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Key histopathological features of liver biopsies that distinguish biliary atresia from other causes of infantile cholestasis and their correlation with outcome: a multicenter study

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Abstract

The liver biopsy guides diagnostic investigation and therapy in infants with undiagnosed cholestasis. Histologic features in the liver may also have prognostic value in the patient with biliary atresia (BA). We assessed the relative value of histologic features in 227 liver needle biopsies in discriminating between biliary atresia (BA) and other cholestatic disorders in infants enrolled in a prospective Childhood Liver Disease Research and Education Network (ChiLDReN) cohort study by correlating histology with clinical findings in infants with and without BA. In addition we reviewed 316 liver biopsies from clinically proven BA cases and correlated histologic features with total serum bilirubin 6 months post-hepatoportoenterostomy (the Kasai procedure, HPE) and transplant-free survival up to 6 years. Review pathologists were blinded to clinical information except age. Semi-quantitative scoring of 26 discrete histologic features was based on consensus. Bile plugs in portal bile ducts/ductules, moderate to marked ductular reaction and portal stromal edema had the largest odds ratio for predicting BA vs non-BA by logistic regression analysis. The diagnostic accuracy of the needle biopsy was estimated to be 90.1% (95% CI: 85.2%, 94.9%), whereas sensitivity and specificity for a diagnosis of BA are 88.4% (95% CI: 81.4, 93.5) and 92.7% (95% CI: 84.8, 97.3) respectively. No histologic features were associated with an elevated serum bilirubin 6 months post-HPE, though it (an elevated serum bilirubin) was associated with an older age at HPE. Higher stages of fibrosis, a ductal plate configuration, moderate to marked bile duct injury, an older age at HPE and an elevated INR were independently associated with a higher risk of transplantation.

Keywords

infant cholestasis; liver biopsy; biliary atresia; large duct obstruction; laterality defects

INTRODUCTION

A pre-laparotomy liver biopsy is the cornerstone of the diagnostic work-up of an infant with undiagnosed cholestasis with an emphasis on timely recognition of biliary obstruction and early hepatoportoenterostomy in biliary atresia (BA) (1). The interpretation of liver biopsies in infantile cholestasis is challenging as the histologic features of many of the disorders causing infantile cholestasis overlap, are dynamic and vary with age (1, 2). The histologic features which characterize BA and the accuracy of liver biopsies in the diagnosis of biliary obstruction in infants have been reported in a number of studies, mostly based in single institutions with interpretation by a limited number of pathologists (3–9), with a reported accuracy rate ranging between 60% and 95%. Whether this variability reflects the pathologists' experience, or depends on the timing of the biopsy relative to the course of the disease, either too early (1, 10) or too late (9) is unclear. Comparison of these studies is also difficult either because of variation in disease mix in different parts of the world (5), or because of a difference in mean age of the patients at the time of biopsy (8, 11). Once the diagnosis of BA is established, the primary treatment is a hepatoportoenterostomy (the Kasai procedure, [HPE]), which results in successful bile drainage in half the patients with BA (12). Even with successful drainage, most patients will ultimately require liver transplantation (12). A number of studies have attempted to correlate histologic features in

the liver with clinical outcome, again largely based in single institutions and with variable, often conflicting results. Some investigators have reported a worse outcome with increasing fibrosis (13–15), whereas others have determined that lobular inflammation (16), degree of biliary proliferation (17), or the ductal plate malformation (18), are associated with a worse clinical outcome. Other investigators have found no correlation between liver histology and outcome (19–21).

The resources of the National Institutes of Diabetes and Digestive Kidney Diseases (NIDDK)-supported Childhood Liver Disease Research and Education Network (ChiLDReN) provided the opportunity to evaluate liver biopsies from a large number of jaundiced infants from multiple institutions enrolled in a prospective clinical database. We thus carried out a two part study in which we sought to answer the following questions: a) which histologic features, alone or in combination, are most closely associated with BA and best distinguish between BA and non-BA cases; b) whether any histologic features vary with respect to clinical parameters such as age, presence or absence of extrahepatic malformations, or with the surgical assignment of the anatomic BA subtype (Ohi classification) (22) in clinically confirmed cases of BA; c) whether any histologic features help discriminate between various non-BA causes of cholestasis, such as Alagille syndrome (ALGS), idiopathic neonatal hepatitis (INH), and alpha-1 antitrypsin deficiency (A1AT); and d) whether any histologic features correlate with clinical outcome in BA patients post-HPE. We attempted to answer the first three questions using needle biopsies, which is the standard of practice in most North American pediatric institutions, and the last using a combination of needle and wedge liver biopsies obtained at or close to the time of HPE.

METHODS

Study Participants

Infants with cholestasis were enrolled in a prospective longitudinal study of the ChiLDReN Network (PROBE; Clinicaltrials.gov NCT00061828). A subset of PROBE patients with BA were also enrolled in a randomized treatment trial of steroids post-portoenterostomy (START; Clinicaltrials.gov NCT00294684). Eligible subjects were 180 days old or less at the time a liver biopsy was performed and presented at one of 15 participating clinical centers with cholestasis, defined as a serum direct or conjugated bilirubin greater than or equal to 2 mg/dL and greater than 20% of total bilirubin. Infants who had received parenteral nutrition, who were very low birth weight (<1500 g), who had acute liver failure or cholestasis associated with shock or sepsis, or who had undergone previous HPE or other hepatobiliary surgery were not eligible for enrollment. The clinical diagnosis of BA at the enrolling site was based on an intraoperative cholangiogram and/or examination of the excised biliary remnants. Subjects with clinical BA were divided into three clinical groups as follows: no significant extrahepatic anomaly (isolated BA, group 1), a major extrahepatic anomaly not associated with a laterality defect (group 2), or a major anomaly associated with a laterality defect (syndromic BA, group 3) (23). The diagnosis of all non-BA cases was established on clinical, laboratory, or genetic grounds with adequate follow-up, as defined below, to confirm the absence of BA. A diagnosis of INH was made when characteristic histologic features (lobular cholestasis, giant cell transformation,

extramedullary hematopoiesis) were present on a liver biopsy in the absence of an identifiable cause. Cases without characteristic features of neonatal hepatitis on a liver biopsy, without an identifiable cause, and in whom the cholestasis resolved during the period of observation were labelled “cholestasis indeterminate”.

Data Collection

All enrollments occurred between June 1, 2004 and November 2, 2014 and required informed consent from participant’s parents or guardians. The protocol was carried out with local institutional review board approval.

Baseline data included demographics, medical history, laboratory, and radiographic studies and were collected by research coordinators and clinical investigators. Operative information was recorded by the attending surgeon, and included assessment of the gross appearance of the liver and biliary tree, the latter classified by the surgeon into Ohi main types and subtypes (22). Clinical follow-up information from 316 BA patients who underwent HPE consisted of total serum bilirubin six months post-HPE and transplant-free survival for up to six years post-HPE.

Histologic study

To assess the accuracy of the biopsy diagnosis of BA, needle liver biopsies obtained at the participating centers at the time of enrollment in PROBE were reviewed centrally by the ChiLDReN pathologists. Wedge biopsies were excluded from the correlation of histology with clinical diagnosis as it was believed the wedge biopsy would bias the pathologists towards a diagnosis of BA. To evaluate the correlation of histologic features with clinical outcome in patients with BA, either wedge biopsies obtained at the time of HPE or needle biopsies obtained within one week of HPE (to ensure that histologic features were comparable in the two types of biopsies) were also reviewed centrally. In each case, hematoxylin and eosin (H&E) and Masson-trichrome stained slides were examined. The pathologists were blinded to clinical information except for age and used consensus to complete a semi-quantitative evaluation of 26 discrete histologic features, as previously described (24). Bile duct paucity was defined as a ratio of interlobular bile ducts to portal tract ≤0.5 in a minimum of five well-defined portal tracts. Portal fibrosis was staged using the Batts-Ludwig modification of the Scheuer system (25). Bile duct proliferation (bile ducts are defined as having a readily identifiable lumen and completely surrounded by connective tissue) was graded as either absent (one or two bile duct profiles per portal tract); mild (3 to 5 duct profiles per portal tract); or moderate/marked (> 5 duct profiles per tract), in either a focal (<50% of portal tracts) or generalized (>50% of portal tracts) distribution. Ductular reaction was defined as the presence of increased ductular profiles at the limiting plate or interface (26) and graded as either absent, mild (involving less than half the circumference of the portal tract), or moderate/marked in a focal (involving <50% of portal tracts) or generalized distribution. Portal stromal edema was characterized by clearing and separation of the extracellular matrix resulting in expansion of the portal tract. The ductal plate configuration (or malformation, DPM) was defined as a circinate arrangement of double-layered duct profiles around a central fibrovascular core similar to that observed during normal bile duct development in the fetal liver (27). Evidence for bile duct injury was

defined as cytoplasmic vacuolization, cellular necrosis and/or nuclear pleomorphism, or loss of polarity within interlobular bile ducts. Absence of a score for any feature was recorded as "missing". Each case was also assigned to one of three histologic categories: 1) consistent with large bile duct obstruction (LDO), 2) indeterminate for LDO, or 3) not consistent with LDO. Features that were considered by the group of pathologists to be consistent with large duct obstruction included portal expansion, bile duct proliferation, bile plugs in bile ducts and a ductular reaction. The assignment "not consistent with LDO" indicated that histologic features of obstruction were absent, and other features (for example, bile duct paucity) suggested an alternate diagnosis. The assignment "indeterminate for LDO" was applied to cases in which histologic features were deemed insufficient for a definitive assignment to the category "consistent with LDO". Age was used in assigning the biopsies to one of those categories, as it is the one element of clinical information always available to the pathologist when evaluating a biopsy.

Statistical Analysis

Descriptive data were summarized as the mean ± standard deviation (SD) for continuous variables and as percentages for categorical variables. Pearson correlation coefficients were calculated for pairs of histologic features to characterize their associations. Univariate and multivariable logistic regression were used to assess the relationship between histologic features and BA. Chi-square tests and Wilcoxon two-sample tests for discrete and continuous variables, respectively, were used to assess the relationship between histologic features and serum bilirubin at six months post-HPE. Univariate Cox proportional hazards models and log-rank tests were used to assess the relationship between histologic features and transplant-free survival. Multivariable logistic regression models were built based on univariate results, entering all variables with $p<0.05$ and using a step-wise model selection procedure with a threshold of $p=0.1$ for removal and entry from the model, while maintaining age at biopsy, sex, race, and ethnicity as covariates. Chi-square or Fisher's exact tests were used to compare histologic features consistent with LDO and indeterminate or not consistent with LDO among clinical BA subjects. Sensitivity, specificity, and positive and negative predictive values were calculated with histologic assignment as the test condition, and the clinical diagnosis (BA or non-BA) considered as the true disease state of each patient. Diagnostic accuracy was calculated as the sum of true positive and true negative cases over the total number of cases. Survival with the native liver with 95% confidence intervals (CI) was calculated using the Kaplan-Meier method. All slides were reviewed by a group of pathologists at consensus sessions requiring a minimum of 5 pathologists. Test-retest or "intra-rater" reliability (since there was no inter-rater assessment) was assessed using a random subset of cases and a select group of features. The slides were re-read using the same process and forms as the original reads. For 42 subjects the Kappa values (chance-corrected concordance measure) for bile duct paucity was 0.80, for DPM 0.81, for ductular reaction 0.56, for bile duct proliferation 0.63 and for visible duct/ductal bile plugs 0.90. All analyses were performed using SAS/STAT (SAS Institute Inc. 2008. SAS/STAT® 9.2 User's Guide. Cary, NC: SAS Institute Inc).

RESULTS

The histopathologic features of 227 needle biopsies from subjects obtained at enrollment were evaluated and compared with the clinical diagnosis as determined at each of the participating institutions. There were 136 BA and 91 non-BA cases (Table 1). For the correlation of histologic features with clinical outcome in BA, 206 wedge liver biopsies obtained at the time of HPE were evaluated in addition to 110 of the needle biopsies that had been obtained within one week of HPE, to ensure that the histologic features between needle and wedge biopsies were comparable. Only one biopsy (needle or wedge) from each subject was evaluated. The median age at the time of the needle biopsy was 58 days (SD 29) and 63 days (SD 25) at the time of HPE. Most BA patients had no major extrahepatic anomalies (group 1), with smaller numbers of patients with either a non-laterality associated defect (group 2), or a disorder associated with laterality defects (group 3). In agreement with published studies (22, 28), the biliary tree of most BA patients was classified as Ohi type III at surgery, with smaller numbers classified as either Ohi type I or type II. One hundred ninety-two BA patients post-HPE were part of the observational study only, whereas 124 BA patients were also enrolled in the START study, 62 having received steroids post-HPE, and 62 placebo only (12). At a median follow-up of 3.9 years, 15 (4.7%) of the BA subjects died, and 129 (40.8%) were transplanted. The diagnoses for the non-BA cases are listed in Table 2.

A comparison of histologic features in BA and non-BA

Table 3 presents the grading and distribution of the histologic features between BA and non-BA cases. There was no difference in age at the time of biopsy between clinically diagnosed BA (median 56 days) and non-BA cases (median 60 days). One hundred ninety-six biopsies (86.3%) had more than five evaluable portal tracts. Considerable variation in the severity of histologic findings was noted within the BA cases, and overlap was observed with non-BA cases. Most (72%) BA cases were classified as stage 1 or stage 2 portal fibrosis (modified Scheuer), while 23.6% were stage 3 or 4. Bile duct proliferation was absent in 22.8% of BA cases. The ductular reaction was mild in 53% of BA cases, moderate to marked in 39.7%, and involved <50% of portal tracts in 17%. A mild ductular reaction was also noted in 38.5% of non-BA cases. Duct/ductular bile plugs were absent in 25% of BA cases and conversely noted in 15% of non-BA cases. Some features such as bile duct injury, portal stromal edema and the ductal plate configuration were observed mainly in BA cases (Figure 1). On the other hand, bile duct paucity was noted mainly in non-BA cases (38.5% vs 9.6% BA cases). Most of the other features had a similar distribution between BA and non-BA cases. Within the non-BA group of cases, prominent giant cell transformation was most prevalent in INH cases, moderate or marked interlobular bile duct injury distinguished cases of Alagille syndrome from other non-BA cases, and subjects with indeterminate cholestasis were typically older at biopsy.

Main histologic predictors of BA and non-BA

Eight histologic features were found to be significant predictors of BA by univariate analysis: duct/ductular bile plugs, generalized moderate to marked ductular reaction and bile duct proliferation, portal stromal edema, higher stages of portal fibrosis (stages 3 and 4),

prominent pseudorosette formation, moderate to marked peribiliary neutrophilic infiltrates, and interlobular bile duct injury. Conversely, four histologic features, namely bile duct paucity, common or prominent giant cell transformation, macrosteatosis, and lobular extramedullary hematopoiesis (EMH) were most significantly associated with the non-BA group of cases. After adjusting for the presence of other histologic features and clinical variables such as age at biopsy, five histologic features remained independent histologic predictors of a diagnosis of BA by multivariate analysis: bile plugs in ducts/ductules (OR=13.65; 95% CI: 5.90 to 31.60, p<0.001), portal stromal edema (OR = 10.20; 95% CI 1.92 to 53.96, p = 0. 0.063), no bile duct paucity (OR=4.26; 95% CI: 1.64 to 11.09, p=0.0029), absent to rare giant cell transformation (OR=2.20; 95% CI: 0.98 to 4.63, p=0. 0.054), and absent to rare EMH (OR=3.49; 95% CI: 1.41 to 8.62). These five features when observed together in a biopsy resulted in a concordance index of 0.89 (95% CI: 0.84, 0.93) for the diagnosis of BA vs non-BA.

Variation of histologic features of BA with age and clinical features

Four histologic features were significantly associated ($p < 0.05$) with older age in BA: moderate to marked ductular reaction, duct/ductular bile plugs, higher stages of portal fibrosis, and prominent pseudorosette formation. Progression of fibrosis was observed with age at biopsy (Figure 2). EMH was associated with a younger age at biopsy. None of the other histological features, including bile duct proliferation, portal stromal edema, and bile duct paucity, showed variation with age. None of the histologic features were associated with the presence of major extrahepatic anomalies, including the ductal plate configuration.

Comparing the clinical diagnosis with the pathologists' diagnoses

Table 4 compares the final clinical diagnosis (BA or non-BA) with the assignment of the biopsies by the pathologists into three histologic categories (consistent with, indeterminate or not consistent with LDO). Of the 136 clinically confirmed cases of BA, 107 biopsies (78.6%) were correctly assigned as consistent with LDO, 14 (10.3%) were classified as "indeterminate", and 14 (10.3%) were deemed not consistent with LDO. Assignment to one of these categories did not vary with the age of the subject at the time of biopsy. Eleven biopsies of clinically confirmed BA cases were performed in infants less than 30 days of age, and 10 of these biopsies were correctly assigned as consistent with LDO. To ascertain the accuracy of the needle biopsy in arriving at a confident diagnosis of either large duct obstruction or not, we used only those cases in which we felt there was a clear-cut histologic distinction between "consistent with large duct obstruction" or "not consistent". If cases assigned to the indeterminate category are omitted from this calculation, the sensitivity of the needle biopsy for a diagnosis of BA is 88.4% (95% CI: 81.4, 93.5), the specificity is 92.7% (95% CI: 84.8, 97.3), and the diagnostic accuracy is 90.1% (95% CI: 85.2%, 94.9%).

Seventy-six (83.5%) of the non-BA cases were classified as not consistent with LDO, eight (8.7%) were indeterminate, and six (6.5%) were found to be consistent with LDO. The diagnoses of the latter 14 cases are outlined in Table 5.

BA cases assigned as not consistent with LDO

Table 6 outlines in greater detail the reasons for the discrepant assignment of 14 clinically proven BA cases to the category “not consistent with LDO”. Ages at biopsy for these subjects ranged from 21 to 127 days. In five subjects the needle biopsies showed few portal tract changes in association with prominent lobular features - extensive hepatocellular giant cell transformation (in two), extensive steatosis (in two), and eosinophilic micro-abscesses (in one) that suggested other diagnoses (Figure 3). Six other subjects, aged 30, 41, 47, 53, 78, and 127 days at the time of biopsy were suspected to have bile duct paucity. Of the 13 patients for whom clinical information was available in the study, 8 were described as having pale or acholic stools, in 11 the gallbladder on ultrasound was described as “small/contracted” or “absent/not visualized”, in 8/8 cases no tracer excretion into the duodenum was seen in the hepatobiliary scan despite normal hepatic uptake, and in 2/2 no extrahepatic tree was visualized by MRCP. In addition, 2 patients had polysplenia and other malformations (heterotaxia, intestinal malrotation, preduodenal portal vein) which raised suspicion for syndromic BA. Intraoperative cholangiograms and surgical exploration performed at the participating institutions for clinical suspicion of BA confirmed the diagnosis of BA in all these cases The median interval between biopsy and HPE was 3.5 days (Interquartile range 2,6) in these 14 patients compared to 3 days (Interquartile range 1,5) in the remaining 122; thus there was no significant delay in the appropriate management of these patients despite the inconclusive biopsy results.

The corresponding liver wedge biopsies obtained at the time of HPE were reviewed in 12 of the 14 cases. Nine wedge biopsies had clear obstructive changes, though in three the changes were limited to larger portal tracts not sampled in the needle biopsies. Three other wedge biopsies remained non-diagnostic for obstruction despite an interval of 48 days between the needle and the wedge biopsy in one case. None of the wedge biopsies showed paucity of bile ducts.

Clinical and histologic features in BA cases correlating with clinical outcomes

Table 7 outlines the key histopathologic features identified in liver biopsies from 316 BA patients obtained at the time of HPE and who were followed for up to six years post-HPE. Extensive variability in the severity of the histologic features was noted, as with the needle biopsies. Using a significance threshold of 0.01, an older age at HPE was the only feature significantly associated with poorer bile drainage at six months post HPE (p=0.01), while lobular sinusoidal fibrosis and interlobular bile duct injury were found to be marginally associated with bile drainage at six months post HPE (p=0.03 and p=0.05, respectively).

Degree of fibrosis, interlobular bile duct injury, DPM, age at Kasai, and INR were univariately associated with shorter time to transplant or death (p-values 0.003, 0.009, 0.01, 0.0006, and 0.003, respectively), while ductular reaction, macrosteatosis, BA subtype, and alkaline phosphatase were only marginally associated with outcome (p-values 0.05, 0.03, 0.05, and 0.05, respectively). The hazard of transplant was 1.73 (95% CI: 1.21, 2.48) times greater for patients with stage 3 or 4 fibrosis compared to those with stage 0, 1, or 2 fibrosis, 1.55 (95% CI: 1.11, 2.16) times greater for patients with moderate or marked interlobular bile duct injury versus those with absent or mild interlobular bile duct injury, and 1.53 (95%

CI: 1.09, 2.14) times greater for those with DPM versus those without DPM (Figure 4). The hazard of transplant or death increased by a factor of 1.26 (95% CI: 1.09, 1.46) for every unit increase in baseline INR. In multivariate analyses DPM, INR, and age at HPE remained significant predictors of transplant-free survival, with hazard ratios of 1.57 (95% CI: 1.08, 2.27) for patients with DPM versus those without DPM, 1.25 (95% CI: 1.05, 1.48) for every unit increase in baseline INR, and 1.10 (95% CI: 1.05, 1.16) for every week increase in age at Kasai.

DISCUSSION

This study is the largest review to-date of liver biopsies from cholestatic infants comparing histologic features with clinical diagnosis and outcome. All the subjects were enrolled in a ChiLDReN network prospective longitudinal data base which provided detailed clinical information and follow-up. We sought to identify which histologic features in liver biopsies best characterize BA and optimally discriminate between BA and non-BA, and which features vary with age at biopsy, or are associated with clinical features such as extrahepatic malformations or the anatomic subtype of BA. We also explored possible sources of biopsy misinterpretation as well as whether any histologic features in the livers of BA patients could be predictive of clinical outcome post-HPE, evaluated by total serum bilirubin at six months post-HPE, and survival with the native liver.

All the liver biopsies were reviewed by a panel of pathologists experienced in pediatric liver disease using consensus evaluation of 26 well-defined histological features (24), and incorporating a conventional assessment of the degree of fibrosis (25). We distinguished between ductular reaction and bile duct proliferation (26) and assessed whether these features were focal (involving < 50% of portal tracts) or generalized. We limited the choices to as few grades as possible to enhance interobserver reproducibility (29). Assessment of wedge liver biopsies was limited to correlation with clinical outcome in BA as they might have biased the pathologists towards a diagnosis of obstruction.

This study revealed extensive variability in the severity of the histologic changes in clinically well-characterized cases of BA, contrasting with much of the previously published experience. For example, bile duct proliferation, generally regarded as a key feature of obstruction, was absent in as many as 22.8% of BA cases. Bile duct/ductular plugs were absent in 25% of BA cases. Features seen in BA also overlapped with non-BA cases, as over 40% of the latter had some degree of bile ductular reaction, and bile plugs were present in 15%.

We nonetheless found that duct/ductal bile plugs and portal stromal edema were strong independent histologic predictors of BA, and when these are found in a needle biopsy in association with no bile duct paucity and absent to rare hepatocellular giant cell transformation and EMH, the concordance rate for a diagnosis of BA is 0.89. Using only two routinely-stained slides and omitting cases from the “indeterminate for LDO” category, we estimated the diagnostic accuracy of the needle biopsy to be 90.1%, and the sensitivity and specificity for a diagnosis of BA were 0.88 and 0.93 respectively, similar to a recently published study, but somewhat lower than some previously published studies (1, 8, 11, 30).

We believe this lower diagnostic accuracy comparing needle biopsies only, is more reflective of current clinical practice and is largely explained by the variability we observed in the severity of the histologic features in BA, as well as overlap with non-BA, a finding not well discussed in most previous studies. In practice, an assignment of “indeterminate” would most likely lead to exploratory laparotomy and cholangiography and thus the right diagnosis in those cases.

Fourteen clinically confirmed BA cases (10.3%) were thought at central review to be indeterminate for LDO and fourteen cases (10.3%) were thought to be “not consistent with LDO”. These latter 14 cases were characterized by mild or inapparent bile duct proliferation and ductular reaction, undetectable bile plugs, or had findings in the lobules which deterred us from concluding that LDO was present. The corresponding wedge biopsies of nine of these cases clearly demonstrated features of obstruction. As the interval between the needle and the wedge biopsy was less than one week in most of these cases, progression of the disease is not likely to be a factor in the difference in interpretation. Wedge biopsies may be more representative of changes due to obstruction because they are more likely to contain large portal areas not sampled in the concurrent needle biopsies, as noted in three subjects. Nonetheless, three of the wedge biopsies, from patients aged 69, 80, and 85 days at time of HPE, did not show features of obstruction despite an interval of up to 48 days between the needle and the wedge biopsy in one case. The reason for the lack of more established features of obstruction in these cases remains unclear.

Age was the only item of clinical information available to the pathologists during the assignment of the biopsies to one of the three diagnostic categories, as it is the one item always available to the pathologist in practice. In our study, age was not a significant factor in the assignment of the biopsy to the appropriate category, contrary to previous studies [3, 10], though we did confirm that a number of key features such as duct/ductular bile plugs, ductular reaction, and portal fibrosis vary with the age of the patient. Ten of 11 BA cases less than 30 days of age at biopsy were correctly assigned to the category “consistent with LDO”. This suggests that though the progression of histologic features evolves with age, this progression might not follow the same time course in every patient with BA, and thus age at the time of biopsy should not by itself preclude the appropriate diagnosis. Clinical features which would help support a diagnosis of BA, but to which the study pathologists were blinded, would include acholic stools, a non-excreting DISIDA scan and ultrasound findings of an absent or highly contracted gallbladder. The finding of extrahepatic anomalies such as heterotaxy or aplenia/polysplenia would be suggestive of syndromic BA. In addition, BA is usually associated with an elevated serum gamma-glutamyl transferase (GGT), so the finding of a low or normal serum GGT should steer the diagnosis away from BA.

A cause of misinterpretation of the needle biopsies in BA cases was overestimation of bile duct paucity, a finding initially noted in 13 (9.6%) of the needle biopsies, yet not observed in the corresponding wedge biopsies obtained at HPE. We believe that this was largely due to evaluation of incomplete portal tracts, a significant problem in needle biopsies from small infants. We also underestimated the significance of portal stromal edema in the needle biopsies as a feature of BA, which in multivariable analyses, was found to be highly predictive of obstruction, second only to bile plugs in ducts/ductules. Two cases of alpha-1

antitrypsin deficiency and one case of cystic fibrosis were among the six non-BA cases which were assigned to the category of large duct obstruction. These disorders are well-recognized mimics of BA and are known to be indistinguishable from BA without clinical information and without additional study (1, 3, 31).

One case was diagnosed as Niemann-Pick type C, whereas two other cases resolved clinically without evidence of obstruction, and were clinically categorized as INH and idiopathic cholestasis respectively. Infants with liver injury due to total parenteral nutrition (TPN) or sepsis were not included in the study, as those patients were not eligible for enrollment if they had received TPN, were low birth weight (<1500g), or had cholestasis associated with shock or sepsis. In the authors' experience the use of TPN can result in histologic changes which overlap extensively with those due to BA on a liver biopsy. In those cases, liver biopsy may not be helpful in discriminating between the two and cholangiography may be necessary if there is a high clinical suspicion of BA. Sepsis would be excluded by the appropriate cultures.

When 316 liver biopsies obtained around the time of HPE were assessed, none of the histologic features correlated significantly with drainage at six months post-HPE (serum total bilirubin<or ≥ 1.5), except for sinusoidal fibrosis and bile duct injury, which were only marginally correlated with poorer bile drainage at six months; however an older age at HPE in our subjects did correlate significantly with poorer bile drainage. A lack of correlation of histologic features with post-HPE serum bilirubin was also observed in a recent study (21). We did find that higher stages of portal fibrosis, DPM, bile duct injury, increased INR along with older age at HPE were associated with increased risk of transplantation. Higher stages of fibrosis have been associated with a worse clinical outcome by others (13–15), and with more severe portal hypertension (21). In our study DPM was also associated with an increased risk to transplant, as previously reported (18). On the other hand, DPM was not associated with laterality defects (syndromic BA) in our subjects, as also reported by others (32), and in our experience should not be used as a marker for that disorder.

In summary, we found that duct/ductal bile plugs and portal stromal edema were the strongest independent histologic predictors of obstruction, and when found in a needle biopsy in association with no bile duct paucity and absent to rare hepatocellular giant cell transformation and EMH, the concordance rate for a diagnosis of BA is 0.89. Nevertheless, we noted a wide variation in the severity of the histologic features in our group of BA cases, with the result that about 10% of clinically proven BA cases lacked features of LDO and a further 10% were indeterminate for LDO. In those cases, the wedge biopsies were more clearly representative, as some of the characteristic features were only noted in larger portal tracts not sampled by the needle biopsy. Further, though key histological features such as ductular reaction and duct/ductular bile plugs were found to evolve with age, age alone did not limit recognition of LDO. No histologic features predicted successful bile drainage at six months post-HPE, whereas severity of portal fibrosis, DPM, bile duct injury, and baseline INR were associated with a shorter survival with the native liver.

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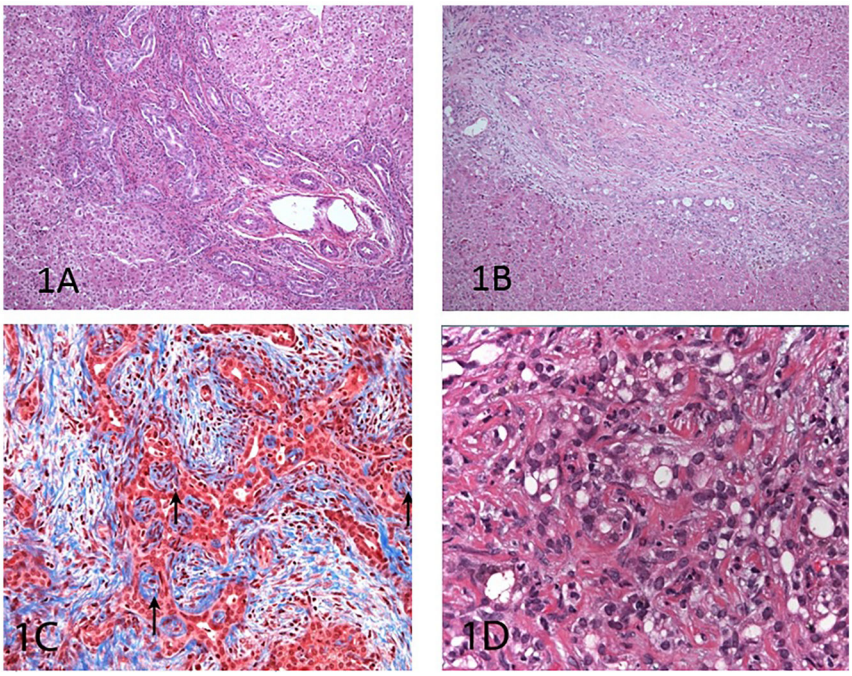

Figure 1.

(A) Liver biopsy from a 38 day-old with BA shows portal tract expansion in association with proliferation of centrally located bile ducts (Hematoxylin-eosin, magnification X200). (B) In contrast, this biopsy from a 55 day-old with BA shows mainly a proliferation of ductular structures at the limiting plate (Hematoxylin-eosin (H+E), X 200). (C) Ductal plate configuration characterized by a complex circinate arrangement of ducts and ductular structures frequently around a small central vessel (arrows) in this 68 day-old with BA, associated with portal stromal edema. Note the prominent bile plugs to the right of the photograph. This child had no extrahepatic malformations (Masson-Trichrome, X200). (D) Extensive bile duct injury manifested by marked vacuolization of the proliferating biliary epithelial cells in this 78 day-old with BA. (H+E, X 400).

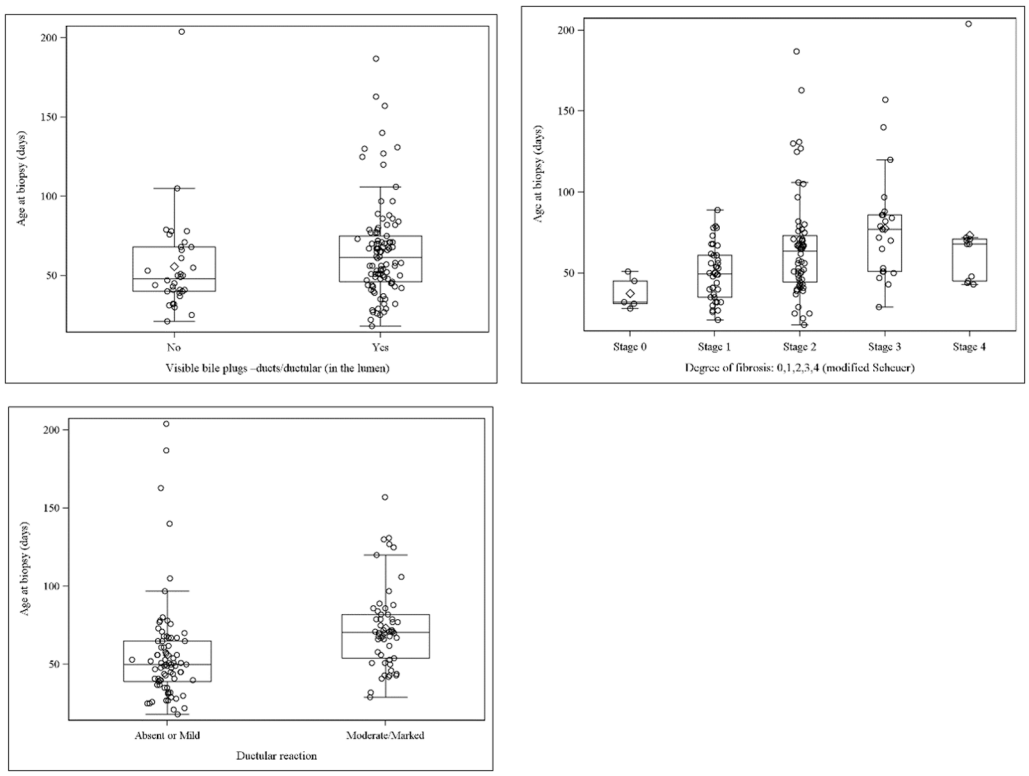

Figure 2.

Distribution of the age in days at the time of biopsy in cases of BA relative to (A) the presence or absence of visible bile plugs in ducts or ductules; (B) the stage of portal fibrosis by the Batts – Ludwig classification (stages 0,1, 2, 3 and 4); (C) the degree of ductular reaction (absent/mild versus moderate/marked). Box and whisker plots overlay the data. The bar within the box represents the median age, the box indicates the 25th and 75th percentiles, and the whiskers span 99% of the data if the data were normally distributed.

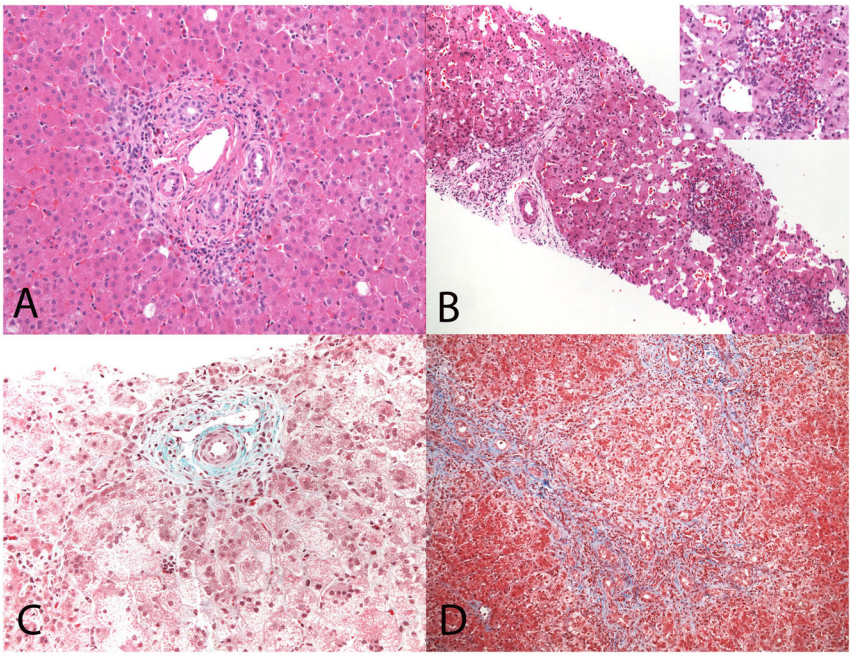

Figure 3.

(A) Liver biopsy from a 29 day-old infant with BA at the time of Kasai portoenterostomy. At central review, this case was considered indeterminate for large duct obstruction as portal tracts are not expanded, and there is no significant bile duct proliferation or ductular reaction(H+E, X200). (B) Liver biopsy from a 21 day-old subject with BA. There is a mildly enlarged portal tract without ductular reaction or bile duct proliferation. Several eosinophilic micro-abscesses are noted in the lobule towards the right of the photograph, and at higher magnification in the inset. This case was classified not consistent with LDO at central review(H+E, X100). (C) 78 day-old infant with BA and bile duct paucity. Though the features in this biopsy were considered to be not consistent with obstruction, the presence of laterality defects and splenic anomalies raised the clinical suspicion for BA, which was confirmed at the time of laparotomy (Masson-Trichrome, X 200). (D) 67 day-old subject with alpha-1 anti-trypsin deficiency. There is portal expansion with increased cellularity, and marked bile duct proliferation with prominent bile plugs (H+E, X100).

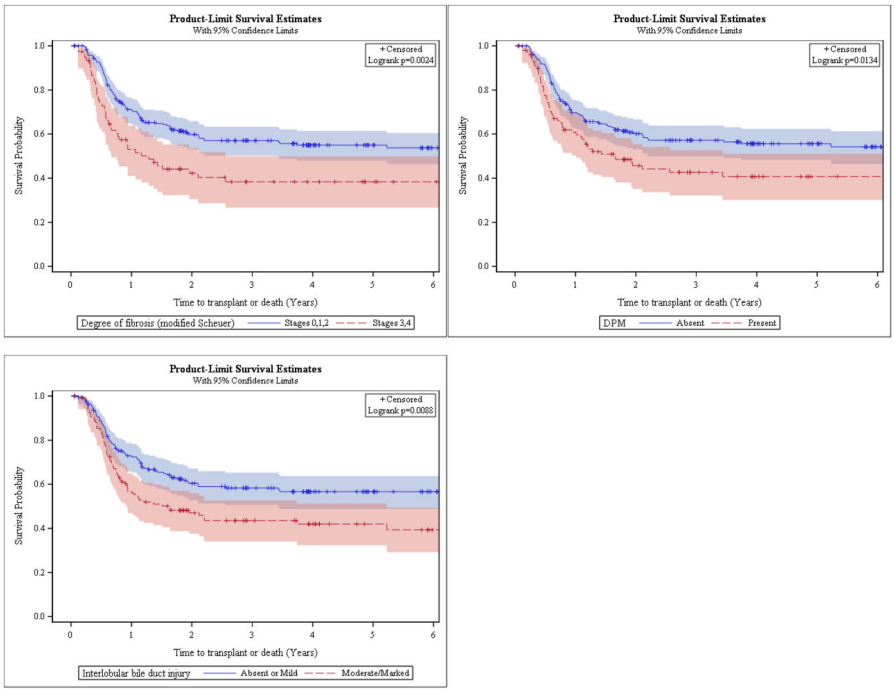

Figure 4.

Kaplan-Meier survival estimates for degree of liver fibrosis, presence of ductal plate configuration (DPM) and interlobular bile duct injury

Table 1

Clinical and demographic features

	Correlation of needle biopsies with clinical diagnosis	Correlation of liver biopsy features in BA with clinical outcome
N	227	316
BA cases	136	316
Non-BA cases	91	
Needle biopsies	227	110
Wedge biopsies		206
Sex (%)	Male (58%)	Female (50.3%)
Race		
White	125 (55%)	180 (57%)
Black	27 (12%)	38 (12%)
Other or missing	75 (33%)	98 (31%)
Median age at biopsy (SD)	58 days (29)	
Median age at HPE (SD)		63 days (25)
Extrahepatic anomalies (BA cases only)		
None	113 (83.0%)	280 (88.6%)
Laterality defect (BASM)	13 (9.5%)	27 (8.5%)
Non-laterality defect	9 (6.6%)	9 (2.8%)
Ohi subtype at HPE (BA cases only)	N= 136	N= 316
Type I	12 (8.8%)	30 (9.5%)
Type II	11 (8.0%)	23 (7.3%)
Type III	111 (81.6%)	260 (82.3%)
Missing	2 (1,4%)	3 (0.9%)
Observational study only		191
START study Placebo		62
START study treatment		62
Transplant		129 (40.8%)
No transplant		187 (59.2%)
Death		15 (4.7%)
Median follow-up (years)		3.9

Abbreviations: BASM, biliary atresia splenic malformation syndrome (syndromic BA)

Table 2

Clinical Diagnoses in cases assessed by needle biopsy

Clinical Diagnosis	Frequency
Biliary Atresia (BA)	136
Non-BA	91
Idiopathic neonatal hepatitis	32
Cholestasis, indeterminate	24
Alpha-1 antitrypsin deficiency	8
Alagille syndrome	11
Non-syndromic bile duct paucity	1
Cystic fibrosis	1
Mitochondrial DNA depletion	1
Panhypopituitarism	2
CMV hepatitis	3
Bile acid synthetic disorder	1
PFIC 1,2 or 3	3
Niemann-Pick type C	1
Neonatal ascites	1
Cholestasis with septo optic dysplasia	2
Total	227

Table 3

Histologic Features, n (%), by Clinical Diagnosis of BA vs Non-BA

Feature	Grading	Clinical Diagnosis BA (N=136)	Clinical Diagnosis Non BA (N=91)
Degree of fibrosis (modified Scheuer)	Stage 0	5 (3.7%)	50 (54.9%)
	Stage 1	38 (27.9%)	26 (28.6%)
	Stage 2	60 (44.1%)	10 (11.0%)
	Stage 3	22 (16.2%)	3 (3.3%)
	Stage 4	10 (7.4%)	2 (2.2%)
	Missing	1 (0.7%)	
Bile duct proliferation	Absent	31 (22.8%)	74 (81.3%)
	Focal, Mild	24 (17.6%)	7 (7.7%)
	Focal, Moderate/Marked	1 (0.7%)	
	Generalized, Mild	41 (30.1%)	6 (6.6%)
	Generalized, Moderate/Marked	37 (27.2%)	4 (4.4%)
	Missing	2 (1.5%)	
Ductular reaction	Absent	8 (5.9%)	50 (54.9%)
	Focal, Mild	23 (16.9%)	17 (18.7%)
	Focal, Moderate/Marked	1 (0.7%)	
	Generalized, Mild	49 (36.0%)	18 (19.8%)
	Generalized, Moderate/Marked	53 (39.0%)	5 (5.5%)
	Missing	2 (1.5%)	1 (1.1%)
Average intensity of peribiliary neutrophilia	Absent	37 (27.2%)	70 (76.9%)
	Focal, Mild	34 (25.0%)	7 (7.7%)
	Focal, Moderate/Marked	3 (2.2%)	
	Generalized, Mild	36 (26.5%)	8 (8.8%)
	Generalized, Moderate/Marked	25 (18.4%)	6 (6.6%)
	Missing	1 (0.7%)	
Visible duct and ductular bile plugs	No	34 (25.0%)	77 (84.6%)
	Yes	102 (75.0%)	14 (15.4%)
Ductal Plate Configuration	Absent	113 (83.1%)	91 (100.0%)

Feature	Grading	Clinical Diagnosis BA (N=136)	Clinical Diagnosis Non BA (N=91)
	Present	22 (16.2%)	
	Missing	1 (0.7%)	
Bile Duct Paucity	No	116 (85.3%)	53 (58.2%)
	Yes	13 (9.6%)	35 (38.5%)
	Missing	7 (5.1%)	3 (3.3%)
Acute cholangitis	Absent	115 (84.6%)	88 (96.7%)
	Rare	13 (9.6%)	1 (1.1%)
	Mild	5 (3.7%)	2 (2.2%)
	Moderate/Marked	2 (1.5%)	
	Missing	1 (0.7%)	
Interlobular bile duct injury	Absent	12 (8.8%)	45 (49.5%)
	Mild	35 (27.5%)	9 (9.9%)
	Moderate/Marked	87 (64.0%)	37 (40.7%)
	Missing	2 (1.5%)	
Periductal fibrosis	Absent	128 (94.1%)	89 (97.8%)
	Present	7 (5.1%)	2 (2.2%)
	Missing	1 (0.7%)	
Portal tract cellular infiltrates	Absent	44 (32.4%)	23 (25.3%)
	Focal, Mild	25 (18.4%)	19 (20.9%)
	Focal, Moderate/Marked	1 (0.7%)	
	Generalized, Mild	57 (41.9%)	36 (39.6%)
	Generalized, Moderate/Marked	8 (5.9%)	12 (13.2%)
	Missing	1 (0.7%)	1 (1.1%)
Portal stromal edema	Not Detected	92 (67.6%)	87 (95.6%)
	Present	41 (30.1%)	4 (4.4%)
	Missing	3 (2.2%)	
Lobular fibrosis	Absent	120 (88.2%)	76 (83.5%)
	Present, Focal	9 (6.6%)	4 (4.4%)
	Prominent	1 (0.7%)	9 (9.9%)

Feature	Grading	Clinical Diagnosis BA (N=136)	Clinical Diagnosis Non BA (N=91)
	Not Evaluable	5 (3.7%)	2 (2.2%)
	Missing	1 (0.7%)	
Hepatocellular swelling	Absent	22 (16.2%)	8 (8.8%)
	Rare (<5%)	37 (27.2%)	25 (27.5%)
	5% to <50%	48 (35.3%)	33 (36.3%)
	>=50%	26 (19.1%)	25 (27.5%)
	Missing	3 (2.2%)	
Macrosteatosis	Absent	123 (90.4%)	74 (81.3%)
	Rare (<5%)	6 (4.4%)	9 (9.9%)
	5% to <50%	3 (2.2%)	7 (7.7%)
	>=50%		1 (1.1%)
	Missing	3 (2.2%)	
Microsteatosis	Absent	121 (89.0%)	73 (80.2%)
	Rare (<5%)	6 (4.4%)	10 (11.0%)
	5% to <50%	6 (4.4%)	7 (7.7%)
	>=50%		1 (1.1%)
	Missing	3 (2.2%)	
Pseudorosette formations	Absent	10 (7.4%)	27 (29.7%)
	Present	37 (27.2%)	22 (24.2%)
	Prominent	86 (63.2%)	42 (46.2%)
	Missing	3 (2.2%)	
Giant cell transformation	Absent	18 (13.2%)	8 (8.8%)
	Rare	25 (18.4%)	10 (11.0%)
	Present	46 (33.8%)	22 (24.2%)
	Common	32 (23.5%)	27 (29.7%)
	Prominent	13 (9.6%)	24 (26.4%)
	Missing	2 (1.5%)	
Lobular extramedullary hematopoiesis	Absent	66 (48.5%)	21 (23.1%)
	Rare	45 (33.1%)	33 (36.3%)

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Feature	Grading	Clinical Diagnosis BA (N=136)	Clinical Diagnosis Non BA (N=91)
	Prevalent	19 (14.0%)	20 (22.0%)
	Extensive	3 (2.2%)	17 (18.7%)
	Missing	3 (2.2%)	
Regional non-zonal variability in hepatocyte cytoplasmic volume	Absent	133 (97.8%)	91 (100.0%)
	Missing	3 (2.2%)	
Giant cell necrosis	Absent	122 (89.7%)	79 (86.8%)
	Present	14 (10.3%)	12 (13.2%)
Lobular mononuclear inflammation	Absent	122 (83.1%)	73 (80.2%)
	Rare	14 (10.3%)	10 (11.0%)
	Focal	5 (3.7%)	3 (3.3%)
	Diffuse	1 (0.7%)	4 (4.4%)
	Missing	3 (2.2%)	1 (1.1%)
Hepatocellular necrosis	Absent/rare	84 (61.8%)	46 (50.5%)
	Few hepatocytes	40 (29.4%)	34 (37.4%)
	Many hepatocytes	10 (7.4%)	11 (12.1%)
	Missing	2 (1.5%)	
Clusters of coarsely granular red hepatocytes	Absent	133 (97.8%)	90 (98.9%)
	Rare		1 (1.1%)
	Missing	3 (2.2%)	
Compact aggregates of bile stained macrophages within portal tracts	Absent	83(61.0%)	59 (64.8%)
	Rare	14 (10.3%)	9 (9.9%)
	Present	36 (26.5%)	23 (25.3%)
	Missing	3 (2.2%)	
Compact aggregates of bile stained Kupffer cells in zone 1	Absent	90 (66.2%)	72 (79.1%)
	Rare	13 (9.6%)	5 (5.5%)
	Present	30 (22.1%)	14 (15.4%)
	Missing	3 (2.2%)	

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Table 4

Clinical Diagnosis vs Histologic Assignment

Histologic Assignment	Clinical Diagnosis		
	BA (%)	Non BA (%)	Total
Consistent with large duct obstruction (LDO)	107 (78.6)	6 (6.5)	
Indeterminate for LDO	14 (10.3)	8 (8.7)	
Not consistent with LDO	14 (10.3)	76 (83.5)	
Biopsy insufficient to render assignment	1 (0.7)	1 (1.0)	
Total	136 (100)	91 (100)	227

Table 5

Non-BA cases assigned as indeterminate or consistent with LDO

Diagnosis	Indeterminate for LDO (n)	Consistent with LDO (n)
INH	4	1
Alpha-1 antitrypsin deficiency		2
Alagille syndrome	1	
Bile acid synthetic disorder	1	
Indeterminate cholestasis	2	1
Niemann-Pick type C		1
Cystic fibrosis		1

Abbreviations: INH, Idiopathic neonatal hepatitis; LDO, large duct obstruction

Table 6

Histologic characteristics of 14 cases clinically diagnosed BA cases interpreted by pathologists as NOT consistent with a large duct obstruction

Age at biopsy (days)	Bile duct/ portal tract ratio	Major histologic findings and reason for discrepant diagnosis
21	0.83	No significant portal expansion or bile duct proliferation. Several eosinophilic micro-abscesses in the lobule
30	0.33	No portal expansion, bile duct proliferation or bile duct plugs. Bile duct paucity suspected.
31	0.93	Minimal portal expansion; no significant bile duct proliferation. No ductal bile plugs
32	0.875	Generally small hypoplastic-appearing portal tracts without significant bile duct proliferation; no bile plugs
41	0.32	Many small portal tracts without bile ducts, suspected bile duct paucity
47	0.38	Minimal bile duct proliferation, no bile duct plugs, prominent giant cell proliferation. Bile duct paucity suspected
45	1.0	Only focal portal expansion and ductular reaction. Significant steatosis
50	1.0	Only focal bile duct proliferation. Extensive hepatocellular giant cell transformation
53	0.375	No significant bile duct proliferation or ductular reaction. No bile duct plugs. Prominent giant cell transformation. Bile duct paucity suspected
54	0.1	Minimal portal expansion. Mild ductular reaction with only rare bile plugs. Suspected bile duct paucity.
78	0.60	Small portal tracts without bile duct proliferation or ductular reaction
78	0.33	No significant fibrosis, bile duct proliferation and no plugs. Suspected bile duct paucity. Extensive Giant cell transformation. Situs inversus increased clinical suspicion for BA and cholangiogram undertaken which was consistent with BA
127	0.50	Moderate portal expansion and ductular reaction. Suspected paucity with decreased bile duct/portal tract ratio. Only rare bile duct plugs
204	>1	Minimal ductular reaction and no bile duct plugs

Table 7

Summary of Key Histopathologic Features in 316 liver biopsies from BA patients

Variable		N (%)
Type of Specimen	Needle	110 (34.8%)
	Wedge	206 (65.2%)
Study/Treatment Group	PROBE Only	192 (60.8%)
	START Placebo	62 (19.6%)
	START Treatment	62 (19.6%)
Number of Portal Tracts	<5 Portal Tracts	8 (2.5%)
	>=5 Portal Tracts	305 (96.5%)
	Missing	3 (0.9%)
Bile Duct Paucity	No	270 (85.4%)
	Yes	10 (3.2%)
	Missing	36 (11.4%)
Degree of fibrosis (modified Scheuer)	Stage 0	8 (2.5%)
	Stage 1	59 (18.7%)
	Stage 2	170 (53.8%)
	Stage 3	49 (15.5%)
	Stage 4	27 (8.5%)
	Missing	3 (0.9%)
DPM	Absent	211 (66.8%)
	Present	102 (32.3%)
	Missing	3 (0.9%)
Ductular reaction	Absent	8 (2.5%)
	Focal, Mild	47 (14.9%)
	Focal, Moderate/Marked	1 (0.3%)
	Generalized, Mild	116 (36.7%)
	Generalized, Moderate/Marked	139 (44.0%)
	Missing	5 (1.6%)
Bile duct proliferation	Absent	34 (10.8%)
	Focal, Mild	34 (10.8%)
	Focal, Moderate/Marked	1 (0.3%)
	Generalized, Mild	103 (32.6%)
	Generalized, Moderate/Marked	140 (44.3%)
	Missing	4 (1.3%)
Mononuclear inflammatory cells in bile duct epithelium	Absent	262 (82.9%)
	Rare	35 (11.1%)
	Common	8 (2.5%)
	Missing	11 (3.5%)
Periductal fibrosis	Absent	298 (94.3%)

Variable		N (%)
	Present	15 (4.7%)
	Missing	3 (0.9%)
Portal tract edema	Not Detected	249 (78.8%)
	Present	58 (18.4%)
	Missing	9 (2.8%)
Lobular fibrosis	Absent	265 (83.9%)
	Present, Focal	30 (9.5%)
	Prominent	8 (2.5%)
	Not Evaluable	10 (3.2%)
	Missing	3 (0.9%)
Hepatocellular swelling	Absent	50 (15.8%)
	Rare (<5%)	74 (23.4%)
	5% to <50%	125 (39.6%)
	>=50%	58 (18.4%)
	Missing	9 (2.8%)
Macrosteatosis	Absent	274 (86.7%)
	Rare (<5%)	26 (8.2%)
	5% to <50%	6 (1.9%)
	>=50%	1 (0.3%)
	Missing	9 (2.8%)
Microsteatosis	Absent	285 (90.2%)
	Rare (<5%)	14 (4.4%)
	5% to <50%	8 (2.5%)
	Missing	9 (2.8%)
Pseudorosette formations	Absent	19 (6.0%)
	Present	68 (21.5%)
	Prominent	219 (69.3%)
	Missing	10 (3.2%)
Giant cell transformation	Absent	34 (10.8%)
	Rare	57 (18.0%)
	Present	111 (35.1%)
	Common	74 (23.4%)
	Prominent	32 (10.1%)
	Missing	8 (2.5%)
Lobular extramedullary hematopoiesis	Absent	112 (35.4%)
	Rare	124 (39.2%)
	Prevalent	51 (16.1%)
	Extensive	20 (6.3%)
	Missing	9 (2.8%)

Variable		N (%)
Regional non-zonal variability in hepatocyte cytoplasmic volume	Absent	307 (97.2%)
	Missing	9 (2.8%)
Giant cell necrosis	Not Stated	255 (80.7%)
	Stated	61 (19.3%)
Lobular mononuclear inflammation	Absent	272 (86.1%)
	Rare	21 (6.6%)
	Focal	9 (2.8%)
	Diffuse	4 (1.3%)
	Missing	10 (3.2%)
Portal tract cellular infiltrates	Absent	60 (19.0%)
	Focal, Mild	46 (14.6%)
	Focal, Moderate/Marked	1 (0.3%)
	Generalized, Mild	167 (52.8%)
	Generalized, Moderate/Marked	38 (12.0%)
	Missing	4 (1.3%)
Visible bile plugs	No	58 (18.4%)
	Yes	258 (81.6%)
Interlobular bile duct injury	Absent	93 (29.4%)
	Mild	99 (31.3%)
	Moderate/Marked	119 (37.7%)
	Missing	5 (1.6%)
Acute cholangitis	Absent	253 (80.1%)
	Rare	38 (12.0%)
	Mild	17 (5.4%)
	Moderate/Marked	5 (1.6%)
	Missing	3 (0.9%)
Hepatocellular necrosis	Absent/rare	147 (46.5%)
	Few hepatocytes	111 (35.1%)
	Many hepatocytes	54 (17.1%)
	Missing	4 (1.3%)
Clusters of coarsely granular red hepatocytes	Absent	306 (96.8%)
	Missing	10 (3.2%)
Compact aggregates of bile stained macrophages within portal tracts	Absent	163 (51.6%)
	Rare	35 (11.1%)
	Present	109 (34.5%)
	Missing	9 (2.8%)
Compact aggregates of bile stained Kupffer cells in zone 1	Absent	182 (57.6%)
	Rare	28 (8.9%)
	Present	97 (30.7%)

Variable		N (%)
	Missing	9 (2.8%)
Average intensity of neutrophils	Absent	82 (25.9%)
	Focal, Mild	71 (22.5%)
	Focal, Moderate/Marked	4 (1.3%)
	Generalized, Mild	101 (32.0%)
	Generalized, Moderate/Marked	55 (17.4%)
	Missing	3 (0.9%)
Gender	Male	157 (49.7%)
	Female	159 (50.3%)
Race	White	180 (57.0%)
	Black or African American	38 (12.0%)
	Other	90 (28.5%)
	Missing	8 (2.5%)
Ethnicity	Non-Hispanic	241 (76.3%)
	Hispanic	74 (23.4%)
	Missing	1 (0.3%)
BASM (biliary atresia splenic malformation, syndromic BA)	BASM 7/11	27 (8.5%)
	Isolated Rotation	9 (2.8%)
	Neither	280 (88.6%)
BA subtype	Type I	30 (9.5%)
	Type II	23 (7.3%)
	Type III	260 (82.3%)
	Missing	3 (0.9%)
Age at Kasai (days)	N	316
	Mean(STD)	63.81 (25.08)
	Median (IQR)	61.00 (29.50)
	Min, Max	16.00, 169.00