health-related tasks, regimen knowledge, and psychosocial adjustment, with patients responding using Likert scales.

It was recently highlighted that almost 80% of adult gastroenterologists believe that the preparation of young people transferred to adult care is inadequate [24] and that patients are not demonstrating transition readiness on arrival in adult care. One practical solution to this scenario would involve the routine use of a measure such as the TRAQ, which would enable professionals to assess a young person's skills, identify and address areas where education is required, and monitor progress through the period of transition. In a recent evaluation of a transition readiness skills program, liver transplant recipients who had participated in the program were more likely to be adherent with medication and to attend their first adult clinic than those who did not participate [25]. Furthermore, better adolescent and parent knowledge about the treatment regimen was associated with better adherence to post-transfer clinic appointments.

Knowledge and skills training for pediatric and adult health-care professionals

Adolescents can be challenging to work with (see McDonagh & Kaufman [26] for an excellent overview of "the challenging adolescent") but the value of the therapeutic alliance between health professionals and young people, whether in adult or pediatric services, should not be underestimated. Adolescents have identified that provider characteristics are significantly more important than the physical environment and process issues in determining their satisfaction with a transitional care program [27], so it is important that staff (both pediatric and adult) receive appropriate training and updates to develop and maintain their skills and knowledge of adolescent health. Training opportunities are becoming more widely available and should cover areas such as adolescent development, the wider impact of chronic illness on the adolescent (physical, psychosocial, environmental), ethics, confidentiality, cultural competencies, dyadic versus triadic consultations, and the differences in the provision of pediatric and adult services. However, training is far from universally available so it is perhaps not surprising that less than half of adult gastroenterologists report feeling competent to deal with developmental issues of adolescents [28].

Pediatric providers can be reluctant to transfer patients to adult care, possibly due to an unwillingness to relinquish care of a young person whom they have cared for over many years or because they are unconvinced that an adult team is able to effectively manage a former pediatric patient. Such ambiguity may be manifested as mixed messages to families, for example related to the timing of transfer and changing previously agreed dates or by indirectly communicating reservations about adult care to families. If this occurs young people and their families are more likely to feel confused and may become increasingly ambivalent themselves about transferring to adult services. Training for pediatric providers should therefore include knowledge about the impact of their own behavior and attitudes on young people and families as well as gaining an understanding of the adult services who will be taking over the care of their pediatric patients and their model of care provision.

A further consideration for adult providers is their unfamiliarity with specific diseases which are usually thought of as only affecting children, due to the poor prognosis previously associated with these conditions and the unlikelihood of children surviving into adulthood. This applies to liver diseases such as metabolic liver disease, biliary atresia, and cholestatic diseases such as Alagille syndrome, where young people are transferring to adult care having completed their education and undergone puberty with conditions which are often "new" for adult hepatologists. Similarly, whilst adult professionals may have a lot of experience of adult transplant recipients, they are less used to those patients who are now adults but were transplanted as children or to the young adult who

now requires transplantation for a chronic condition of childhood, with its associated long-term endocrine and emotional challenges. Gaining appropriate knowledge and training in "pediatric" conditions and their long-term consequences is therefore an important requirement for adult providers of transitional care.

Parents/carers and transition

Part of normal development involves young people becoming more autonomous and parents being able to "let go" and this is also true for transition to adult services, where parents need to be ready for their child to leave pediatric services. Transition to adult services can be an anxiety-provoking and emotional time, particularly when parents have had a long-standing relationship with the pediatric team and have built up trust in them over many years, and there is evidence from other chronic illnesses that parents find the process of emerging adulthood, the acquisition of autonomy, and transfer to adult services challenging [29]. Parents and carers have had a key role in the management of their child's liver condition from diagnosis and therefore have an emotional vested interest. Parents are key facilitators of their child's transition to adulthood and play an essential role in the move towards independent self-management. Often, parents or carers report feeling excluded or sidelined during the transition process, particularly by adult services who adopt a patient-centered model of health care as opposed to the family-centered model usually seen in pediatrics. This perceived rejection can be difficult for parents who may in turn overcompensate and display an unwillingness to "let go."

Rather than excluding parents or carers from the process of transition, they should be viewed as an asset to help facilitate successful transition. A key component of successful transition is the ability to self-manage. Parents and carers play a vital role in supporting their child's acquisition of self-management skills. There is an important balance to strike: withdrawing support too early may result in the young person being unable to manage on their own, resulting in a negative transition experience and poorer health outcomes, but being overprotective may have similar results as the young person is not given the opportunity to acquire positive management skills at the right time, resulting in maladaptive coping strategies later in life. Moreover, overprotective parenting conveys to the young person that their parent does not think they are mature enough or can be trusted to manage their own care. It is therefore important that a balance is found between increased autonomy for young people and appropriate parental involvement.

Parents also experience a transition of their own as their child navigates emerging adulthood. This transition is complicated by the presence of a long-term condition which the parent has historically had a significant role in managing as they are now expected to relinquish some of their parental authority and facilitate their child assuming more responsibility for their own care. Consideration should be given to the impact of a parent's way of coping with transition on their child's experience of transition and their subsequent behaviors and health outcomes. Parents who adopt maladaptive coping strategies may engender similarly maladaptive coping styles in their children, resulting in a negative impact upon the transition experience, but meeting parents' specific information needs, which is an important adaptive coping strategy, can help reduce feelings of anxiety and uncertainty and help parents regain a sense of control [30], with a resulting positive impact on their child. Acknowledgement of the crucial role that parents have in the transition process and providing appropriate support, knowledge, and skills are important components of a successful transition program.

Key components of transitional care programs

Since the previous edition of this book more detailed descriptions of transition programs have appeared in the literature for conditions such as diabetes but recent evidence suggests that formal transition programs are uncommon in adult transplant hepatology clinics [31], with little mention of transition at all in the published literature on non-transplant hepatology. From the generic chronic illness literature and the plethora of policy documents to guide transitional care provision, a number of key elements for successful transition have been identified (Box 35.4).

More recently a benchmark for transition from child to adult services in the UK [32] has been developed, comprising eight factors with statements for best practice and indicators of best practice provided for each factor. The benchmark was developed in an iterative three-stage process involving workshops, focus groups, and interviews with key stakeholders, including young people with long-term health conditions, parents, and health professionals working with young people. The final list of factors in the transition benchmark, together with the corresponding statements of best practice for each factor, are shown in Table 35.2. In the UK, at least, the hope would be to evaluate individual transition programs against the benchmark so that performance can be judged and areas for improvement identified.

Evidence to support transition

Despite the large number of transition programs that have sprung up in recent years there is relatively little published evidence of their effectiveness. In a systematic review, conducted in 2010, addressing the effectiveness of transitional care programs for young people aged 11–25 years with chronic physical

Box 35.4 Key components of an effective transition program.

- Dedicated clinics (either joint pediatric/adult clinics in the pediatric center and/or young adult clinics in the adult center)
- Knowledge and skills training for young people, to include regular evaluation
- Assessment of transition readiness (including transfer not occurring during a medical "crisis" or at other key transition times such as starting university) using a validated tool such as TRAQ
- Written transition policy – developed and agreed with all key players; should include a flexible timeline to accommodate heterogeneity of adolescence, potential impact of chronic illness on adolescent developmental processes, and developmental delay – but transition planning should start early (11+ years)
- Transition program – to meet the needs of young people but also their parents/carers; should include individualized transition plans and use of health passports (see Table 35.1 for websites providing guidance, resources, and examples of templates)
- Transitional care coordinator – diverse role to include clinical expertise, education, leader for change, and "champion" of transition
- Involvement of interested adult service – engagement of adult providers in service development is vital for success of transition; young people want to meet their adult doctors prior to transfer
- Multiagency involvement, including primary care – to ensure provision of holistic care; primary care may be key in providing continuity during the period of transfer and have an important role for addressing broader health needs and encouraging skills for appropriate health-care utilization
- Involvement of young people and families
- Professional training of pediatric and adult staff
- Administrative support and effective communication between providers – the importance of relevant documentation is often underestimated but is crucial for effective transition; fear of poor information exchange is a recognized concern of young people and parents and can hinder the transition process; information to transfer to adult providers should include:
  - diagnosis of primary condition and date diagnosis made
  - diagnosis of secondary or other conditions (including mental health)
  - location and severity of disease; symptoms; impact of symptoms
  - surgery, results of interventions, and any complications
  - medications – doses, duration, efficacy, adverse reactions, monitoring arrangements; any adherence concerns
  - involvement of other specialists (including allied health)
  - role of parents
  - key social or psychological considerations (including any cognitive or developmental issues)
  - primary care involvement
  - any plans for management already discussed with young person
- Evaluation and audit

Table 35.1 Transition websites (including some with example templates of transition plans, health passports, and proformas for information transfer).

Transition guidance/program/resource	Description	Website (all last accessed July 2016)
Published guidance for health professionals		
Royal College of Nursing (UK): Adolescent transition care - guidance for nursing staff	A guide to help practitioners achieve a seamless transfer using a national clinical pathway framework	http://www.rcn.org.uk/__data/assets/pdf_file/0011/78617/004510.pdf
Care Quality Commission (UK): From the Pond into the Sea – Children's transition to adult health services	A review looking at the transition arrangements made for young people with complex health needs as they move from children's to adult services	https://www.cqc.org.uk/sites/default/files/CQC_Transition%20Report.pdf
The Department of Health (UK): Quality criteria for young people friendly health services	Guidance setting out principles to help commissioners and service providers to improve the suitability of NHS and non-NHS health services for young people	https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/216350/dh_127632.pdf
Existing programs and useful resources		
Good 2 Go Transition Program: The Hospital for Sick Children (SickKids), Toronto	A program to support young people through transition based on a shared care model with many useful resources available to view and download (including My Health Passport)	http://www.sickkids.ca/good2go/
The Royal Children's Hospital Melbourne, Australia	A transition program featuring detailed guidance for young people, parents, and health-care professionals and factsheets and tools available to view and download	http://www.rch.org.au/transition/
Ready, Steady, Go transition program: Southampton Children's Hospital (UK)	An easy to use program to help prepare young people to feel confident to move to adult services. This program is available for adoption by other centers with permission from the program lead	http://www.uhs.nhs.uk/OurServices/Childhealth/TransitiontoadultcareReadySteadyGo/Transitiontoadultcare.aspx
General resources		
Got Transition/Center for Health Care (USA)	A project between the Maternal and Child Health Bureau and The National Alliance to Advance Adolescent Health (USA). Contains useful transition information, tools, and resources	http://www.gottransition.org/index.cfm

Table 35.2 Factors and statements of best practice in the transition benchmark.

Factor	Best practice
Factor 1: Moving to manage a health condition as an adult	Young people offered advice and information in a clear and concise manner about how to manage their health condition as an adult
Factor 2: Support for gradual transition	As they progress through the transition process, the young person is gradually prepared and provided with personally understandable information and support
Factor 3: Coordinated child and adult teams	The young person is supported through a smooth transition by knowledgeable and coordinated child and adult teams
Factor 4: Services are "young people friendly"	Young people are provided with care in an environment that recognizes and respects that they are a "young person" not a child or adult
Factor 5: Written documentation	Concise, consistent and clear written document containing all relevant information about the young person's transition is provided to the teams involved in the transition process
Factor 6: Parents	Parents are included in the transition process gradually transferring responsibility for health to the young person
Factor 7: Assessment of "readiness"	The young person's readiness for transition to adult care is assessed
Factor 8: Involvement of the general practitioner	The young person's general practitioner is informed of the plan for transition and is able to liaise with other relevant teams to facilitate services requested/needed by the young person

or mental health or disability, only 10 studies were identified which met the following criteria: involved a health service intervention during the period of transition; evaluated changes in health outcomes following transfer; and compared outcomes either between an intervention or control group or used a before and after design in a single group [11]. Eight of these studies involved patients with diabetes mellitus, one was of patients with cystic fibrosis, and one involved kidney transplant recipients. No studies involved patients with liver disease. Significant improvements in outcomes were shown in six of the studies (all involving patients with diabetes) but overall, studies were of poor methodological quality and involved interventions with multiple components, which made it almost impossible to determine the effectiveness of particular elements. Interventions were broadly defined as focussing on the patient (disease-specific education, generic education/skills training), staff (joint pediatric and adult clinic, named transition coordinator), or service provision/access (separate young adult clinic, enhanced follow-up, out-of-hours telephone support), with patient education strategies and specific transition clinics being the most commonly used strategies. However, it is not clear how generalizable the results are to conditions other than diabetes, nor how sustainable the observed changes in health outcomes are likely to be.

Other studies which do not meet the criteria of the Crowley review but have nevertheless looked at evidence to support transition have identified improvements in follow-up, disease control, documentation of adolescent health issues, satisfaction for young people, and adherence to treatment after transfer. In a recent study, greater satisfaction with transitional care was found to be significantly related to better social and emotional quality of life [33], although follow-up was relatively short (1 year) and the components of transitional care were not described. However, many issues remain unclear, such as the extent to which different models of transitional care result in equivalent medical and psychosocial outcomes, what constitutes "successful" transition and whether most transition issues are generic. Moreover, the field of liver disease lags behind those of diabetes, rheumatology, congenital heart disease, and organ transplantation, amongst others, in evidencing its practice regarding transitional care and the potential benefits of it.

Whilst it is generally acknowledged that monitoring and evaluation should be part of any transition program, identifying appropriate standards and outcome measures has been more challenging. Box 35.5 lists proposed standards and outcomes that should be monitored and evaluated as part of a transitional care service [7].

Box 35.5 Proposed standards and outcomes for monitoring and evaluating transitional care.

Quality standards

- Measure services against quality criteria for young person friendly health services, such as the "You're Welcome" criteria [34]
- Measure services against standards set out in national transition documents
- Include opportunities for the patient to be seen alone, transition planning, and psychosocial screening and transfer summaries

Effectiveness and safety

- Attendance outcomes
- Attended first two appointments in adult service
- Engaged with adult services 1, 2, 3 years after transfer

Clinical outcomes

- Markers of condition/disease control
- Complications of condition/disease
- Unplanned hospital admissions

Non-clinical outcomes

- Living independently
- Education/vocation

Patient-reported outcomes

- Assessment of disease/condition knowledge
- Assessment of self-management and advocacy
- Assessment of quality of life

Experience

- Young people's and their carers' satisfaction with transitional care

Conclusion

The majority of young people with liver disease will now survive into adult life but their ongoing requirements for health care will necessitate their transition from a family-centered pediatric service to the world of patient-centered adult care. Although there has been a rapid growth in transition services for young people with a chronic illness in the last few years, with a strong professional consensus on best practice for successful transition, for many young people transition still remains far from smooth. It is therefore important that attention should continue to be given to ensuring that the process of transition itself does not adversely affect young people in terms of their physical, emotional, social, and vocational outcomes. There has been a recent shift in approach from a focus on finding answers to the question of how best to manage transition (in a medical model paradigm) to an increasing emphasis on how the needs of young people are best met as they move through adolescence into adulthood. This change in approach, which involves a more holistic approach to understanding adolescents, will result in an improvement in outcomes for young people across all areas of their lives, thus allowing them to maximize their potential despite their health condition. Transition services require routine evaluation in order to ensure services are, and continue to be, effective and meet the needs of young people, families and health professionals. As part of this, it is important to capture young people's experiences of transition services as part of ongoing service development, monitoring and evaluation and to ensure that outcomes are reviewed and acted on as appropriate.

Case studies

Case study 1

KC was diagnosed with biliary atresia and had a successful Kasai portoenterostomy in infancy. He remained well throughout childhood; however, liver biopsies in adolescence identified significant cirrhosis. At 15 years old he was transplanted with a whole liver graft due to cirrhosis and portal hypertension. He remained well for 2 years but clinicians became increasingly worried about his psychological health during routine clinic visits based on disclosures by KC and his family of non-adherence to medications, aggressive behavior, excessive drinking, and low mood. As a consequence, he was referred to a psychologist where he was seen for an initial appointment but due to lack of engagement he received no further psychological intervention. Routine blood tests revealed erratic tacrolimus levels and deranged liver function tests indicative of non-adherence; subsequently, he was admitted for an episode of acute rejection. The importance of taking his medications was emphasized by clinicians. At this time KC was 17 and it was decided it would be best to transfer him to adult services in accordance with hospital policy.

KC attended the first few appointments in adult services and tacrolimus levels were initially stable. Subsequently, he failed to attend any outpatient clinic appointments and his general practitioner was unable to contact him to collect blood levels locally. He eventually presented in an accident and emergency department 18 months later with severe jaundice and abdominal pain. He was admitted to the liver ward for 4 days and treated for acute rejection. Over the next 5 months he continued to be followed up in the outpatient clinic every few weeks with intermittent hospital admissions for chronic rejection and abdominal pain during this time. Tacrolimus levels continued to be erratic. Despite having a history of mental health difficulties and significant non-adherence, no attempts were made to refer KC to an adult psychology service. After a 10-day admission for management of end-stage liver disease it was decided that re-transplantation was not an option due to the severity and deterioration of his condition. He was diagnosed with terminal graft failure 2 months later and transferred for end-of-life care and died 3 days later.

Comment

The timing of transfer is a key component of transition planning. The evidence supports an individual approach to the timing of transfer rather than the use of rigid age limits or chronological age as the justification for transfer. Unfortunately, hospital policies and funding arrangements often mean that services are under pressure to transfer young people at 16 when they are deemed "adults." Instead, timing of transition should be dependent on the developmental readiness and health status of the young person. It was not ideal to transfer KC at a time when he was psychologically vulnerable, particularly as this was impacting upon his adherence to medications which had serious consequences for his graft and long-term health. Transfer at a time of any medical instability is ill-advised. It would have been better to attempt to engage KC in a psychological intervention prior to transfer and to put in place some strategies to improve his medication management, particularly as multidisciplinary support in the adult setting can be limited.

At the time of transfer there was no formal transition planning in place to facilitate transition from pediatric to adult services. Consequently, KC received no transition preparation prior to transferring to adult care. Ideally, a transition program designed to prepare young people for adult services and equip them with the skills and knowledge would have begun early in adolescence and incorporated elements to foster self-management skills and independence such as beginning self-medication or attending part of the clinic appointment alone. Non-adherence was highlighted as a problem for KC prior to transfer. Given that non-adherence is the main cause of graft loss after transfer has taken place in this population, a transition program designed to assess and

build self-management skills would have been helpful for KC. Implementing support for the issues surrounding non-adherence while still under pediatric care would have allowed more time to provide support prior to transition taking place.

KC's case presents potential training opportunities for health professionals involved in transitional care. Having a wider understanding of the psychosocial context of young people is essential in order to both build a relationship and understand behavior. Health professionals can become impatient with young people who they suspect are not taking their medications and who they often label as "non-adherent" or "non-compliant" with little investigation regarding the reasons why. In the current case there were some important clues which could have helped to predict an unsuccessful transition which, with hindsight, would have been helpful to identify. We know, for example, that psychological vulnerability is a potential risk factor for transition. Highlighting psychosocial issues at an early stage to allow time for intervention and the knowledge and skills required for transition readiness to be acquired is vital. Furthermore, recognition of the importance of psychological health in the context of overall health and wellbeing is essential in order to ensure good long-term outcomes for young people with liver conditions.

Case study 2

DG was diagnosed with biliary atresia and had a successful Kasai portoenterostomy at 12 weeks old. He had a whole graft liver transplant at 4 years old and was then maintained on tacrolimus and prednisolone. His 15-year post-transplant assessment showed normal hepatic function with no evidence of autoimmune disease and good renal function.

As DG reached adolescence he was seen in the adolescent clinic where he had regular contact with the designated transition nurse at his outpatient appointments. During these appointments he would spend time alone with the transition nurse and worked through a planned transition program, designed to help prepare young people for transition.

When he was nearly 18 years old discussions were held with DG and his parents regarding transfer to the adult liver unit. At the time he was medically stable and had successfully worked through the transition program. However, he was due to start university that year which involved moving away from home and living independently from his family for the first time. It was therefore decided that it would be best for DG to wait until he had begun university before transferring to adult services. He was transferred to adult services at the age of 19 where he attended a handover clinic in the outpatient department of the adult hospital. This handover clinic was led by his new consultant but was also attended by both his pediatric consultant and the transition nurse, whom he had seen regularly in pediatric care, to facilitate transition preparation. Following transfer he continued to be seen in the "transition clinic" where he would be followed up by the same transition nurse until the age of 25. Clinically there were no concerns following transfer and he routinely attended all of his appointments. He continued to study at university.

Comment

This second case study was chosen to highlight how a planned, coordinated transition program which supports the medical, educational, vocational, and psychosocial needs of the patient can impact positively not only on the transition experience but also on patient outcomes.

The evidence supports the need for formal transition planning as set out by a written transition policy. Successful transition planning involves starting the process early, to maximize the opportunity to acquire the skills and knowledge necessary to manage transition. DG transferred to adult services at a time when a transition program had been implemented into the service based on evidence regarding best practice. This program included designated adolescent clinics, encouraging young people to be seen independently of parents to encourage autonomy, a key worker or transition nurse working across sites to facilitate continuity of care, transition readiness documentation to assess knowledge and skills and joint handover clinics in the adult setting. The timing of transfer was carefully considered by the multidisciplinary team and the importance of education and psychosocial wellbeing was highly valued. Acknowledging that DG had other significant changes happening at the time of intended transfer meant that transfer was planned for a time which was more appropriate and therefore maximized the potential for a positive transition experience.

DG's case demonstrates the importance of incorporating key workers to facilitate transition and provide continuity of care. Using this approach means that young people can become gradually familiarized with adult services with the security of clinicians they know who gradually withdraw involvement as the young person adapts and enters adulthood. Staff working across pediatric and adult settings can also be an important resource for imparting knowledge and training about adolescent health and transitional care.

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CHAPTER 36

Pediatric Liver Disease: Surviving to Adult Life

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Key points

- Advances in therapy mean that significant numbers of children with previously fatal liver disease are surviving into adult life.
- Adult physicians need to be aware of the clinical management and complications of diseases originating in infancy.
- Adult physicians need to be familiar with the long-term consequences of liver transplantation in childhood.
- Survivors of childhood illness require a different approach to other young adults.
- Developing adequate transitional care for these young people is based on effective collaboration at the pediatric–adult interface.

Biliary atresia

Extrahepatic biliary atresia is a disease of unknown etiology with no proven genetic basis. It occurs in approximately 1 : 15,000 live births. There are two clinical phenotypes, a syndromic form (20%) that is associated with other congenital anomalies including situs inversus, cardiac anomalies, and the absence of the inferior vena cava. The acquired form is more frequent and accounts for 80% of cases. The disease is characterized by a progressive inflammatory obliterative process involving the extrahepatic bile ducts. Untreated the disease leads to liver failure and ultimately death in the early months of life. Surgical techniques such as the Kasai operation and liver transplantation have led to improved outcomes in this condition.

The hepatoportoenterostomy or Kasai procedure was first performed in 1959 [1]. It involves removing any extrahepatic bile ducts, exposing the porta hepatis and attaching a segment of the small intestine to this area. This allows drainage of the intrahepatic ducts into the small intestine but depends on the extent of damage to the intrahepatic bile ducts. Age at surgery, histology of the excised bile duct, and surgical expertise are all factors that influence the outcome of surgery.

Outcomes

Center volume impacts on outcome after the Kasai procedure. The UK now has centralized management of biliary atresia after a paper demonstrated significantly differing short- and long-term outcomes associated with case load [2]. In a recent review of UK practice the outcome of 148 infants with biliary atresia was audited [2]. Between 1999 and 2002, 148 patients with biliary atresia were treated. A Kasai was performed in 142 and primary liver transplant in five. Jaundice cleared in 57% of cases after Kasai and of the 135 patients that survived 62% still had their native liver with 38% having received a liver transplant. Overall 4-year estimated survival was 89%, an improvement on the pre-centralized era.

Most articles describing long-term outcomes describe only small case series of patients. A recent article grouped 14 studies together and thereby included 184 above the age of 20 years [3]. Of note 88% were still alive with their native liver but 61% were suffering from complications related to their liver. The majority of survivors have cirrhosis and portal hypertension and complications included cholangitis, variceal hemorrhage, and HCC (HCC). The majority of patients with complications suffered from attacks of cholangitis and half had a variceal bleed whereas HCC was uncommon affecting only one patient in the cohort. Surviving patients with their native liver have normal fertility.

Preventing and managing complications

Despite undergoing a Kasai procedure many adult patients have a secondary biliary cirrhosis. This means that they are at risk of episodes of cholangitis. Patients should be warned of this and advised to attend hospital for intravenous antibiotics

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if an attack occurs. Prophylactic antibiotics can be a helpful strategy in patients with recurrent attacks of cholangitis. All patients with cirrhosis ought to be screened for varices on a 3-yearly basis. If found primary prophylaxis should be commenced either in the form of non-selective β-blockade or variceal band ligation.

Finally there is not enough evidence to describe the true incidence of HCC in this cohort therefore one would advise an ultrasound and α-fetoprotein (AFP) in cirrhotic patients on a 6-monthly basis. Some patients who have survived to adult life with their native liver may require liver transplantation.

When to consider transplant assessment in adulthood

There is little to guide the clinician when it comes to timing of transplantation in this cohort, due to the relative novelty of their survival to adulthood. However it is known that this group has excellent long-term outcomes after transplantation therefore early assessment and listing is crucial. The standard indications for liver transplantation are used (see Chapter 31) and assessment is required in patients with persisting jaundice and recurrent episodes of cholangitis, decompensated cirrhosis or malnutrition.

Autoimmune liver disease

Autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC) are all autoimmune diseases that differ in their presentation, epidemiology, and response to treatment. They have a variable but usually slow natural history and as they are rare diseases are challenging to study (see Chapter 11). Unlike the other autoimmune diseases PBC is rarely seen in childhood.

Autoimmune hepatitis

AIH is characterized by an elevation of immunoglobulin G (IgG), typical antibodies, and histological features of interface hepatitis. There are two typical types seen in childhood: type 1 with antinuclear and antismooth muscle antibodies, and type 2 with anti-liver–kidney microsomal antibodies. There is a wide clinical spectrum seen in childhood ranging from acute liver failure entailing transplantation to a very mild form of the disease. Type 2 AIH presents more aggressively, at a younger age, and commonly with IgA deficiency. However, duration of symptoms prior to diagnosis, family history of autoimmunity, presence of associated autoimmune disorders, response to treatment, and prognosis are similar in the two groups [4]. Of note type 2 AIH is present in 20% of cases of the autoimmune polyendocrinopathy candidiasis ectodermal dystrophy syndrome.

The adult system of diagnosing AIH is not suitable for children due to lower titers considered to be positive in this population and the requirement for cholangiography to exclude sclerosing cholangitis. Most patients respond well to immunosuppression even in the presence of cirrhosis.

Once in adulthood patients should be managed as per adult guidance with regular review of liver function tests and immunology. Any cirrhotic patients should undergo HCC and variceal surveillance as per international guidelines. Finally there are now a number of reports of patients with classical type 1 AIH diagnosed in childhood evolving into PSC over time. Therefore poor response to treatment, cholestatic liver tests, and the onset of inflammatory bowel disease (IBD) should trigger investigations of the biliary tree. If PSC is diagnosed then cautious reduction in immunosuppression is recommended.

Primary sclerosing cholangitis

PSC is a progressive inflammatory/obliterative disease affecting the bile ducts. At present there are no effective treatments for this disorder other than liver transplantation. It primarily affects adults but cases are reported in children. The majority of patients will have associated IBD and even patients without symptoms of IBD should undergo a screening colonoscopy with biopsies. Once the patient reaches adulthood the management is similar to adult patients with the disease.

Patients are at a higher risk of biliary tract malignancy and bowel cancer. It is therefore recommended that patients undergo a yearly ultrasound and that any suspicious lesion within the gallbladder is removed if the liver disease permits [5]. Furthermore due to the high risk of bowel cancer all patients should be offered a yearly screening colonoscopy. For those patients who have undergone colonoscopy under general anesthetic as children the transition to sedation-led procedures in the adult arena can be challenging.

Autoimmune sclerosing cholangitis

An overlapping syndrome of AIH and sclerosing cholangitis has been reported in children. In many cases the AIH comes first with PSC presenting later. In contrast to adult PSC there seems to be a female preponderance and a response to immunosuppression [6].

As patients reach adulthood resistance to immunosuppression or the development of cholestatic liver function tests suggests that the disease has progressed to PSC. This can be evaluated by up-to-date cholangiography. By this stage a considered reduction in immunosuppression is recommended as it may no longer have a role and could be counterproductive due to the malignancy and cholangitis risk.

Alagille syndrome

Alagille syndrome is an autosomal dominant syndrome that arises from mutations in the *JAG1* gene. The diagnosis of this eponymous syndrome relies on the identification of the

characteristic clinical features. These include typical facies, cholestasis with bile duct paucity, congenital heart disease, and renal, neurological, and skeletal disease. These clinical manifestations occur variably in affected individuals and patients can have the *JAG1* mutation but only partial or no clinical features of the syndrome (see also Chapter 8). Over time some patients may develop cirrhosis and 20–30% of patients require transplantation in childhood.

Alagille syndrome in adulthood

Examination of adult patients with the *JAG1* mutation has demonstrated that the phenotype can vary from the full clinical syndrome to no features at all. In adulthood patients may have cholestasis, pruritus, and malnutrition. Management therefore needs to focus on nutritional support, fat-soluble vitamins, and on rare occasions enteral feeding. Ursodeoxycholic acid is prescribed to improve bile flow and protect against the toxic effects of cholestasis. Itch may be troublesome, but is less severe than in childhood and is managed with cholestyramine and rifampicin. A high cholesterol level is observed but there is no evidence that it affects long-term cardiovascular risk so treatment is not warranted.

Young adults with significant cardiac disease may develop pulmonary hypertension or require further corrective surgery, with balloon dilatation or surgical correction of the pulmonary valve or pulmonary artery stenosis surgery. Renal disease requires specific management or renal transplantation as required.

Intracranial or abdominal vascular anomalies are also described and may be more common in adult life. They include: stenosis of the celiac, superior mesenteric or carotid arteries, and aneurysms of the hepatic or cerebral arteries.

Ocular abnormalities include optic drusen, retinal demyelination, or optic atrophy secondary to intracranial hypertension, all of which will require ophthalmological attention.

Any patients planning to have a family should undergo preconception genetic counseling as they have a 50% chance of an affected child. Although prenatal diagnosis is now possible, termination is not usual because of the varied phenotype.

Liver failure is rare in adult life, but over time patients may develop a secondary biliary cirrhosis requiring transplantation and the indications are analogous to the adult patients with cirrhosis. Their assessment needs to consider associated cardiovascular and renal abnormalities.

Progressive familial intrahepatic cholestasis

PFIC describes a rare group (1 in 100,000 births) of autosomal recessive disorders caused by a defect in bile secretion. On the basis of presentation, laboratory findings, liver histology, and genetic defect, it is divided into PFIC type 1, PFIC type 2, and PFIC type 3, and more recently PFIC type 4. The defect is impaired bile salt secretion in PFIC1/2; in PFIC3, it is impaired biliary phospholipid secretion and in PFIC4, the abnormality is in the tight junction protein 2. The basic treatment involves nutritional support and treatment of pruritus in some cases with biliary diversion to relieve pruritus (see also Chapter 8). Ultimately most patients will require transplantation in childhood.

There are limited data describing the outcomes of patients in adulthood. In a small series many young adults were below average height and suffered with symptomatic gallstone disease. Furthermore there is an association between PFIC2 and the risk of developing HCC. Therefore the priorities in young adults with PFIC who have not undergone transplantation should be managing nutrition, treating vitamin deficiencies, controlling pruritus, and screening for HCC. Also of note, recurrence of PFIC after liver transplantation is a possibility due to alloimmunization of the recipient against the affected protein (FIC1, BSEP, or MDR3).

Congenital hepatic fibrosis and Caroli disease/syndrome

There are several hepatic fibrocystic syndromes but liver disease in association with autosomal recessive polycystic kidney disease is the most commonly recognized in childhood (see also Chapter 14). The liver disease tends to present later in childhood and is a spectrum including abnormalities of the biliary tree and liver fibrosis. If bile duct abnormalities are combined with liver fibrosis patients are classified as having Caroli syndrome but those with bile duct abnormalities alone are described as having Caroli disease.

Most patients reach adulthood without requiring liver transplantation. However management of both portal hypertension and cholangitis can be challenging [7]. All patients should be screened for varices and undergo primary prophylaxis with β-blockade if grade 2 varices are found. The main indications for liver transplantation in this group are either synthetic failure or recurrent cholangitis. However it is important to consider the extent of renal disease at assessment and whether a combined approach is required. This is also the case when renal grafting is under consideration. All patients with Caroli disease and renal disease require assessment by a hepatologist as there is a risk of complications such as ascending cholangitis and liver failure, suggesting the need for a combined liver and renal transplant (see also Chapters 14 and 33).

Cystic fibrosis

CF has an incidence of 1 in every 3000 live births. The gene defect is an abnormality in the CF transmembrane conductance regulator (CFTR) located on chromosome 7q31. CF-associated liver disease occurs in around 30% of patients and usually presents before adulthood. Liver failure only accounts for 2.5% of overall CF mortality (see also Chapter 16).

CF is a complex disease to manage due to the multisystem nature of the disease. The combination of chest disease, sarcopenia, portal hypertension, and hepatic dysfunction necessitate a multidisciplinary clinic approach. Holistic management of CF in young adults includes:

- Standard management of pancreatic deficiency and diabetes if present.
- Counseling about adolescent issues, fertility, and lifestyle. Most women are fertile, but menarche and conception may be delayed due to malnutrition and ongoing chronic disease. About 98% of males are infertile due to failure of the vas deferens, and should be appropriately counseled.
- Managing the combination of CF liver and lung disease, portal hypertension, and hypersplenism. This requires a multidisciplinary approach from both respiratory and hepatology teams for optimum care based on standard adult management.
- Making a decision about timing for liver transplantation.

The indications for liver transplantation include malnutrition unresponsive to nutritional support, intractable portal hypertension, and hepatic dysfunction. A number of studies have indicated good initial survival, stabilization of pulmonary function and nutritional parameters [8].

Tyrosinemia type I

Tyrosinemia type I is an autosomal recessive disorder due to a defect of fumaryl acetoacetase (FAA).

The clinical phenotype is heterogeneous. Acute liver failure is a common presentation in infants, while older children present with chronic liver disease, rickets, a hypertrophic cardiomyopathy, renal failure, or a porphyria-like syndrome. Renal tubular dysfunction and hypophosphatemic rickets can occur at any age (see also Chapter 9).

Management is with a phenylalanine and tyrosine-restricted diet and nitisinone which prevents the formation of toxic metabolites and allows normal growth and development [9].

The long-term outcome of children and young adults who have tyrosinemia type I treated with nitisinone is unknown, but there are emerging concerns about neurocognitive function due to the high levels of tyrosine in nitisinone. They require long-term monitoring and follow-up with 6-monthly imaging and AFP for screening of HCC.

Liver transplantation is now only indicated for the development of liver failure unresponsive to nitisinone, or the development of HCC.

Wilson disease

Wilson disease is due to genetic defect of *ATP7B* inherited in an autosomal recessive manner. This gene functions as a transporter of copper within hepatocytes. This in turn leads to copper accumulation within the brain, liver, and other tissues. Almost half of patients present with liver disease either with the symptoms of cirrhosis or with acute liver failure. The neuropsychiatric presentation tends to occur later in life and is associated with a worse treatment response. The mainstay of therapy is chelating agents such as penicillamine or zinc. However some patients may require liver transplantation (see also Chapter 20).

Wilson disease is rare and therefore long-term outcome data are limited. Most patients with hepatic Wilson disease will achieve a good treatment response if they adhere to chelating therapy [10]. Interestingly adherence is a problem in patients with Wilson disease and even in those who have undergone transplantation. Some experts have suggested that this may be related to the complex neuropsychiatric changes associated with the disease. Adherence should be monitored in all patients with adherence questionnaires and yearly urinary measurements of copper and zinc if appropriate.

Liver disease and the Fontan circulation

Congenital cardiac defects that lack two effective ventricles require operations to correct this, leading to the eponymously named Fontan circulation. After a Fontan procedure the systemic venous return is connected to the pulmonary arteries without the interposition of an adequate ventricle, and all shunts on the venous, atrial, ventricular, and arterial level are interrupted. The consequence of this operation is chronic systemic venous hypertension and a reduction of cardiac output. In patients with Fontan circulation, chronic venous congestion of the liver associated with chronic hypoxemia secondary to reduced cardiac output may lead to liver cirrhosis.

Fontan-associated liver disease (FALD) is now well recognized, however it is not clear which patients will progress to fibrosis, and develop portal hypertension and cirrhosis. If cirrhosis develops, there is a risk of HCC [11]. Therefore it is recommended that all patients with the Fontan circulation are screened for cirrhosis with a combination of imaging and non-invasive marker of fibrosis. Those who are cirrhotic should undergo surveillance for HCC and varices. Finally patients who may require transplantation for synthetic liver failure or development of a HCC should be referred to a center with an adult congenital heart disease team.

Impact of liver disease on adulthood

Pregnancy

Previously pregnancy in patients with liver disease was discouraged; however this is now no longer the case. Therefore it is not uncommon to manage pregnant patients with

chronic liver disease or post-liver transplant (see also Chapter 7). This brings a new set of challenges for both patients and clinicians.

Ideally patients should undergo counseling prior to conception with both a hepatologist and obstetrician familiar with high-risk cases. The purpose of the consultation is to discuss the effect of pregnancy on underlying disease, effect of disease on pregnancy outcomes, medications during pregnancy, their potential teratogenicity, expected complications, and their subsequent management. However in patients with severe liver disease and a Model for End-Stage Liver Diseases (MELD) score greater than 10, a different discussion needs to take place [12]. This group is a greater risk of both poor maternal and fetal outcomes. The patient should be encouraged to postpone pregnancy until their disease is treated or until after transplantation. In some cases pre-pregnancy counseling is not possible, so termination should be considered in patients with decompensated liver disease although this decision will ultimately rest with the patient.

Variceal hemorrhage is a real risk during pregnancy for patients with pre-existing portal hypertension [13]. Portal pressures increase during pregnancy due to the systemic and intra-abdominal changes during gestation. Therefore pregnant women with portal hypertension should be offered variceal surveillance in the form of an upper gastrointestinal endoscopy during their second trimester. The options for primary prophylaxis against variceal bleeding include variceal band ligation and β-blockade. Although β-blockers have been associated with intrauterine growth restriction, bradycardia, and hypoglycemia their benefits will outweigh any potential risks.

Growth nutrition and endocrine function

Linear growth impairment occurs in children with chronic liver disease due to fat malabsorption, abnormal nitrogen metabolism, and increased energy expenditure. Patients do catch up after successful liver transplantation but 25% will have a height less than 5% for their age [14]. This height deficit is particularly common in patients transplanted for metabolic disorders such as urea cycle defects or α1-antitrypsin deficiency.

End-stage liver disease leads to endocrine complications including pubertal delay, growth failure, and hepatic osteodystrophy. As children grow abnormalities in bone metabolism can lead to fractures, rickets, and decreased bone density. Therefore all patients should have calcium, phosphate, vitamin D, and parathyroid levels monitored. Adequate calcium intake in conjunction with vitamin D replacement is crucial and this should continue after liver transplantation until normal values return. Adolescent patients with chronic liver disease are often affected by pubertal delay but normal function resumes in most cases after transplantation.

Neurocognitive function and psychosocial development

Liver disease in infancy is associated with impaired neurological development. This can be reversed in many cases by transplantation but regular assessments should be carried out to determine any special needs. Post-traumatic stress has been described in both patients and parents affected by liver disease. Furthermore anxiety around treatment and medications is common. Therefore early recognition of mental health issues is crucial as is referral and involvement of appropriate psychological services particularly in adult life.

Long-term outcomes after liver transplantation in childhood

Liver transplantation has transformed the survival of children with liver disease. Patients transplanted as children are now moving into adulthood with a 10-year patient and graft survival of 80% and 65%, respectively [15]. The transition from pediatric services to adult services has traditionally been a time of concern due to the adherence issues associated with adolescence and concerns about differing providers' priorities (see also Chapter 35). However a recent publication has highlighted good long-term outcomes after transition with patient and graft 10-year survival rates of 90% and 88%, respectively. Factors associated with poor outcome after transition were increasing age at transplantation, poor liver function at transition, and evidence of poor attendance and adherence prior to transfer [16]. This suggests that patients who are at risk can be and should receive a focussed approach with intensive clinical and psychological support within a structured transition clinic and in adult services.

Renal function

Acute kidney injury is common after transplantation and impacts on the development of chronic kidney disease in the liver transplant recipient, particularly as the calcineurin inhibitors (CNIs) used post-transplant are associated with nephrotoxicity (see also Chapter 31). A US registry study demonstrated that 18% of liver transplant recipients had stage IV–V chronic kidney disease (stage IV estimated glomerular filtration rate (eGFR) of 15–29 mL/min/1.73 m^2; stage V = end-stage renal disease) [17]. This was the second highest prevalence for any form of solid organ transplantation. The incidence in children is less well described and estimates of renal dysfunction vary between 24% and 70%.

Transplant recipients should undergo an eGFR assessment at every clinic review. Direct measures of GFR are available (chromium ethylenediaminetetraacetic acid (EDTA), inulin), but are costly and impractical to use regularly. Therefore, in clinical practice creatinine-based approximations of GFR are used to measure GFR. The most accurate

formula in the post-transplant population is the MDRD4 (modification of diet in renal disease). An albumin : creatinine ratio (ACR) should be checked on a yearly basis. An ACR ratio of >30 mg/mmol reflects significant parenchymal renal disease. It should initiate a search for the underlying cause. In patients with diabetes, microalbuminuria (ACR >2.5 mg/mmol in men or >3.5 mg/mmol in women) is also clinically significant.

A number of strategies have been employed to try to preserve renal function late after liver transplantation these include CNI minimization with or without additional immunosuppression, CNI withdrawal and substitution either with mycophenolate mofetil (MMF) or MMF and corticosteroids, and CNI withdrawal and substitution with m-TOR inhibitor. Very few studies in this area are randomized controlled trials, and all studies are heterogeneous with differing end points. Renal-sparing regimes can be considered when the calculated GFR is <70 mL/min/1.73 m^2.

Post-transplant lymphoproliferative disorder

Post-transplant lymphoproliferative disorder (PTLD) is the most common malignancy after solid organ transplantation. It is a spectrum of diseases without a reliable united classification system. Children have the highest risk of PTLD related to their lack of exposure to Epstein–Barr virus (EBV) whereas adult patients are at greatest risk of PTLD in their first transplant year. Therefore patients transplanted in childhood should undergo yearly monitoring of EBV viral loads and any symptoms such as unexplained anemia, diarrhea, or fever should be evaluated for possible PTLD (see Chapter 31).

Recurrent disease

The majority of indications for children are either congenital or inherited defects. However there are diseases that may recur including autoimmune liver disease and hepatoblastoma while multisystem disease such as CF persists in the other affected organs. In adult recipients recurrent PSC is reported in 20–30% of grafts at 3–5 years and leads to graft loss in 10%. This is associated with coexisting IBD after transplantation as colectomy seems to have a protective effect. Recurrence of AIH is more common than PSC and is prevented by continuation of steroids. It can be challenging to diagnose due to the histological overlap with rejection.

Transition

The transition from pediatric services to adult services is a challenging time for patients, families, and clinicians. Several studies have identified an increase in non-adherence to medication and hospital visits following transfer to adult clinics leading to graft loss. However the most recent study in a large cohort did not identify the transition period as a risk but found that young adults were at greater risk of graft loss and death [15]. This suggests that services should not only be focussed on the transition of patients but also young adults within their program. Therefore all young patients within an adult service should be seen in a multidisciplinary clinic with the aim of promoting self-management and promoting healthy living.

Conclusion

New advances in medicine, in particular transplantation have resulted in children surviving previously fatal liver diseases. This cohort has now reached adulthood and this provides a number of challenges to the adult liver physician. The team needs to be aware of the different liver diseases that affect children and their associated co-morbidities. Furthermore this cohort requires a multidisciplinary approach during the transition from pediatric to adult care. Finally this group will need careful study and evaluation as they develop to inform management in the future.

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