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The Role of Liver Biopsy in Investigation of Cholestatic Liver Disease in Infancy

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Abstract

Objectives: The objective of this study was to assess the diagnostic yield and impact on management of liver biopsy in infants with cholestatic jaundice.

Methods: A retrospective cohort study of infants with cholestasis who underwent liver biopsy before one year of age between December 2002 and December 2013 at the Montreal Children's Hospital was conducted. Biopsies were reviewed by a single pathologist. The diagnostic yield of the biopsy was assessed in terms of its role in establishing a diagnosis, excluding an important diagnosis or changing management.

Results: Seventy-nine biopsies were performed within the time frame outlined, with 58 fulfilling inclusion criteria. Liver biopsies were found to add novel information in 21 cases (36.2%). The diagnostic yield of the biopsy was unrelated to the severity of direct hyperbilirubinemia, age at biopsy, age at admission, co-morbidities, stool color at presentation and TPN exposure. Among infants under 90 days of age, 21 also underwent cholangiography, the results of which were consistent with biopsy findings. There were four (6.9%) documented complications from biopsies, including bleeding and accumulation of free fluid in the peri-hepatic area.

Conclusions: Liver biopsy is an invasive test used with other clinical modalities to determine the etiology of neonatal cholestasis. These results suggest that biopsy added novel information to the diagnostic workup in 36.2% of cases with a complication rate of 6.9%. Consequently, the role and timing of liver biopsy need to be reassessed to determine which patients would most benefit from this procedure.

Keywords: *Biliary atresia; Cholangiography; Jaundice; Neonates*

Cholestatic jaundice affects one in every 2500 infants and is characterized by an elevated serum conjugated bilirubin (1, 2). The differential diagnosis of cholestatic jaundice in infants is broad and includes biliary atresia (BA), other structural abnormalities, metabolic disorders and infection (3). Biliary atresia is the most common cause of extrahepatic cholestasis in infants (4). Timely diagnosis is central to optimal management in this condition because surgical intervention beyond the age of 45 to 60 days is associated with a worse prognosis, decreased survival and increased risk of liver transplantation (5–7). The liver biopsy, used in combination with other clinical modalities, is a cornerstone of the diagnostic workup of infants with cholestatic jaundice to rule out other conditions before considering surgical exploration and operative cholangiography for biliary atresia (8). While the North American Society of Pediatric Gastroenterology and Nutrition (NASPGHAN) recommends liver biopsy for cholestasis of undetermined etiology to clarify the diagnosis (3, 9), biopsies may show nonspecific changes and do not always determine an underlying etiology. Conventional use of liver biopsies can differentiate extrahepatic

biliary atresia from other conditions and help determine the need for surgical exploration in 90% to 95% of patients (3). More recently, alternative imaging modalities allowing the visualization of the biliary system have become more accessible, such as ultrasound-guided percutaneous cholangiography. At our institution, liver biopsies are commonly done at the time of percutaneous cholangiography when the latter is normal or inconclusive, given the infant is already under general anesthesia; however, the diagnostic yield and appropriate timing of the liver biopsy in relation to a cholangiogram have yet to be clarified.

MATERIALS AND METHODS

This study was a retrospective cohort review of all infants less than one year of age who were evaluated for cholestasis at the Montreal Children's Hospital and who underwent a liver biopsy. A total of 70 patients were identified with a total of 79 biopsies, analyzed by a single pathologist (seven patients underwent two biopsies and one patient underwent three biopsies under the age of one year).

Inclusion Criteria

All patients less than one year of age who underwent a liver biopsy for the assessment of cholestatic jaundice between December 2002 and December 2013 were included in the study. Cholestatic jaundice was defined as an elevation of serum direct bilirubin greater than 17 μmol/L.

Exclusion Criteria

Biopsies that were done for reasons other than to determine the etiology of cholestasis were excluded.

Clinical Data

Medical history was extracted and included the following variables: perinatal history, consanguinity, family history of metabolic or liver disease, cystic fibrosis or early neonatal unexplained deaths. Information was obtained regarding the presenting illness, stool color, presence of failure to thrive and type of feeding (breastmilk or formula). Timing and duration of medication exposure, including parenteral nutrition, was also recorded. Physical examination data documented included presence of dysmorphism, hepato-splenomegaly and cardiac abnormalities.

The laboratory investigations before the liver biopsy, the date and indication for liver biopsy, and the age of the patient at the time of biopsy were recorded. Measured outcomes included the success of the procedure, complications, whether a cholangiogram was performed and the findings, if applicable.

Sample Size and Feasibility

Based on an estimate of 10 liver biopsies being performed annually for neonatal cholestasis, it was estimated that a 10-year review would yield 100 subjects. Based on clinical experience, we hypothesized that the liver biopsy would impact diagnosis and management in approximately 20% of cases. Using a point estimate of 0.2 and a confidence level of 0.95, a sample size of 73 cases would be necessary to obtain a precision of 0.05 around the estimate.

Main Outcome Measures

The main outcome measure of interest was the diagnostic yield of the liver biopsy. The yield was categorized into the following subcategories: 1) defined a specific diagnosis, 2) ruled out an important diagnosis that could not be obtained by another investigation, and 3) led to the implementation of a specific treatment or influenced the management of the patient.

Collected data was anonymized and documented in a password-protected electronic Excel database. Analysis of clinical variables involved the use of descriptive statistics, with means for continuous data (or medians for variables not normally distributed) and proportions for categorical data. The proportion of biopsies deemed to add novel diagnostic information was calculated. Exploratory univariate analyses using t-tests for continuous variables and Chi-square for proportions were used to identify potential clinical predictors in the yield of the liver biopsy. Variables assessed include age at presentation, age at the time of the biopsy, severity of the direct hyperbilirubinemia, presence of pale stools, presence of medical co-morbidity and absence of other positive etiologic testing.

RESULTS

The charts of 70 patients were reviewed, with a total of 79 biopsies (eight patients underwent multiple biopsies) having been performed on these patients who were all under the age of one year. Twenty-one biopsies were excluded as the patients were not cholestatic (Figure 1). As a result, the diagnostic yield of 58 biopsies performed in 53 patients was assessed. Among these biopsies, 45 were performed by interventional radiologists under ultrasound guidance, and 13 were intra-operative biopsies at the time of an intra-operative cholangiogram or Kasai procedure or other surgical procedure (n=13). Demographic data and baseline laboratory characteristics for the patient population are shown in Tables 1 and 2. Biopsies were done at a mean age of 87.66 (14–360) days. Mean bilirubin was 76.1 μmol/L at the time of biopsy. Forty-five biopsies were done under 90 days at a mean age of 56 days, and the remaining 13 biopsies were done at a mean age of 197 days.

Fourteen of 45 biopsies performed under 90 days of age confirmed a diagnosis of biliary atresia. Of these, three patients had two biopsies each (diagnostic and then intra-operative); thus in this cohort, 11 patients (26.2%) had biliary atresia. The final clinical diagnoses for each biopsy (n=58) are shown in Table 3 stratified by age at biopsy. Overall, the most common clinical diagnoses by patient (n=53) was TPN cholestasis (n=17),

followed by idiopathic neonatal hepatitis (n=16) and biliary atresia (n=12).

Of the 58 biopsies, four (6.9%) had documented complications. Two patients had bleeding related complications, with one patient requiring transfusion and another requiring a two-day admission in the pediatric intensive care unit post-biopsy. Another patient had an accumulation of free fluid in the perihepatic area post-biopsy and was the only patient out of the four who did not have a biopsy done under ultrasound guidance. The fourth documented complication following liver biopsy was fresh blood in the stool of the patient.

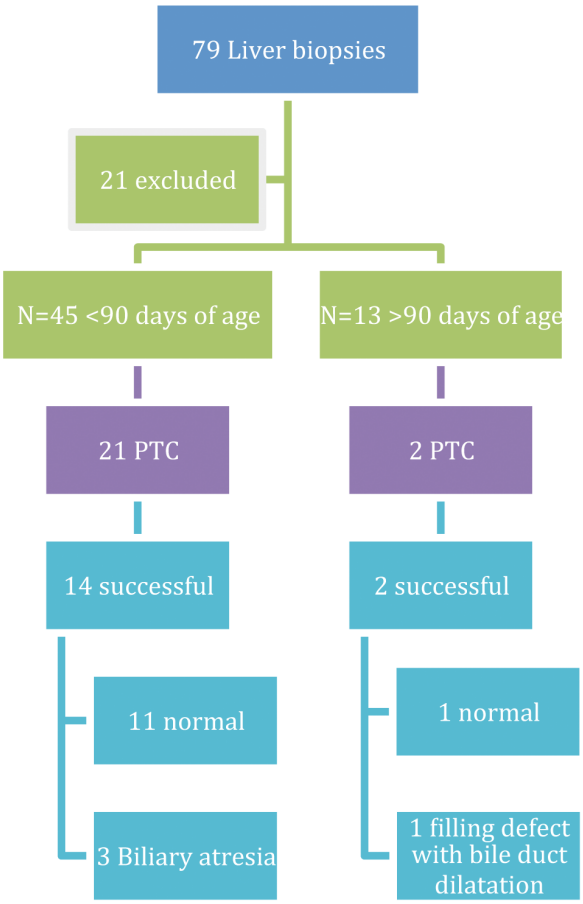

Figure 1. Biopsy breakdown. PTC, percutaneous cholangiogram.

Among infants undergoing liver biopsy under 90 days of age, 21 had an attempted percutaneous cholangiogram (PTC), with 14 of the 21 being successful (Figure 1). Of these, 11 were normal and three were suggestive of biliary atresia. Biopsy findings and PTC were in agreement in all cases. Two patients over 90 days of age also underwent PTC at the time of liver biopsy. One was normal, and the other demonstrated a filling defect in the extrahepatic biliary tree with intrahepatic bile duct dilatation. There were two of 23 (8.7%) documented complications during these attempts. The first was a puncture of the hepatic artery that did not cause any bleeding, and the artery was intact post-puncture. The second complication involved the injection of a small amount of contrast into the peritoneal space that did not lead to any clinical adverse outcome for the patient.

Assessment of the diagnostic yield of the biopsies showed that 21 of the 58 (36.2%) had a direct impact on clinical management. Among biopsies done in patients 90 days of age, 18 out of 45 (40%) added novel information to the diagnostic workup. In this subgroup, the biopsy ruled out BA in nine patients in whom this was not excluded based on PTC (either not done or not successful), confirmed BA in three patients who could not be confirmed via PTC; in two patients, the biopsy was suggestive of bile duct paucity. In addition, the biopsy was ruled out a suspicion of metabolic/storage disease in two patients. In another patient, the biopsy confirmed a clinical suspicion of cholangitis, and in another, the biopsy raised the suspicion of BA, prompting further workup which was subsequently normal (Table 4). Among the patients who underwent liver biopsy over 90 days of age, three of 13 (23%) added additional information to the diagnostic workup as they ruled out a diagnosis (graft versus host disease, glycogen storage disease and liver abscess), and in the remainder, the biopsies were not deemed to have added novel information.

DISCUSSION

Neonates with cholestatic liver disease undergo extensive testing to rule in or out diagnoses such as biliary atresia, which require timely intervention for optimal outcomes. Our results suggest that liver biopsy, an invasive test commonly included

Table 1. Clinical features of the 58 liver biopsies performed by age at time of biopsy

Presenting symptoms:	N (%)	Age ≤90 days) N=45	Age >90 days N=13
Jaundice	55 (98.3%)	44 (97.8%)	11 (84.6%)
Elevated LFTs	28 (48.3%)	20 (44.4%)	8 (61.5%)
Pale stools	33 (56.9%)	29 (64.4%)	4 (30.8%)
Hepatomegaly	22 (37.9%)	12 (26.7%)	10 (76.9%)
	Unknown 4 (6.9%)		
TPN exposure	38 (65.5%)	29 (64.4%)	9 (69.2%)

Table 2. Mean Laboratory investigations (Range) at the time of liver biopsy

Labs	Normal Range	All patients	Biopsy age ≤90 days	Biopsy age >90 days
Direct Bilirubin (μmol/L)	0–3	76.15 (21–277.3)	80.4 (21–277.3)	67.5 (26.5–181)
Total Bilirubin (μmol/L)	<11.3	126.88 (25–487)	135.0 (25–487)	109.3 (34–272)
INR	0.9–1.1	1.10 (0.77–2.84)	1.1 (0.77–2.02)	1.25 (0.81–2.84)
PTT (sec)	31.2–46.8	39.6 (23.4–303)	40.5 (23.4–303)	39.7 (25.8–79.5)
AST (U/L)	<67	171.1 (30–400)	173.1 (49–400)	177.2 (47–386)
ALT (U/L)	0–23	127.6 (29–416)	121.7 (29–416)	157.3 (56–378)
ALP (U/L)	116–450	488.3 (73–1724)	458.6 (73–1724)	624.1(269–1495)
GGT (U/L)	14–114	318.2 (37–1178)	332.0 (49–1097)	295.9 (37–1178)
Albumin (g/L)	26–49	32.5 (22–42)	33.6 (22–71)	31.7 (26–41)

Table 3. Clinical diagnosis classified by age at biopsy

FINAL CLINICAL DIAGNOSIS	N (biopsies)	<90 days	>90 days
TPN cholestasis	17	10	7
Biliary atresia	15 (12 patients)	14 (11 patients)	1
Idiopathic neonatal hepatitis	16	16	0
Possible GVHD	1	0	1
Common hepatic duct obstruction (not BA)	1	1	0
Choledochal cyst	1	0	1
Mitochondrial disorder	1	0	1
CMV vs. TPN cholestasis	1	1	0
Idiopathic neonatal hepatitis vs. TPN cholestasis	2	2	0
Biliary atresia (known) with acute cholangitis	1	1	0
Biliary atresia (known), no evidence of cholangitis	2	0	2

in the battery of tests used in cholestatic patients, adds novel information to the diagnostic workup or management in less than half of patients. This suggests that the role of liver biopsy needs to be re-evaluated in the context of its diagnostic yield in patients with neonatal cholestasis. In this cohort, among biopsies done under 90 days of age (the time frame in which a diagnosis of BA would be considered), the biopsy confirmed a specific diagnosis of BA in 26.2% of patients, and the remainder of biopsies showed mainly intra- or extrahepatic cholestasis with or without giant cell transformation, leading to a clinical diagnosis of either TPN cholestasis or idiopathic neonatal hepatitis. In some of our patients, stools were pigmented; therefore, this may impact the results in terms of the diagnosis of BA but would not impact the role of the biopsy in attempting to identify other causes of neonatal cholestasis. Liver biopsies are invasive and, in this cohort, impacted diagnosis or patient management in 36.2% of patients. No significant difference was found between the groups in whom the biopsy added novel information versus those in whom it did not, in terms of severity of direct hyperbilirubinemia, age at biopsy, age at admission, co-morbidities, stool color and TPN exposure. It is possible that in infants with significant TPN exposure, the pretest probability of identifying another etiology for liver disease is low, and thus in this subgroup, liver biopsy may not be helpful other than for staging purposes.

One possible limitation of the results obtained is the retrospective nature of this study, which depended on the accuracy and detail with which information was documented in the patient charts. In addition, the assessment of the yield of the biopsy was linked to the information provided on the pathology requisition. Thus, if the requisition asked a particular question, this may have skewed the determination of the diagnostic yield. Moreover, it is notable that we had no patients in our cohort with alpha-one antitrypsin deficiency, Alagille syndrome or metabolic diseases diagnosed based on liver biopsy. It is unclear as to why there were no patients with such diagnoses in our study population. One reason could be that patients with these conditions were diagnosed by means other than liver biopsy. As a result, it is difficult to make any conclusions on the diagnostic role of liver biopsy in the context of those specific diagnoses.

Table 4. Diagnostic role of liver biopsy in this cohort

Role of Biopsy	No. of biopsies	Specific Examples
Ruled in a diagnosis	10	Confirmed biliary atresia
		Confirmed ductopenia
		Confirmed cholangitis
Ruled out a diagnosis	6	Ruled out glycogen storage disease
		Ruled out biliary atresia
		Ruled out clinical suspicion of metabolic disease /storage disorder
Changed management	2	Avoided intraoperative cholangiogram (ruled out BA)
		Led to PTC because of biopsy (raised suspicion of BA)
Total	18	

To date, liver biopsy remains an integral part of the evaluation of neonatal cholestasis (10) despite its inconsistency in the diagnosis of biliary atresia (11). Many diseases responsible for cholestasis tend to yield similar findings on histology when biopsy is done early on, such as nonspecific inflammation, giant cell transformation and extra medullar hemopoïesis (4), further limiting the diagnostic role of liver biopsy (12). Moreover, some conditions associated with cholestasis may have limited histological changes early on in the course of disease, such as alpha-one antitrypsin deficiency and Alagille syndrome (8), and the diagnosis may become more apparent on histology with time (9). However, liver biopsy is a useful tool in certain metabolic diseases and can be used for staging of fibrosis and assessing the severity of liver injury. In our cohort, we aimed to assess the yield of the biopsy in the clinical evaluation and management of the patients rather than prognostication.

We found biopsy to commonly yield nonspecific results in the context of neonatal cholestasis, with 36 of 54 (66.7%) patients having no definitive diagnosis following their workup. Biopsy interpretation is also pathologist-dependent, and previous reports state that it can incorrectly be suspicious for biliary atresia in up to 46% of cases (9). Additionally, among pathologists that participated in the Biliary Atresia Research Consortium on histological assessment of biliary atresia, 86% of readings identified signs of obstruction in biliary atresia patients (13). However, 9% to 31% of readings had histological findings on biopsy consistent with biliary atresia in patients with diagnoses other than biliary atresia, and 12% to 19% of readings identified obstructive findings in patients without biliary atresia (14). Additionally, according to the NASPGHAN guidelines, biopsies performed prior to the age of six weeks in infants may need to be repeated if results of the initial biopsy are ambiguous, thereby increasing procedure-related risk to that infant. Thus, while liver biopsy is recommended in the NASPGHAN guidelines to be performed prior to surgical intervention to diagnose biliary atresia (3, 9), it may be worth also considering other diagnostic strategies.

Availability of percutaneous cholangiography has led to its increasing use in the evaluation of the cholestatic jaundice, in particular to rule out biliary atresia (14). In our study, the percutaneous cholangiograms were all performed between 2007 and 2013. In our institution, PTC and liver biopsy are often done at the same time to minimize the need for multiple general anesthetics. However, whether a biopsy should routinely be done in the context of a normal PTC is not clear. In our study population, PTC successfully ruled out biliary atresia in 12 of 15 patients, thus avoiding surgical exploration, but biopsies were performed nonetheless. Although cholangiograms can be invasive particularly if performed operatively, there have been complication rates as low as 4.1% (15) reported in the literature compared to 7.6% to 9% for percutaneous liver biopsy (16, 17). Similarly in this study, cholangiograms had a lower reported complication rate compared with liver biopsy, although our sample size is small. While all these patients also underwent liver biopsy, the question remains as to whether it was necessary to do so if the diagnosis of BA was ruled in or out by the PTC. If the cholangiogram demonstrated a suspicion for biliary atresia, the biopsy was used to confirm the results; yet biopsy in those cases was still not deemed to have added any additional or new information. Jensen et al. have demonstrated that percutaneous transhepatic cholecysto-cholangiography in combination with simultaneous liver biopsy can be used in the workup of cholestatic infants to effectively rule out biliary atresia and reduce the rate of intra-operative cholangiograms (14). Nwomeh et al. have shown that successful percutaneous cholangiograms can reduce the negative laparotomy rate by 47% (18). On the other hand, studies have suggested that liver biopsies are not necessary in deciding whether or not to perform a diagnostic laparotomy on a cholestatic patient, and in some cases, the decision can also be made without percutaneous cholangiography (8). Our findings, as well as those of Jensen et al. and Nwomeh et al., suggest PTC as a good first diagnostic test in cases where biliary atresia is suspected and suggest that a liver biopsy should not be systematically done solely because the child is undergoing general anesthesia. Furthermore, with the advent of more advanced genetic testing, if biliary atresia is not a clinical suspicion, consideration may be given to the use of laboratory testing to try to identify a genetic disorder leading to cholestatic liver disease.

CONCLUSION

Liver biopsy is an invasive test used in combination with other clinical modalities to determine the etiology of neonatal cholestasis. Our data suggest, however, that the liver biopsy only contributes additional information to the management of the patient in about one-third of patients. Further study is needed to determine the indications and appropriate timing for this test in the workup of infants with cholestatic liver disease. Furthermore, percutaneous cholangiography may avoid the need for liver biopsy in a subgroup of patients being evaluated for possible biliary atresia.

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ZC contributed to data collection, data analysis and drafting of the manuscript. NA and SF contributed to the study design, interpretation of data and revision of the manuscript. VHN contributed to the review of all biopsies and revision of the manuscript. The authors have no conflicts of interest to declare.

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