Factors Influencing Time-to-Diagnosis of Biliary Atresia

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Abstract

Objectives—Diagnosing biliary atresia (BA) quickly is critical, because earlier treatment correlates with delayed or reduced need for liver transplantation. However, diagnosing BA quickly is also difficult, with infants usually treated after 60 days of life. In this study, we aim to accelerate BA diagnosis and treatment, by better understanding factors influencing the diagnostic timeline.

Methods—Infants born between 2007–2014 and diagnosed with BA at our institution were included (n=65). Two periods were examined retrospectively: P_1, the time from birth to specialist referral, and P_2, the time from specialist referral to treatment. How sociodemographic factors associate with P_1 and P_2 were analyzed with Kaplan-Meier curves and Cox proportional hazard models. In addition, to better characterize P_2, laboratory results and early tissue histology were studied.

Results—P_1 associated with race/ethnicity, with shorter times in non-Hispanic white infants compared to non-Hispanic black and Hispanic infants (p=0.007 and p=0.004, respectively). P_2 associated with referral age, with shorter times in infants referred after 30, 45, or 60 days of life (p<0.001, p<0.001, and p=0.001, respectively). One potential reason for longer P_2 in infants referred 30 days is that aminotransferase levels were normal or near-normal. However, despite reassuring laboratory values, tissue histology in early cases showed key features of BA.

Conclusions—Our findings suggest two opportunities to accelerate BA diagnosis and treatment. First, to achieve prompt referrals for all races/ethnicities, universal screening strategies should be considered. Second, to ensure efficient evaluations independent of age, algorithms designed to detect early features of BA can be developed.

Introduction

Biliary atresia (BA), the most common indication for pediatric liver transplantation, is a serious liver disease of infancy progressing from cholestasis to cirrhosis to end-stage liver disease in the first year of life.[1] One intervention—the Kasai portoenterostomy (KP)—has been proven to slow BA's rapid course. Importantly, the age at which the KP is performed is critical.[2–8] Infants receiving the KP before 30-45 days of life (DoL) have the best chances of delaying or avoiding need for liver transplant.[3,4] In contrast, infants receiving the KP after 90-120 DoL are less likely to benefit.[9] Hence, an important goal in pediatric hepatology is diagnosing BA quickly to optimize timing of the KP.

Unfortunately, diagnosing BA is difficult, with the average age of KP in the United States greater than 60 DoL.[9,10] Reasons for later diagnoses have focused on the challenges primary care providers (PCPs) face in identifying and referring infants.[11] For example, many PCPs schedule newborn visits in the first two weeks and then again at two months of life, in accord with current recommendations. As a result, PCPs may not see infants within this interval, when concerning symptoms such as pale stools typically develop.[12] In addition, PCPs may not always suspect BA initially in infants with persistent jaundice. This is because BA is rare (incidence 1:10,000-18,000 births) compared to benign physiological jaundice affecting as many as 15% of all healthy newborns.[13]

The objective of this study is to identify opportunities to accelerate BA treatments. To accomplish this, we sought to identify additional factors influencing the timing of the BA diagnosis. We focus our analysis on two sequential periods: P_1, the time in which PCPs recognize and refer infants, and P_2, the time in which specialists such as pediatric gastroenterologists and surgeons evaluate infants and perform the KP.

Methods

Patient selection

This study was approved by the institutional review board at Baylor College of Medicine. All infants born between 2007-2014 and diagnosed with BA at Texas Children's Hospital (TCH), a large pediatric specialty center in Houston, Texas, were eligible. In subjects receiving a KP, the BA diagnosis was established by intraoperative cholangiogram at the time of KP and/or pathological examination of bile duct remnants after KP. In subjects not receiving a KP, the diagnosis of BA was inferred based on the clinical picture, liver biopsy at presentation, absence of alternative diagnoses explaining persistent cholestasis, and pathological examination of the explant after transplant. The decision to not perform a KP was made on a case-by-case basis, and was influenced by the subject's age, fibrosis stage on liver biopsy, and/or signs of portal hypertension already present at the time of presentation.

Patients diagnosed with BA elsewhere and referred to TCH for further care were not eligible for the study.

Data Acquisition

The BA diagnostic timeline was divided into two sequential periods: P_1, the time from birth to specialist referral, and P_2, the time from first specialist visit to KP (Figure, Supplemental Digital Content 1). To calculate these intervals and analyze associations between sociodemographic variables, the following were collected retrospectively from each subject's electronic medical record: birth date, date of first specialist appointment at TCH, occurrence of prior specialist care before referral, date of KP, gender, race, ethnicity, preferred language, insurance type, zip code (to determine distance from TCH), birth era, and birth season. Race and ethnicity were combined and categorized as non-Hispanic white, non-Hispanic black, non-Hispanic Asian, or Hispanic. Distance was designated as 50 miles and >50 miles from TCH, to distinguish those who could and could not travel easily between home and hospital during the evaluation. Birth era was either 2007-2010 or 2011-2014, to help detect any changes in P_1 or P_2 as advances in the field were made. Birth season was divided into Winter (January-March), Spring (April-June), Summer (July-September), or Fall (October-December).

To correlate initial laboratory results at TCH with age, values and the dates they were drawn were obtained retrospectively from each subject's electronic medical record. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AlkP), and gamma glutamyltransferase (GGT) values were measured using standard techniques. Unconjugated bilirubin (Bu) and conjugated bilirubin (Bc) were measured using direct spectrophotometry with the VITROS analyzer (Ortho Clinical Diagnsotics, Raritan, NJ). Total and direct bilirubin were not measured. Reference intervals for AST, ALT, Bc, AlkP, and GGT were derived by the TCH laboratory with the middle 95% of values from age-matched populations.[14] Reference intervals for Bc ratios were borrowed from the existing literature, with a Bc ratio >0.20 signifying liver disease and a Bc ratio 0.20 marking red blood cell turnover and Bu processing.[15]

To analyze histopathology in early BA cases, liver and biliary remnant tissue were obtained from the TCH Department of Pathology archives. Samples were stained with the following: hematoxyline and eosin (H&E), to study morphology and identify bile plugs; trichrome, to measure the amount of fibrosis; anti-Cytokeratin 7 (CK7) antibodies, to outline bile duct/ductule morphology and number; and anti-CD56 antibody, to detect reactive bile duct/ductules.[16–18] Liver samples were evaluated by an experienced pathologist (MJF) based on common findings in BA as outlined by ChiLDReN.[19] Bile duct proliferation was categorized as none, focal, or generalized. Portal fibrosis was graded as absent/some tracts, most tracts, focal bridging, marked bridging, or cirrhosis. Bile plugs were rated as absent, canalicular only, bile duct only, or bile duct and canalicular.

Statistical Analysis

For the time-to-event analyses, two outcomes were studied: (1) first specialist appointment, where P_1 represented time-to-event and was calculated as ([date of first specialist

appointment at TCH] — [birth date]); and (2) KP, where P_2 represented time-to-event and was calculated as ([date of KP] — [date of first specialist appointment at TCH]). Kaplan-Meier curves and the log-rank test were used to characterize P_1 or P_2 relative to subject attributes, while univariate Cox proportional hazards models were used to determine the direction of associations between P_1 or P_2 and subject attributes. We tested the proportional hazard assumption for our variables of interest by evaluating the Schoenfeld residuals after fitting each model, and also by using the time varying covariates option in Stata. In these analyses, the proportional hazards assumption was not violated. Multivariable analyses were not performed because of limited sample sizes.

For analysis of laboratory values, Bc ratios were calculated as ([Bc]/[Bc+Bu]) based on the equation suggested by Moyer and colleagues.[15] Spearman's rank correlation coefficients (r_s) with one-tailed p-values were used to describe correlations between test results and age, based on assumptions that laboratory data is non-parametric, right-skewed (away from the zero value), and expected to increase with age if a correlation exists. All calculations were performed using Microsoft Excel (Seattle, WA), GraphPad Prism (La Jolla, CA), and Stata 14.0 (College Station, TX) software.

Results

There were 65 subjects born between 2007-2014 and diagnosed with BA at TCH (Table, Supplemental Digital Content 2). This represents the majority of predicted cases in the region, assuming an incidence of 1:10,000-18,000 births and reported birth rate of 90,000/year.[20] More subjects were female than male, as has been reported previously in some BA studies. In addition, more subjects were Hispanic than non-Hispanic white, non-Hispanic black, or non-Hispanic Asian, reflecting the racial/ethnic composition of children in the study city. Most subjects were born to parents who spoke English, had public insurance, and lived within 50 miles of the study hospital. Approximately the same number of subjects were born between 2007-2010 and 2011-2014, and subjects' birth dates were evenly distributed among the seasons.

We first focused on the beginning of the diagnostic timeline, when PCPs identify infants and refer them to specialists for further evaluation (denoted as P_1 in this study). For this analysis, 12 subjects referred directly from the nursery before the first PCP visit and nine subjects referred to specialists at other institutions before evaluation at our center were excluded (Figure, Supplemental Digital Content 1). The mean P_1 time was 72.9±39.9 days for the remaining 44 subjects (Table, Supplemental Digital Content 2). Race/ethnicity was associated with age at referral, with non-Hispanic white subjects having significantly shorter P_1 times than Hispanic and non-Hispanic black subjects (p<0.01) (Figure 1). These associations were confirmed with Cox analyses, with non-Hispanic black (HR 0.23, 95% CI 0.08-0.67, p=0.007) and Hispanic (HR 0.30, 95% CI 0.13-0.68, p=0.004) subjects having a lower likelihood of early referral (Table, Supplemental Digital Content 3). No significant differences in P_1 were found among other attributes such as gender, preferred language, insurance type, distance from our center, birth era, or birth season (Table, Supplemental Digital Content 3; Figure, Supplemental Digital Content 4).

We next focused on the second part of the diagnostic timeline, when specialists such as pediatric gastroenterologists and surgeons evaluate and perform the KP (denoted as P_2 in this study). For this analysis, 16 subjects referred too late for the KP and one subject with congenital heart disease precluding further operations were excluded (Figure, Supplemental Digital Content 1). The mean P_2 time was 15.6±23.0 days for the remaining 48 subjects (Table, Supplemental Digital Content 2). Shorter P_2 times were strongly associated with older ages at referral. Figure 2a is a scatter plot demonstrating an inverse relationship between evaluation time and age, and Figures 2b-d show significant differences in P_2 times for subjects referred before or after 30, 45, and 60 DoL, respectively. These associations were confirmed with Cox analyses, with subjects presenting after 30 DoL (HR 15.47, 95% CI 4.43-53.99, p<0.001), 45 DoL (HR 6.94, 95% CI 2.72-17.71, p<0.001) and 60 DoL (HR 3.12, 95% CI 1.55-6.25, p=0.001) having a higher likelihood of faster specialist evaluation and treatment (Table, Supplemental Digital Content 5). Shorter P_2 times were also associated with non-summer birth months and living beyond 50 miles of the study hospital (Table, Supplemental Digital Content 5; Figure, Supplemental Digital Content 6).

We hypothesized that one reason for longer P_2 times in younger infants is that laboratory results may be equivocal in early stages of disease. To test this, initial AST, ALT, Bc, AlkP, GGT, and Bc ratios were plotted versus age for all 65 subjects. In accord with our hypothesis, initial AST and ALT were within laboratory reference intervals in infants less than 30 DoL, a result which could be falsely reassuring (Figure 3). Initial AST and ALT were higher in older infants, with a positive correlation with age (r_s=0.77, 95% CI 0.64-0.85, p<0.0001 and r_s=0.79, 95% CI 0.67-0.87, p<0.0001, respectively). In contrast, initial Bc was elevated in all ages. Initial AlkP and GGT were less predictable, and were normal or elevated at different ages (Figure, Supplemental Digital Content 7). Finally, initial Bc ratios were elevated at all ages except when calculated in the immediate post-natal period, consistent with a previous report.[21]

Another potential reason for longer P_2 times in younger infants is that specialists may wait to allow time for histological changes to occur and disease to become more apparent. To investigate this concept further, samples from the six subjects undergoing liver biopsy before 30 DoL were studied (Table, Supplemental Digital Content 8). Contrary to our expectations, liver tissue from all six cases showed signs of extrahepatic obstruction, including bile duct proliferation (highlighted best with cytokeratin and CD56 immunohistochemistry), portal fibrosis, and bile duct plugging (Figure 4a-d). In addition, despite pigmented stools in five of six cases, biliary remnants from all six cases had areas devoid of patent ducts (Figure 4e-f). These results suggest that even at early ages there can be complete obliteration of the biliary tract.

Discussion

We analyzed the BA diagnostic timeline to identify factors which correlate with how quickly BA is diagnosed. For the first period (P_1), non-Hispanic white infants were referred sooner than non-Hispanic black and Hispanic infants. PCPs could be detecting jaundice more readily in light-skinned infants, which could explain later referrals in non-Hispanic black infants and some Hispanic infants. Another possibility is that parents from certain cultures,

races, or ethnicities may be more likely to access the health care system and/or attend scheduled well-child visits. Based on our findings, such a culture effect would be independent of language spoken, insurance status, or travel distance from a specialty center. A third explanation is that PCPs may have implicit biases leading to uneven treatment across populations. Implicit biases have been recognized in other pediatric disease, but their role in BA has not been explored.[22]

For the second period (P_2), infants have longer evaluation times when referred earlier and vice versa. This may be because specialists could be less suspicious of BA in younger infants who have normal or near normal aminotransferase levels. Alternatively, specialists may purposely postpone evaluations in early referrals to allow time for the disease to progress. Such an approach avoids "over-testing" infants whose cholestasis would otherwise self-resolve. It also theoretically minimizes the potential of false-negative biopsy results, which are assumed to occur in early stages of disease.[23] Shorter P_2 times were also found in infants born in non-summer months and living further from the treatment center. The former could reflect reduced availability of pediatric gastroenterologists, interventional radiologists, pathologists, and/or pediatric surgeons during summer vacation months, whereas the latter suggests that diagnostic tests are consolidated for those who cannot easily travel back and forth between home and hospital.

One important question and potential limitation is whether our results are applicable to other regions. Our study population may be atypical, with high percentages of Hispanic infants (54%) and of infants identified too late to receive the KP (17/65 or 26%). However, a number of previously published findings suggest our observations may be similar in other parts of the United States. First, older KP ages for Hispanic and/or African American infants have been reported in single- and multiple-hospital studies.[9,24,25] Second, large variations have been described in the time North American specialists take to evaluate infants, though the reasons for these variations were not explored.[3,9] Third, guidelines for specialists caution against liver biopsies in younger infants, to avoid false-negative results (see below).[23] Hence, specialists following these guidelines would be expected to delay evaluations in younger infants. Future prospective studies in different regions can further examine the applicability of our findings.

Assuming our results are applicable to other regions, specific policy solutions could accelerate the diagnosis and treatment of BA. For P_1, screening programs could be applied universally independent of race/ethnicity. Two such programs were discussed in a recent American Academy of Pediatrics technical report.[26] The most studied approach is the stool color card program.[27–30] This program relies on PCPs and/or parents identifying infants who may have BA based on the pale-colored stools which develop over time. A second approach still under investigation is newborn direct/conjugated bilirubin screening.[31–34] This approach capitalizes on elevated direct/conjugated bilirubin levels shortly after birth in infants with BA.[21]

For P_2, efforts to help specialists appreciate the urgency of evaluations—even in infants less than 30 DoL—should be prioritized. To help accomplish this, specialists need diagnostic algorithms which account for findings in very young infants. Current algorithms have been

derived from older infants who already have markedly elevated AST and ALT levels.[35,36] In young infants, however, AST and ALT levels should be normal or close to normal, and, perhaps counter-intuitively, do not exclude BA. Current algorithms also require infants to have high Bc levels, such as above 2.5 mg/dL in one study.[36] While young infants with BA will have Bc levels above laboratory reference intervals, their levels may not always exceed those found in older infants.[21,37] Similarly, some algorithms use Bc ratios greater than 0.2 to indicate cholestasis.[15] Most measurements from younger infants fit this rule, except in some circumstances in the few days after birth.

Our findings suggest that liver biopsies could be helpful in diagnostic algorithms to detect early BA. The utility of such early biopsies has been controversial. On one hand, current guidelines caution against early biopsies, because of potentially false-negative results if sampling occurs before disease has had time to progress.[23] On the other hand, both a meta-analysis and large consortium study found no correlation with young age and false-negative biopsy results. [38,39] Interestingly, reports which caution against early biopsies usually cite a case series which featured an infant with BA who had a normal biopsy at three weeks of life. [40] However, the same infant had normal biopsies at five and nine weeks of life as well, raising the possibility that the false-negative results may have been related to other factors rather than age at sampling.

Finally, the issue of "over-testing" young infants who may or may not have BA deserves special mention. Waiting potentially protects infants from the risks of diagnostic tests, such as pain from blood draws, radiation exposure from imaging, and/or side-effects of anesthesia. However, waiting also potentially exposes infants to risks of progressing liver disease. We found complete obliteration of bile ducts in the earliest ages sampled, suggesting that waiting would lead to worsening bile back-up and resulting liver injury. Furthermore, the high-grade fibrosis found in some early cases argues strongly against delaying evaluations simply based on age. This interpretation agrees with data from centers around the world, which correlate an earlier KP with the best chance of delaying or preventing need for liver transplantation.[2] For example, a KP performed before 30 DoL in a Canadian study correlated with the highest 10-year liver transplant-free survival rates.[3] Similarly, a KP performed before 45 DoL in a French study was associated with 12.1% higher transplant-free survival rates after 15 years, and a KP performed before 30-45 DoL correlated with reduced need for liver transplant in Swiss and United States studies.[4–6]

Conclusion

By examining factors influencing the time-to-diagnosis in BA, this study identifies opportunities to accelerate the timeline and potentially improve outcomes. In the period from birth to specialist referral, infants of certain races/ethnicities are referred sooner. To ensure even referral times for all races/ethnicities, PCPs could be aided by universal screening programs such as the stool color card and/or newborn direct/conjugated bilirubin measurements. In the period from specialist referral to KP, infants referred later receive faster evaluations. To ensure even evaluation times independent of referral ages, specialists could follow diagnostic algorithms designed for younger infants. Such algorithms would

recognize normal aminotransferase in cases   30 DoL, and not wait for disease progression if liver biopsy is used as a diagnostic test.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

BA	biliary atresia
KP	Kasai portoenterostomy
DoL	day of life
PCP	primary care physician
TCH	Texas Children's Hospital
AST	aspartate aminotransferase
ALT	alanine aminotransferase
AlkP	alkaline phosphatase
GGT	gamma glutamyltransferase
Bu	unconjugated bilirubin
Bc	conjugated bilirubin
H&E	hematoxyline and eosin
CK7	cytokeratin 7

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What is Known

- Infants with biliary atresia (BA) must be identified and treated quickly to delay or avoid need for liver transplant.

- Unfortunately, early diagnoses before 30-45 days of life are uncommon, for reasons which have not been fully explored.

What is New

- We identify factors which correlate with the length of time from (i) birth to specialist referral and (ii) specialist referral to diagnosis and treatment.

- Policy makers can use our findings to design programs which accelerate the BA diagnostic timeline.
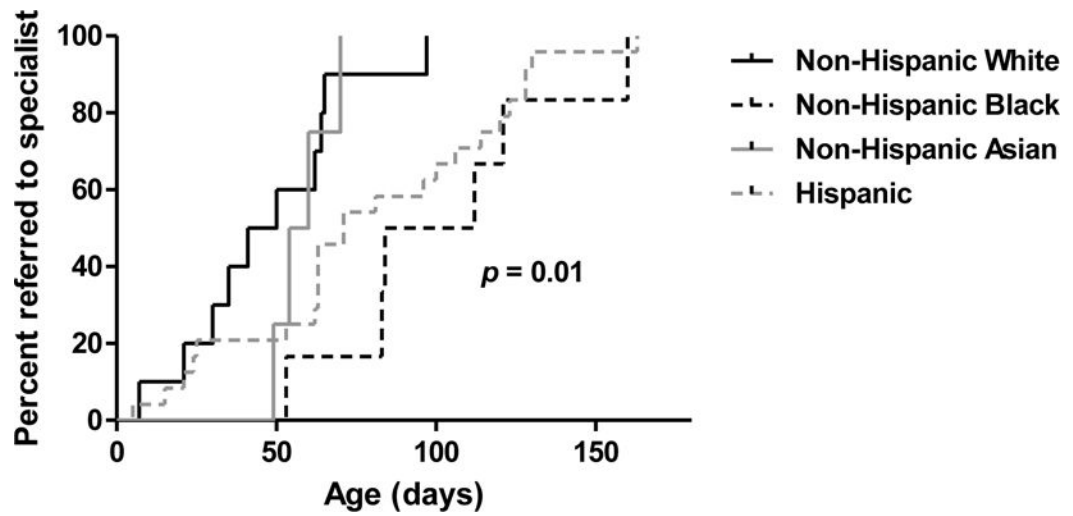
Figure 1. Later referrals for non-Hispanic black and Hispanic subjects
Kaplan-Meier curves for time to referral based on race/ethnicity. Differences among groups were calculated using the log-rank test.
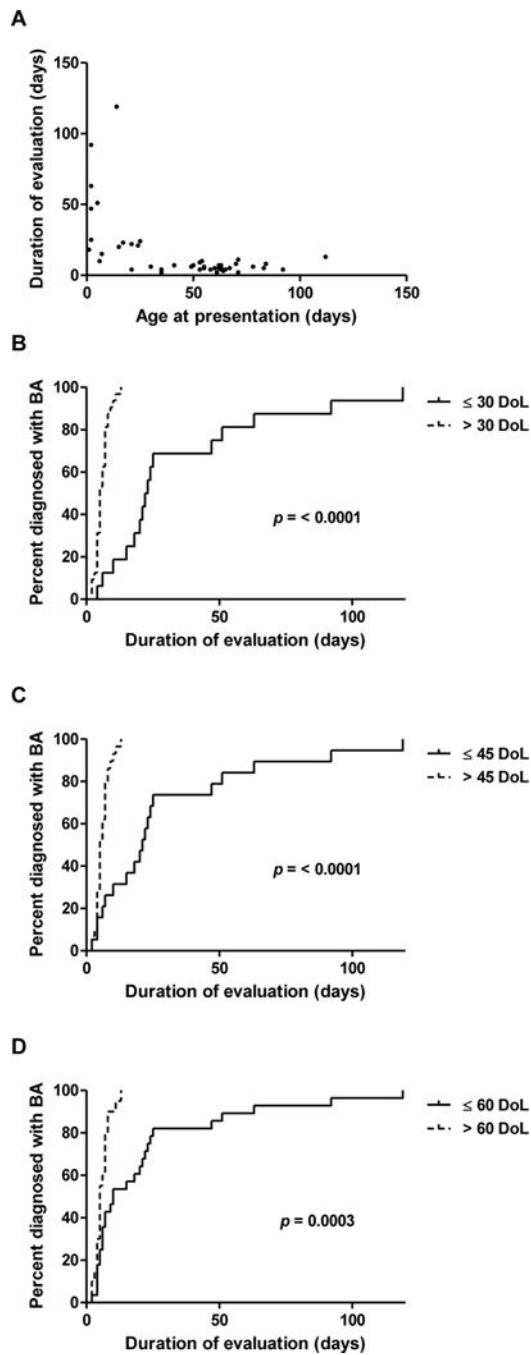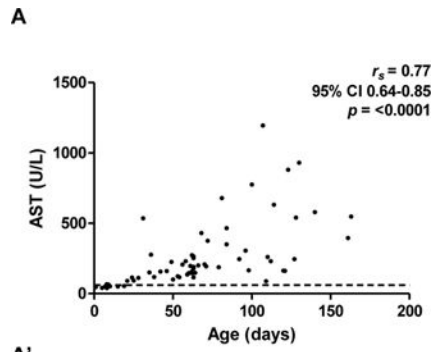
Figure 2. Evaluation times vary inversely with age at referral

(A) Scatter plot of P_2 times and age at presentation demonstrating an inverse relationship. (B-D) Kaplan-Meier curves for time to KP after specialist referral, based on age at presentation. Differences between groups were calculated using the log-rank test.
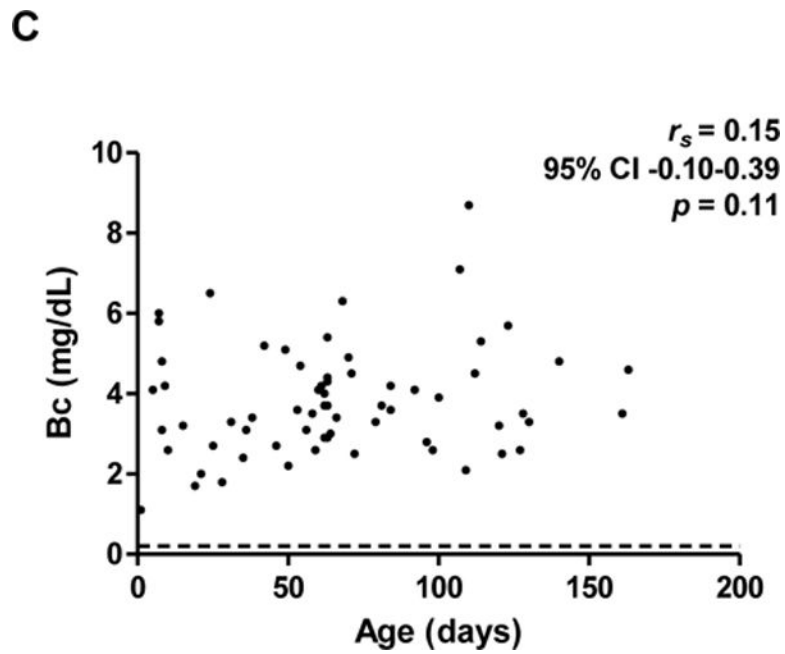
A

A'

B

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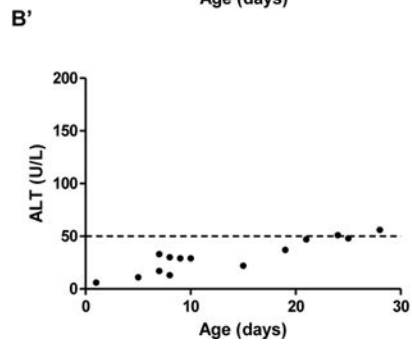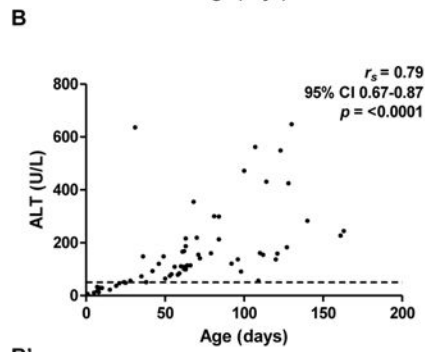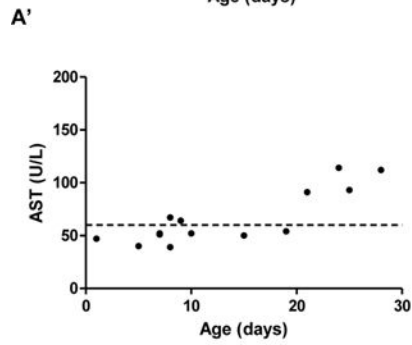
Figure 3. Normal/near-normal transaminases, but elevated Bc, in first 30 DoL
Initial AST (A) and ALT (B) levels, with insets (A′) and (B′) highlighting first 30 DoL. (C)
Initial Bc levels. Dashed lines denote upper limits of normal (reference intervals: AST 20-60
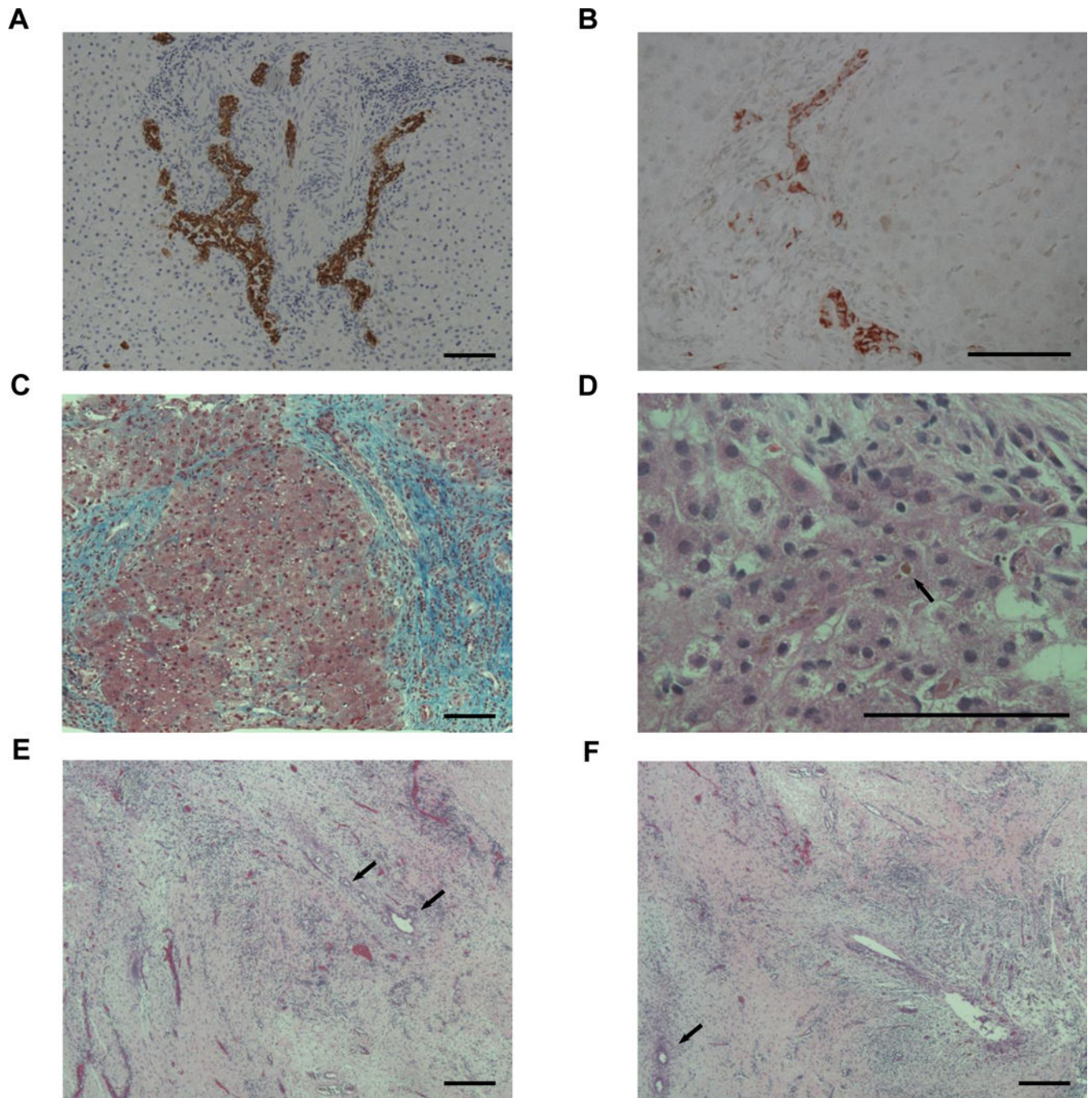U/L; ALT 6-50 U/L; Bc 0-0.2 mg/dL).

Figure 4. Abnormal early histology in liver and biliary remnants
(A-D) Liver. (A) Duct proliferation (brown) at DoL 15 (case 1, 100X, anti-CK7). (B)
Reactive ducts/ductules (red) at DoL 30 (case 6, 200X, anti-CD56). (C) Bridging fibrosis
(blue) at DoL 21 (case 4, 100X, trichrome). (D) Canalicular bile plugging (arrow) at DoL 24
(case 5, 400X, H&E). (E-F) Biliary remnants. Areas of left (E) and right (F) hepatic duct
tissue devoid of large, epithelial-lined ducts at DoL 16. Some small duct branches with

epithelial cells loss, distorted lumens, and circumferential fibrosis are seen (arrows) (case 2, 100X, H&E). Bar=100 uM.