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The Liver Biopsy in Modern Clinical Practice: A Pediatric Point-of-View

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Abstract

Liver biopsy remains the foundation of evaluation and management of liver disease in children, although the role of the liver biopsy is changing with development of alternative methods of diagnosis and advancement of hepatic imaging techniques. The indications for liver biopsy are evolving as current knowledge of etiologies, noninvasive biomarker alternatives and treatment options in pediatric liver disease are expanding. The procedure can often be complicated in children by technical difficulties, cost and smaller specimen size. Communication and partnership of clinicians with pathologists experienced in pediatric liver diseases are essential. DNA sequencing, novel imaging modalities, non-invasive biomarkers of fibrosis and apoptosis, proteomics, and genome-wide association studies offer potential alternative methods for evaluation of liver disease in children. This review presents specific indications, considerations, methods, complications, contraindications, and alternatives for pediatric liver biopsy.

Keywords

liver biopsy; pathology; indications; contraindications; complications; pediatric; children; adolescent

Liver biopsy remains the foundation of evaluation and management of liver disease in children despite the evolving development of other less invasive diagnostic techniques. Histological assessment of the liver remains an essential tool in establishing the diagnosis in numerous pediatric diseases in combination with various clinical and laboratory data. Specific histological features can help differentiate patterns of hepatitis, cholestatic liver diseases, steatosis, vascular abnormalities, infectious diseases, and infiltrative or storage diseases (1,2). Liver biopsy is especially valuable in cases of overlap syndromes, in cases with atypical clinical presentation or in cases when a histological specimen can assist in a diagnostic dilemma and guide therapy.

The role of liver biopsy has also evolved into a prognostic tool in a variety of liver diseases providing information such as histological grades of inflammation and staging of fibrosis. Finally, a liver biopsy may serve as a significant method in assisting clinicians in therapeutic management decisions.

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Indications for Liver Biopsy in Children

Liver histopathology remains an essential tool for the evaluation and management of children with liver disease. The indications for liver biopsy are numerous and are evolving as current knowledge of etiologies, molecular basis, and treatment options in pediatric liver disease is expanding. This section discusses some specific indications, special circumstances, and associated controversies of the liver biopsy in children.

Neonatal cholestasis

Neonatal cholestasis is a serious condition that requires urgent investigation. The most common cause of neonatal cholestasis is extrahepatic biliary atresia (BA), a condition where timely surgical management relates to outcome of the Kasai procedure (3,4,5).

Liver biopsy remains the single most helpful informative examination in neonatal cholestasis and can yield >90% diagnostic accuracy for BA in experienced hands (6). Typical features of biliary atresia include ductular reaction, bile plugs within bile ductules, portal tract edema, and portal fibrosis (Figure 1). However, when the biopsy is done early in the course (before 6 weeks of age), these features may not all be present and repeat biopsy or an intra-operative cholangiogram to rule out BA may be required. Liver biopsy can also be diagnostic for other specific conditions such as alpha-1 antitrypsin deficiency and Alagille syndrome, or can reveal unexpected findings that can guide further diagnostic work-up. One such example is microvesicular steatosis, suggesting a possibility of metabolic liver disease. Other complementary techniques, such as immunohistochemical methods, electron microscopy and biochemical and molecular assays can improve diagnostic yield. A liver biopsy is recommended for investigation of neonatal cholestasis and should be interpreted by an experienced pathologist (7).

Abnormal Liver Tests of Unknown Etiology

The role of liver biopsy in the evaluation of asymptomatic patients with otherwise unexplained elevated liver enzymes is not well established. Liver biopsy has long been considered an important diagnostic adjunct in the evaluation abnormal liver tests of unknown etiology after a thorough history, physical examination, biochemical, serological, and imaging investigation have failed to establish a diagnosis. Available data from adult studies demonstrate that in a proportion of patients liver histology will point to a specific diagnosis (8) and can lead to a change in patient management (9). In one adult study, histological liver biopsies were performed in 354 patients to investigate abnormal liver tests; 64% of biopsies revealed diagnosis of non-alcoholic fatty liver disease (NAFLD), while other diagnoses included cryptogenic hepatitis, drug-induced liver injury, primary and secondary biliary cirrhosis, autoimmune hepatitis (AIH), alcohol-related liver disease, primary sclerosing cholangitis (PSC), hemochromatosis, and amyloid and glycogen storage disease. Only 6% of patients had a normal liver biopsy, whereas 26% were found to have some degree of fibrosis and 6% of patients had cirrhosis. Patient management was modified in 18% of patients after liver biopsy, and three families were entered into a screening program for heritable liver disease (9). However, in another study of adult patients with persistently elevated liver tests, histological findings after a liver biopsy changed the diagnosis in only 14 % of cases, and rarely altered management (10).

In the setting of the abnormal liver tests of unknown etiology, the risks and benefits of a liver biopsy in a child should be carefully evaluated, and the decision to perform a biopsy should be considered individually after a thorough non-invasive investigation has failed to elucidate a diagnosis.

Autoimmune Hepatitis

Diagnosis of AIH is based on a series of positive and negative criteria developed by the International Autoimmune Hepatitis Group (11,12). Liver biopsy is necessary to establish the diagnosis. Typical features include a dense mononuclear and plasma cell infiltration of the portal areas, interphase hepatitis with destruction of the hepatocytes at the periphery of the lobule and disruption of the limiting plate, parenchymal collapse expanding from the portal area into the lobule, and hepatic regeneration with “rosette” formation. In addition to the typical histology, other positive criteria include elevated serum transaminase and immunoglobulin G (IgG) levels, and presence of positive autoantibodies such as ANA, SMA, or LKM-1. Seronegative AIH has typical appearance of AIH on histology, responds to immunosuppression, but lacks detectable autoantibodies (13). This is a rare form of AIH in adults, but its prevalence and clinical characteristics remain to be defined in children.

The optimal duration of immunosuppressive treatment for AIH is unknown. Treatment withdrawal is successful only if there is histological resolution of inflammation in addition to normal liver function tests, normal IgG levels, and negative or low titer autoantibodies. Therefore, a liver biopsy is performed when the cessation of treatment is considered.

Sclerosing Cholangitis

The role of liver biopsy in Primary Sclerosing Cholangitis (PSC) is controversial. The diagnosis of PSC is usually established on the basis of a cholangiogram, when abnormal bile ducts demonstrate beading, irregularity and narrowing. Magnetic resonance cholangiogram (MRC) is non-invasive method with high sensitivity and specificity for detection of PSC in adults, 0.86 and 0.94, respectively (14). While technically more challenging in children, endoscopic retrograde cholangiopancreatography (ERCP) can be used if MRC images are suboptimal, although it is usually reserved for sampling in the presence of a dominant stricture and for intervention to relieve biliary obstruction. Liver biopsy may show histological features diagnostic of PSC, such as “onion-skin” fibrosis, but findings are often nonspecific due to the patchy and focal nature of the disease (15) and comprise a variable degree of portal inflammation, typically without significant interface hepatitis, as well as features of biliary tract disease, including ductular reaction, cholestasis, and swelling of periportal hepatocytes with accumulation of copper and copper binding protein (best identified with the aid of special stains, such as Victoria Blue, Rhodanine, and Orcein stains). Therefore, in the presence of an abnormal cholangiogram, liver biopsy is considered to yield little extra information, except in diagnosing suspected variants of PSC such as an overlap syndrome with autoimmune hepatitis. In pediatrics, this entity is often clinically described as autoimmune sclerosing cholangitis (ASC), a variant of PSC associated with strong autoimmune features, including typical autoimmune features on liver biopsy and serological features identical to AIH. It may be as prevalent as AIH in childhood, but it affects boys and girls equally (16). It is important to make the diagnosis, as the parenchymal damage of ASC may respond to immunosuppressive treatment, although bile duct disease tends to progress (16).

Metabolic Liver Disorders

Alpha-1 Antitrypsin Deficiency (A1AD)

Alpha 1-antitrypsin deficiency is an inherited metabolic disorder in which mutations in the coding sequence of the serine protease inhibitor, alpha 1-antitrypsin, prevent its export from the hepatocyte. The diagnosis of A1AD does not require a liver biopsy and is established by serum phenotype determination. Diagnostic histology reveals periodic acid-Schiff-positive, diastase-resistant globules of hepatocytes in the endoplasmic reticulum. In part, the

pathogenesis of A1AD in the liver is thought to be based on abnormal accumulation of the glycoprotein in hepatocytes resulting in programmed cell death, hepatic inflammation, fibrosis, and cirrhosis (17). In infants who are less than 13 weeks of age, the diagnostic A1AD globules may not be sufficiently evident on routine microscopy (18), making phenotype determination even more important.

Cystic Fibrosis

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 (19). Thus neonatal screening for CF has become routine in many countries. The sweat chloride test remains the primary test for the diagnosis of CF in the post-natal period; DNA analysis using a CFTR multi-mutation method can be utilized for clarification and confirmation. Liver Disease associated with cystic fibrosis (CFLD) is a well known complication of this multiorgan disease. No specific CFTR mutations have been associated with the presence and severity of liver disease. It is suggested that environmental factors or modifier genes might be important in the development of CFLD since the liver phenotype in CF patients with the same CFTR genotype is variable. One of the suspected genes, *SERPINA1* Z allele was found to be strongly associated with CFLD and portal hypertension (20, 21, 22).

The typical hepatic lesion of CF, related to the CFTR defect in cholangiocytes, is focal biliary cirrhosis, which results from biliary obstruction and progressive periportal fibrosis; this initially focal fibrogenic process may progress to multilobular biliary cirrhosis (23). Steatosis is also frequently seen and has been considered a benign condition in CF, without a proven relationship to the subsequent development of cirrhosis. Abnormalities of the intrahepatic bile ducts compatible with sclerosing cholangitis have been reported in children with CF (24).

Histological assessment of CFLD may provide important information on the predominant type of lesion (steatosis or focal biliary cirrhosis) and the extent of portal fibrosis (25). However, because of the patchy distribution of lesions in CFLD, liver biopsy may underestimate the severity of lesions and is not a routine investigation in many CF centers.

Familial Intrahepatic Cholestasis Syndromes

Progressive familial intrahepatic cholestatic (PFIC) diseases is a heterogeneous group of autosomal recessive hereditary diseases usually presenting in infancy or childhood with cholestasis of hepatocellular origin. Recently, understanding and diagnosis of this group of diseases have been enhanced by substantial clinical, biochemical, and molecular studies.

FIC1 deficiency (previously PFIC type 1) is caused by mutations of the ATP8B1 gene, encoding FIC1 protein. Benign Recurrent Intrahepatic Cholestasis (BRIC), which presents later in life, also has a defect in FIC1, but probably to a lesser extent. Because BRIC and PFIC1 were found to share the same mutations, they are both currently referred to as FIC1 deficiency. Liver biopsy if done early in life demonstrates bland canalicular cholestasis, although mild degree of hepatocellular ballooning, acinar pseudorosettes, and giant cell transformation may be seen focally (26). Small-sized hepatocytes have been reported in FIC1 (27). Fibrosis is not a characteristic finding initially but can be seen later in the course of the disease and may eventually result in cirrhosis. Currently, no specific antibody can detect the lack of the FIC1 protein by immunohistochemistry (IHC). To differentiate other etiologies of PFIC, IHC for BSEP and MDR3 can demonstrate that these proteins are well maintained along the hepatocytic canalicular membranes. To date, the most specific

pathologic finding is provided by electron microscopy, which shows the characteristic coarse, particulate, and granular “Byler bile” in dilated bile canaliculi (28, 29).

BSEP (bile salt export pump) deficiency (previously PFIC type 2) is caused by a mutation in ABCB11 gene, which encodes a protein that transports bile salts across the canalicular membrane. The histopathology of BSEP deficiency may vary according to the age of the patient. In infants, the most frequent pathologic finding is giant cell hepatitis similar to idiopathic neonatal hepatitis, but usually with minimal inflammatory component. Hepatocellular apoptosis, giant cell transformation, hepatocellular as well as canalicular cholestasis can be seen. Other histologic findings observed are ductular reaction and paucity of interlobular bile ducts. Eventually, cirrhosis associated with bile duct proliferation is the predominant feature. The use of IHC for BSEP, in most instances, allows a definitive pathologic diagnosis. Lack of expression of BSEP by IHC, in the proper clinical setting and with the use of adequate controls, is diagnostic (30). However, the presence of BSEP expression does not rule out a functional BSEP deficiency as BSEP expression can vary in some ABCB11 mutations (31). Hepatocellular carcinoma is a recognized complication of BSEP deficiency; the first series of 11 patients included 7 patients diagnosed before 2 years of age (32).

MDR3 (class III multidrug resistance p-glycoprotein) deficiency (previously PFIC type 3) is caused by mutations in the ABCB4 gene, which encodes a flippase required for biliary phosphatidylcholine secretion. Clinically, the serum γ -glutamyl transpeptidase (gGT) level is elevated, in contrast with FIC1 and BSEP deficiencies. Early biopsies in this disease show portal fibrosis and ductular proliferation. Cholestasis is present as diffuse hepatocellular cholestasis, but occasionally canalicular and ductular cholestasis can be seen. Among the PFIC diseases, the liver histology of MDR3 deficiency in the young infant is the one most closely resembling extra-hepatic biliary obstruction. Later, the liver biopsies show biliary cirrhosis with preserved bile ducts. MDR3 IHC staining can help guide the diagnosis before performing a molecular analysis of the MDR3 gene. IHC staining for MDR3 is negative in those patients who had an MDR3 gene mutation leading to a truncated protein, whereas weak or normal MDR3 canalicular expression can be observed in patients with missense mutations (33,34).

Bile Acid Synthesis Disorders

Inborn errors of bile acid synthesis usually present in infancy as life-threatening cholestatic liver disease and later in childhood or in adult life as progressive neurological disease. Both types of disease can often be treated very effectively with bile acid replacement therapy and it is therefore important to diagnose these disorders as early as possible. The cholestatic disease in infancy is characterized by conjugated hyperbilirubinemia, elevated transaminases but normal gGT. A liver biopsy is not diagnostic and usually shows giant-cell hepatitis; steatosis and extramedullary hemopoiesis may also be present. The most useful screening test for many of these disorders is analysis of urinary cholanooids (bile acids and bile alcohols) and can be achieved by electrospray ionisation tandem mass spectrometry (35).

Wilson Disease (WD)

Hepatic copper content $\geq 250 \mu\text{g/g}$ dry weight remains the best biochemical evidence for WD (36). The major problem with hepatic parenchymal copper concentration is that in later stages of WD, distribution of copper within the liver is often inhomogeneous. In extreme cases, nodules lacking histochemically detectable copper are found next to cirrhotic nodules with abundant copper. Thus, the concentration can be underestimated due to sampling error. In a pediatric study, sampling error was common enough to render this test unreliable in patients with cirrhosis and clinically evident WD (37). In younger patients, the measurement

of hepatic parenchymal copper concentration is especially important, since hepatocellular copper is mainly cytoplasmic and thus may be undetectable by routine histochemical methods. Copper quantification can be obtained using an adequate paraffin-embedded liver biopsy specimen. In general, the accuracy of measurement is improved with ample specimen size: at least 1–2 cm of biopsy core length should be submitted (38). Technical problems associated with obtaining a liver biopsy in a patient with decompensated cirrhosis or severe coagulopathy may be overcome by performing a transjugular liver biopsy.

Glycogen Storage Disease

Glycogen storage diseases (GSD) are a unique group of diseases that vary in age of onset of symptoms, morbidity, and mortality and affect primarily the liver, skeletal muscle, heart, and sometimes the central nervous system and the kidneys. Glycogen storage diseases are classified according to their individual enzyme deficiency affecting synthesis or degradation of glycogen (39).

Liver biopsy for evaluation of glycogen content and structure of liver tissue may be the initial step in determining subsequent enzyme analysis necessary to provide a definitive enzyme assay diagnosis (40) (Figure 2). Electron microscopic (EM) examination of biopsy material from child with suspected GSD can help differentiate GSD from another metabolic storage disease or a mitochondrial disorder, and can further assist in narrowing the range of diagnostic tests that need to be performed on a limited amount of biopsy tissue (41). Enzyme activity on liver tissue can be performed for GSD types 1a, III, IV, VI, IX (42).

Appropriate specimen allocation is the most important aspect for optimal evaluation of a suspected metabolic disease. For assessment of GSD, tissue should be obtained for routine histology (formalin fixation), histochemical stains (frozen and/or alcohol fixated), EM (glutaraldehyde), and genetic/molecular evaluation (frozen at -70°C). It is especially important to maintain optimal preservation of glycogen with freezing and/or alcohol fixation, allowing for quantitative evaluation by analytical techniques (frozen tissue) and qualitative assessment by histochemical staining (PAS, PAS-diastase). Quantitative analysis of the suspected enzyme responsible for a specific GSD or assessment of gene mutation and sequencing of the gene responsible for the enzyme defect require frozen tissue (42).

Mitochondrial Respiratory Chain Disorders

The diagnosis of mitochondrial respiratory chain deficiency is usually made by analysis of mitochondrial respiratory chain activity in muscle biopsy. The enzyme activities in skeletal muscle biopsies from these patients can be normal or equivocal. The importance of mitochondrial respiratory chain enzyme analysis in liver, in addition to muscle, may play a role even in cases where the primary clinical deficit is neurological and there is no liver disease (43).

Neonatal Hemochromatosis

Neonatal hemochromatosis (NH) is clinically defined as severe neonatal liver disease in association with extrahepatic siderosis in a distribution similar to that seen in hereditary hemochromatosis (44,45). Due to abnormal accumulation of iron in liver and other tissues, it was considered a neonatal iron storage disease until recently. NH is now best classified as congenital alloimmune hepatitis (46) given significant evidence that the pathology and liver injury of the disease is due to maternal alloimmunity directed at the fetal liver (47).

Pathological descriptions of NH have primarily been obtained from autopsy specimens. Severe acute and chronic inflammation may be seen, fibrosis is pronounced, particularly in the lobule and around the central vein, and cirrhosis is evident in nearly all cases (48). The

residual hepatocytes may exhibit either giant cell or pseudoacinar transformation with canalicular bile plugs, and in some cases almost no hepatocytes remain. The siderosis is coarsely granular in contrast with the hazy iron staining of normal newborn liver (49).

It is not recommended to evaluate hepatocyte siderosis for the purpose of diagnosing NH. The normal newborn liver contains ample stainable iron, and therefore siderosis is not diagnostic. In addition, pathologic hepatic siderosis has been described in a number of neonatal liver diseases; although extrahepatic siderosis has never been demonstrated. The diagnosis of NH also cannot be ruled out by absence of stainable iron in the liver since hepatocytes that normally contain iron might be completely gone. Furthermore, performing a liver biopsy in a severely coagulopathic infant may be hazardous and contraindicated. Demonstration of extrahepatic siderosis in suspected NH can be achieved by tissue biopsy or by MRI (50). Biopsy of the oral mucosa is a clinically useful approach to obtain glandular tissue in which to demonstrate siderosis (51,52).

Nonalcoholic fatty liver disease (NAFLD)

NAFLD is the most common cause of liver disease in children and its rise has been linked to the increasing prevalence of obesity. NAFLD is defined as excessive deposition of fat in the liver leading to steatosis in the absence of significant alcohol consumption. Nonalcoholic Steatohepatitis (NASH) forms part of a histological spectrum of NAFLD and involves hepatic inflammation and hepatocellular damage (53,54). While hepatic steatosis is thought to be a relatively benign entity, NASH can lead to progressive liver injury resulting in cirrhosis and the development of hepatocellular carcinoma (HCC).

Liver biopsy remains the only accepted technique to diagnose NASH, establish the presence of fibrosis (1), exclude potentially confounding factors such as AIH or drug toxicity, and identify other comorbid liver diseases. Several systems have been proposed for the histological evaluation of NAFLD, of which the most widely used is the NAFLD activity score (NAS) (55), which is based on the degree of steatosis, lobular inflammation, and hepatocyte ballooning, with an additional score for fibrosis. NASH has a distinct histopathology in children. Whereas adults have predominantly perisinusoidal fibrosis (Type I), children have increased inflammation and fibrosis in the portal tracts, greater amount of steatosis, with much less frequent findings of Mallory hyaline bodies or hepatocyte ballooning (Type II). Features of both Type I and Type II NAFLD are found in 32 to 83% of children (53,56) (Figure 3).

Acute Liver Failure

A liver biopsy may provide essential clinical information in cases of acute liver failure (ALF) (57). In pediatrics, approximately 40% of ALF is of unknown etiology. Unfortunately, as many patients are significantly coagulopathic, the biopsy may be precluded unless a transjugular approach is attainable (58). If feasible, a liver biopsy may provide a definitive diagnosis that can guide therapy (such as AIH, WD, infectious hepatitis, or metabolic disorder).

Liver tumors

Liver tumors are rare in children and present a large variety of differential diagnoses. Appropriate management of lesions noted on imaging often depends on obtaining an accurate diagnosis. In one review of 44 pediatric patients who underwent fine needle aspiration, 26 (60%) were found to have neoplastic lesions, and malignancy accounted for 21 (87.5%) of these lesions (59). Other studies have similarly reported malignancy in about two-thirds of pediatric liver tumors that were biopsied (60, 61).

Hepatoblastoma (HB) is the most common malignant tumor of liver in children. The average age at diagnosis is 18 months, and only 5% of cases are diagnosed in children older than 4 years (62). Histologically, HB is classified as epithelial with subtypes (fetal/embryonal/small cell undifferentiated); or mixed epithelial/mesenchymal. Histology is necessary for diagnosis and may have a significant prognostic importance with small cell undifferentiated tumors having poor response to chemotherapy and worse outcome (63).

Hepatocellular carcinoma (HCC) is the second most common liver malignancy in childhood. Approximately 65% of all HCCs occur in children older than 10 years (64). Unlike in adults, where HCC is usually seen with underlying liver disease, only 20–35% children with HCC children have underlying liver disease (65). Some childhood disorders predisposing to HCC are biliary atresia (66), BSEP deficiency (32), GSD type I (67), tyrosinemia (68), α 1-antitrypsin deficiency (69), and Alagille syndrome (70). Fibrolamellar HCC is a distinct pathologic variant usually affecting adolescents and young adults without an underlying liver disease; better survival in this entity is presumably due to the young age of the patients and the lack of cirrhosis making aggressive surgical resections possible (71).

In patients with underlying liver disease, especially cirrhosis, nodules larger than 1 cm that have a typical appearance suggestive of HCC (hypervascular in the arterial phase with washout in the portal venous or delayed phase) on a dynamic CT scan or contrast enhanced MRI studies, should be treated as HCC (72). A biopsy of HCC carries a significant risk of needle-track seeding (1.6–5%) (73, 74, 75) and should only be considered if the diagnosis cannot be made on radiological studies (72). In children, where most cases of HCC occur in non-cirrhotic livers, biopsy still plays an essential role.

Liver Transplantation

Liver biopsy and histological assessment following liver transplantation in children is a fundamental aspect of management in this patient population. It is often important to make a specific diagnosis in the setting of abnormal liver tests to investigate allograft rejection, bile duct injury or obstruction, viral infection, recurrence of the original disease, or drug-induced liver injury. In cases of auxiliary liver transplants, a biopsy may be used as a guide to withdrawal of immunosuppression. Some liver transplant programs perform liver biopsy on a protocol basis after transplantation (e.g., annually), even in those patients with normal liver tests, although evidence to support this approach is lacking. On the other hand, there is good evidence suggesting in some persistent diseases, such as Hepatitis C, fibrosis progression may be predicted by using liver histology in patients following transplantation (76,77). In children, hepatitis C is a rare indication for a liver transplant, and risks and benefits of protocol biopsies should be carefully considered by pediatric transplant centers. Central perivenulitis (CP), which encompasses dropout of zone 3 hepatocytes, red blood cell extravasation, and perivenular mononuclear inflammation, can be seen in up to 27% of pediatric allograft biopsies (78). Although most commonly associated with portal rejection, it also carries a significant risk for the development of zone 3 fibrosis and a trend toward the development of ductopenic chronic rejection (78,79,80).

Hepatitis B

Liver biopsy is recommended in most children with compensated liver disease prior to initiation of therapy for Hepatitis B (81). Histologic findings from a liver biopsy are used to define the grade of inflammation and the stage of fibrosis, which in turn can guide treatment decisions in a patient with persistently elevated liver enzymes and evidence of viral replication. Presence of moderate to severe necroinflammation, and/or anything more than mild portal fibrosis, supports initiation of antiviral therapy. In contrast, the benefit of treatment has not been established for patients with minimal to mild necroinflammation and/

or fibrosis. The exception to this may be family history of HCC which puts a child at a higher risk of developing HCC in the future; some experts consider such a family history as adequate cause to lower the histologic threshold for treatment (82). Liver biopsy is also helpful in excluding cirrhosis before considering interferon (IFN) treatment in children as liver function can decompensate (83). Although there is a significant interobserver variability in interpreting liver biopsies from HBV-infected children (84), histologic findings can help predict response to treatment and prognosis. Greater degrees of histologic activity correlate with higher likelihood of response to treatment with both IFN-alfa (85) and nucleoside analogues (86). For these reasons, most experts would agree that only those children with moderate inflammation or at least moderate fibrosis should be considered for treatment.

Hepatitis C

The diagnosis of chronic hepatitis C is no longer based on liver histology but on serological and virological tests. However, liver biopsy remains the gold standard for assessing the severity of inflammation and fibrosis in chronic Hepatitis C. In addition, a liver biopsy is helpful in elucidating diagnosis in the case of associated autoimmune markers (especially LKM1 autoantibodies), steatosis or co-infection with other viruses; these conditions may influence the outcome of the disease and the efficacy of treatment (87). If cirrhosis cannot be ruled out on clinical grounds, liver histology can exclude cirrhosis before initiation of a new drug or combination of drugs.

Intestinal Failure Associated Liver Disease (IFALD)

Liver disease has been well described as a complication of intestinal failure and long-term parenteral nutrition (PN) and develops in 40–60% of children and 15–40% of adults (88). The pathophysiology of IFALD is multi-factorial. The absence of enteral feeding, prematurity and low birth weight, reduced enterohepatic circulation, early and recurrent sepsis, length of bowel remnant, deficiencies or toxic components of PN solution have all been postulated as contributing factors to IFALD. The histopathological changes of IFALD present a spectrum from hepatic steatosis to biliary cirrhosis. Hepatic steatosis is more common in adults and may develop without evidence of inflammation, cholestasis, or hepatocyte necrosis (89). Steatosis is less common in infants who are more likely to present with centrilobular cholestasis, portal inflammation, and necrosis. More advanced liver disease has been described in children who are being evaluated for combined liver and small bowel transplantation and include portal fibrosis (100%), pericellular fibrosis (95%), and bile ductular proliferation (90%). Pigmented Kupffer cells (81%) and portal bridging (86%) were also prominent features. Cholestasis is not always present (90). Biliary cirrhosis is a late development (91). A liver biopsy has a role in assessing disease severity, notably fibrosis, when a patient is being considered for intestinal or multivisceral transplantation.

Other Indications for Liver Biopsy

Liver biopsies continue to play an essential role in evaluation of hepatosplenomegaly, cryptogenic cirrhosis, portal hypertension, drug toxicity, assessment of other metabolic disorders such as Gaucher disease or lysosomal acid lipase deficiency. Liver biopsy obtained during the assessment of hepatic dysfunction in patients with sickle cell disease, infiltrative malignancies, as well as bone marrow transplant or heart transplant recipients can serve as an important diagnostic tool with a significant impact on the clinical management of these patients. However, detailed discussion of these conditions is beyond the scope of this review.

Special Considerations in Pediatric Liver Biopsy

Liver biopsy can frequently be more complicated and more expensive in children. Procedures are often done in the endoscopy suite or the operating room and require general anesthesia due to lack of patient cooperation. Furthermore, overnight admissions after the biopsy may be required for observation of young infants or children with comorbid conditions. In addition to parental consent, issues of patient assent must be considered. There are technical difficulties with obtaining appropriate specimen due to the size of the patients. The use of wide or long biopsy needles is often prohibitive due to the size of the patient and/or the liver.

A standard liver biopsy represents only about 1/50 000th of the entire liver, and thus sampling error is a significant problem. Sampling error can approach 20–30%. In numerous diseases affecting children, such as PSC or CFLD, findings in the liver may be focal and misrepresented on a small biopsy sample. For accurate diagnosis, it has been estimated that at least 11 complete portal tracts are required in adults (92) with a biopsy length of at least 15–25 mm. A study has demonstrated that for reliable staging of fibrosis in hepatitis C patients, a 25 mm biopsy length was adequate to overcome variation due to sampling (93). In children, this is not consistently achieved due to size of the patients. In such circumstances, one has to carefully evaluate the value of additional passes that may increase the risk of complications.

Depending on the clinical indication of the biopsy, liver tissue may be required for numerous purposes. Allocation of tissue must be optimized by the clinician; it is especially important in pediatrics, when the specimen size is scarce. Most of the specimen is fixed in formalin for routine histochemical and immunohistochemical analysis. In addition, extra specimen allocation may be needed for a quantitative copper analysis in case of potential Wilson's disease, or for quantitative iron assessment if iron overload is suspected. Although electron microscopy is of limited use in most adult cases, it has a special value in pediatric liver biopsies when metabolic disorders are suspected; several 1–2 mm cubes of liver tissue are required to be fixed in glutaraldehyde for processing. Likewise, a small amount of tissue may also be requested to be snap-frozen for genetic or molecular studies. Rarely, an allotment of the tissue may need to be used for culture or PCR if bacterial, fungal, viral, or mycobacterial infection is suspected. It is important that adequate tissue is obtained to perform all necessary tests for an appropriate diagnosis to guide future therapy and to avoid repeat biopsy.

One of the most important factors for successful diagnosis is interpretation by a pathologist experienced in pediatric liver diseases. A partnership with a clinician who is caring for the child is required. Diagnostic errors by pathologists without specialty experience have been reported in greater than 25% of patients evaluated at an academic center (94). Neonatal jaundice is one of the diseases affecting children that frequently present as a challenge to both clinicians and pathologists; missed opportunity for the diagnosis of biliary atresia can result in loss of important time before surgical correction. However, when interpreted by experienced pathologists, liver biopsy had a very high sensitivity (99%) and specificity (92%) for the diagnosis of biliary atresia (95). The possibility of interobserver and intraobserver variations in assessment of liver biopsy specimen is well recognized and may be a major potential limiting factor in liver biopsy interpretations (96,97). The statistical variation of agreement between pathologists, expressed as kappa coefficient, has also been demonstrated to be considerable (kappa as low as 0.4) in the setting of evaluation of post-transplant liver biopsy specimens (98). The importance of communication of the clinical team with the pathologist regarding critical clinical details and differential diagnoses for appropriate tissue triage and diagnostic tests should be emphasized.

The role of liver biopsy remains more controversial in children because numerous liver diseases are rare and underinvestigated, the natural history data for pediatric liver diseases is deficient, and the knowledge of etiopathogenesis is frequently lacking. There is a well-recognized paucity of evidence-based guidelines and approved therapies for liver diseases in children; treatment decisions are often based on anecdotal evidence or insufficiently powered studies. In addition to diagnostic studies, obtaining liver tissue for research investigations is imperative in pediatrics. Histology as a primary endpoint should be considered a gold standard for pediatric treatment trials.

Methods for Liver Biopsy

Percutaneous liver biopsies are considered a safe and time-efficient method for obtaining liver tissue. A percussion-guided transthoracic approach is the classic percutaneous method.

Ultrasound guidance has been used to guide liver biopsies both in real-time or via a prebiopsy marking technique of the site. The benefit from using ultrasound to help determine biopsy site remains debatable. Potential liver biopsy sites marked by percussion were changed in between 3 and 15% of patients after ultrasound was performed (99,100). A large, randomized, prospective trial found that ultrasonography use lowered the rate of post-biopsy minor complications (such as pain) and hospitalizations but did not lower rates of hypotension and bleeding (101). On the other hand, a retrospective study demonstrated that in ultrasound-guided biopsies performed in radiology department, the risk of major bleeding was similar to nationally published figures (102). Thus, the role of ultrasonography to direct percutaneous liver biopsy remains controversial, unless image guidance is necessary for focal lesions or alternative transplant grafts.

A transjugular approach to liver biopsy is often used in patients with a contraindication to percutaneous biopsy, or when concomitant hepatic venous pressure gradient (HVPG) measurements or Transjugular Intrahepatic Portosystemic Shunt (TIPS) are planned. Although the biopsy specimen may be smaller and more fragmented than that acquired from a percutaneous approach, it is generally diagnostic (103,104). In pediatrics, a transjugular approach presents technical limitations due to size of the patient and may not be a plausible approach for smaller children.

A plugged biopsy is a modification of the percutaneous method in which a biopsy track is plugged with collagen, thrombin, or a comparable material as the cutting needle is removed from a sheath (105) and may be safer than a standard percutaneous approach in patients with increased risk of bleeding (106). In one study, the plugged liver biopsy was more time-efficient and obtained longer specimen but had higher rate of hemorrhage than the transjugular liver biopsy (107).

Intraoperative or laparoscopic liver biopsy allow visualization of the peritoneal cavity and liver surface. This type of biopsy can be performed with a typical needle device or by a wedge resection. Wedge resection method may overestimate the degree of liver fibrosis due to its proximity to the capsule. Surgical liver biopsies have the advantage of obtaining tissue from grossly visible lesions and can offer immediate and effective control of bleeding.

Suction, cutting, and spring-loaded cutting needles with triggering mechanisms have all been safely used for the purpose of liver biopsy. The cutting needle devices pass into the liver parenchyma using a troughed needle before an outer sheath slides over the core of tissue; this generally yields a more reliable specimen in advanced fibrosis with least amount of fragmentation.

Complications of Liver Biopsy

Although the liver has an abundant vascular supply, complications associated with a liver biopsy are rare. Minor complications after a liver biopsy include localized and temporary pain at the biopsy site as well as mild, transient hypotension likely related to a vasovagal reaction. Transient and localized abdominal pain and/or right shoulder discomfort can be expected in up to 20% of patients. Severe pain not responding to appropriate analgesia and/or instability of vital signs should trigger an evaluation of potential bleeding. In most cases, bleeding can be managed conservatively with fluids, pain control, and occasionally a blood transfusion. Seldom hepatic artery embolization or laparotomy are required for control of bleeding. Mortality associated with a liver biopsy is usually related to hemorrhage. Although the risk of mortality greatly varies in the literature, the most commonly quoted mortality rate (in adults) is less than or equal to 1 in 10,000 liver biopsies (1, 108, 109, 110). Other rare complications including pneumothorax, hemothorax, bile peritonitis, perforation of viscous organs, infection, hemobilia, and neuralgia have been reported after liver biopsy (1).

Any clinician who performs a liver biopsy should have a complete understanding of potential complications and should be equipped to recognize red flags, evaluate the patient, and appropriately manage significant events. The risks of liver biopsy should be well communicated to the family and the patient prior to procedure; in select cases age-appropriate language and terminology must be used to obtain assent.

Contraindications to Liver Biopsy

The most common contraindication for a liver biopsy in adults, i.e. an uncooperative patient, is virtually eliminated in children with general anesthesia. Although there is no specific cutoffs for laboratory parameters for impaired hemostasis, frequently INR > 1.5 and platelet count < 60,000/ml are utilized to indicate an increased risk of bleeding after a liver biopsy. History of spontaneous mucosal bleeding or unexplained bleeding after a surgical procedure may indicate a presence of a true bleeding diathesis. In patients with clinically evident ascites, a transjugular approach is generally recommended, although removal of ascites by drainage prior to biopsy may allow for a safe percutaneous alternative (especially important in small children when a transjugular approach is not technically feasible). Although biopsy of infectious lesions is generally safe, the presence of echinococcal cyst may be associated with fatal anaphylaxis and represents a contraindication to a biopsy. Other potential contraindications to percutaneous liver biopsy are morbid obesity, possible vascular lesions, extrahepatic biliary obstruction, bacterial cholangitis, unavailability of blood products for transfusion. Although not specifically quantified but clinically relevant, the size of a premature infant may present as a contraindication to liver biopsy.

Alternatives to Liver Biopsy in Children

There is a great emphasis to development of non-invasive methods to replace liver biopsy in evaluation of liver disease given the invasiveness of this procedure. Advances in serologic testing, enzyme analysis, DNA sequencing, and conventional imaging techniques have reduced the need for liver biopsy. Novel imaging studies and biomarkers hold promise as noninvasive means of both establishing the diagnosis and following the disease course.

DNA sequencing has changed approach to diagnosis of some liver diseases in children. To detect the most common mutations of inherited syndromes of intrahepatic cholestasis, a sequencing chip was developed that identifies disease-causing mutations in the genes *SERPINA1* (Alpha 1-antitrypsin deficiency), *JAG1* (Alagille syndrome), *ATP8B1* (PFIC1), *ABCB11* (PFIC2), and *ABCB4* (PFIC3) (111). This technological tool is commercially available and has simplified the diagnostic algorithm for children with chronic cholestasis.

Genome-wide association studies (GWAS) have allowed the detection of single nucleotide polymorphisms (SNPs) in association with hepatobiliary diseases. The first large-scale GWAS conducted on 536 patients with primary biliary cirrhosis (PBC) detected strong associations for variants in the *HLA* class II region, most notably *HLA-DQB1*, as well as coding sequence variants related to interleukin-12 α (*IL12A*) and the IL12 receptor β 2 (*IL12RB2*) (112). Another study of 1020 patients with chronic HCV identified seven gene polymorphisms associated with cirrhosis (113). In a pilot GWAS in patients with NAFLD, SNPs were detected in association with liver fibrosis and lobular inflammation (114). Although the findings of these studies need to be validated prospectively, integration GWAS-derived genetic scores carry the promise of risk stratification and personalized medicine.

Routine imaging modalities—ultrasound, computed tomography, and magnetic resonance imaging (MRI)—are generally capable of detecting advanced disease from the signs of portal hypertension but are typically insensitive to fibrosis. Novel imaging techniques have been studied in determination of liver fibrosis. Transient elastography (TE) can measure liver stiffness by using a probe that emits a low-frequency vibration and calculates the speed of the propagating mechanical wave. In adult studies, TE shows sensitivity and specificity values close to 90% in detecting advanced fibrosis (115). TE has also been evaluated in limited pediatric studies (116,117), and was found to accurately discriminate patients without fibrosis from those with severe fibrosis or cirrhosis. The limitation of TE signal that only penetrates 25 to 65 mm excludes its use in obese patients or those with ascites (118). Magnetic resonance elastography (MRE) is a similar technique that quantifies liver stiffness by propagating mechanical waves. MRE can diagnose severe fibrosis and cirrhosis with high accuracy (119) and can also be utilized in obese patients; however it is not currently available for clinical use.

Due to the increasing prevalence of NAFLD, much attention has also been focused on imaging studies that help detect clinically significant steatosis in children. Ultrasound shows typical echogenicity only when 30% or more of liver is steatotic (120). MRI has been demonstrated to accurately quantify fat content in the liver in limited pediatric studies (121,122) but is an expensive modality and may require general anesthesia for younger children.

Noninvasive biomarkers of chronic liver diseases encompass those that measure liver fibrosis and hepatocyte apoptosis. Various scoring systems have been developed for predicting and staging fibrosis in chronic liver disease. “Fibrotest,” which combines alpha-2 macroglobulin, haptoglobin, GGT, apolipoprotein A1, and total bilirubin (123) has been validated in several (including limited pediatric) cohorts (117, 124, 125). As the test incorporates total bilirubin and haptoglobin, false-positive results may be caused by hemolysis, Gilbert’s syndrome and cholestasis. Another marker, the enhanced liver fibrosis test (ELF) includes a panel of hyaluronic acid, amino terminal propeptide of collagen type III (PIIINP), and tissue inhibitor of metalloproteinase (TIMP-1) combined in an algorithm to predict liver fibrosis (126). When evaluated in a study of 112 pediatric patients with biopsy proven NAFLD, ELF was found to be highly predictive of fibrosis, although only a few patients in this study had moderate or severe fibrosis (127). While numerous other biomarker models have been validated in adults and show promise, most fail to differentiate between early stages of fibrosis and can primarily distinguish cirrhosis from no or minimal fibrosis.

Cytokeratin-18 (CK-18) is a major intermediate filament protein in hepatocytes that is cleaved during apoptosis and has been extensively evaluated as a biomarker. CK18 has been found to both predict the presence NASH and its severity with the area under the receiver

operator curve (AUROC) at 0.83 (0.74, 0.91) for the diagnosis of NASH in 139 patients with biopsy proven NAFLD versus 150 healthy controls (128). Serum levels of CK-18 were found to correlate with the severity of liver steatosis in both adult and pediatric patients with chronic HCV (129,130). In a recent prospective biopsy-controlled study, CK-18 was shown to discriminate different fibrosis stages from healthy controls as well as differentiate between minimal (<10%) and higher grades of steatosis (>10%) in 121 patients with chronic liver diseases (131). Although not routinely utilized in clinical practice at this time, CK-18 is a promising marker of apoptosis that may become a valuable non-invasive marker for following patients with steatosis and fibrosis.

Proteomics is the systematic large-scale study of all proteins in an organism, and numerous novel proteins have been studied in association with fibrosis in chronic liver diseases (132). Blood protein peak signatures identified by mass spectrometry have been demonstrated to be highly predictive (AUC > 0.85) of fibrosis in a range of liver diseases in adult studies (133,134,135). Subsequent identification of unique proteins can allow novel algorithms to be created which may be more applicable clinically.

Delaying the diagnostic biopsy can also be viewed as an alternative. In infants with jaundice, a liver biopsy may not be time-sensitive if the HIDA scan ruled out biliary atresia, and cholestasis may improve in numerous etiologies of neonatal jaundice before the biopsy is undertaken. Waiting may also be warranted if unidentified viral infection is suspected. If there is a potential no-risk therapy for potentially reversible condition, a liver biopsy may be avoided. One such example is initiating a trial of dietary and lifestyle intervention in an obese teenager with insulin resistance, echogenic liver, and elevated ALT. Biopsy of alternative sites may be preferable to higher risk liver biopsy in patients where coagulopathy is of clinical concern. One such example is a biopsy of salivary gland in neonatal hemochromatosis to demonstrate the degree of iron deposition.

Conclusions

In summary, histologic assessment of liver tissue remains the cornerstone of evaluation and management of liver disease in children, although the indications for performing a liver biopsy have undergone substantial changes in the last decade. The role of liver biopsy depends on the specific situation, but it should be considered when the treating physician and the family feel that a biopsy would help to clarify situations where there is sufficient uncertainty about diagnosis, severity of disease, prognosis, and treatment decisions.

There is considerable need for development of alternative diagnostic methods for evaluation of liver fibrosis and architecture to replace or supplement the invasive liver biopsy, and it is expected that these alternative tests will continue to improve and become validated for use in clinical practice. Over the next decade, novel imaging modalities, biomarkers, proteomics, and GWAS investigations are likely to further change the role of liver biopsy in children.

Glossary

A1AD	alpha-1 antitrypsin deficiency
AIH	autoimmune hepatitis
ALF	acute liver failure
ANA	anti-nuclear antibody
AFP	a-fetoprotein

ASC	autoimmune sclerosing cholangitis
BA	biliary atresia
BRIC	benign recurrent intrahepatic cholestasis
BSEP	bile salt export protein
CF	cystic fibrosis
CFLD	liver disease associated with cystic fibrosis
CFTR	cystic fibrosis transmembrane regulator
CK-18	cytokeratin-18
CP	central perivenulitis
CT	computed tomography scan
ERCP	endoscopic retrograde cholangiopancreatography
FIC1	familial intrahepatic cholestasis type 1
GGT	gamma-glutamyl transpeptidase
GSD	glycogen storage disease
H&E	hematoxylin and eosin
HBV	hepatitis B virus
HCV	hepatitis C virus
HCC	hepatocellular carcinoma
HIDA	hepatobiliary iminodiacetic acid scan
HVPG	hepatic venous pressure gradient
IFN	interferon
IFALD	intestinal failure associated liver disease
IgG	immunoglobulin G
IHC	immunohistochemistry
LKM-1	liver kidney microsomal type 1 antibody
MDR3	multidrug resistance protein 3
MRC	magnetic resonance cholangiogram
MRI	magnetic resonance imaging
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NH	neonatal hemochromatosis
PAS	periodic acid-Schiff
PAS-D	periodic acid-Schiff-diastase
PFIC	progressive familial intrahepatic cholestasis
PN	parenteral nutrition
PSC	primary sclerosing cholangitis

SMA	anti-smooth muscle antibody
TIPS	transjugular intrahepatic portosystemic shunt
WD	Wilson disease

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Figure 1.

In this case of biliary atresia (A–B) there is marked expansion of portal tracts by fibrosis as well as portal edema and prominent ductular reaction (part B), highlighted by cytokeratin 7 immunohistochemistry (B, inset). In cases of idiopathic neonatal hepatitis (C), portal fibrosis is less conspicuous and ductular reaction, if present, is inconspicuous. Lobular changes, including giant cell transformation (C, inset) are pronounced in this example. Alagille's syndrome is characterized by prominent bile duct loss. A portal tract containing a hepatic artery but no accompanying bile duct is shown here (D). A, hematoxylin and eosin (H&E) (superior core) and Masson's trichrome (inferior core), 20x. B, Masson's trichrome, 100x; C–D, H&E, 40–100x.

Figure 2.

Type I glycogen storage disease showing diffuse deposition of glycogen material within hepatocytes (A). Presence of glycogen is further supported by positive Periodic Acid Schiff (PAS) (B) and negative PAS-diastase staining (C).

Figure 3.

In typical cases of early-stage NASH (adult pattern), portal tracts are usually normal (A–B) and centrilobular/perivenular sinusoidal fibrosis is present (C). Hepatocyte ballooning and Mallory bodies are characteristic features (the latter may be highlighted by ubiquitin immunohistochemistry, C-inset). In contrast, type II (pediatric pattern) NASH is characterized by portal-based fibrosis, often associated with portal inflammation (D–E), while central veins are spared (F). A and D, hematoxylin and eosin stain; B, C, E, and F, trichrome stain (original magnification 100–200 $\times$).