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# Biliary Atresia: Do Histopathological Insights Predict Clinical Outcomes?

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## Abstract

# Purpose

Biliary atresia (BA) is a progressive fibroinflammatory cholangiopathy of infancy and remains the leading indication for pediatric liver transplantation. Although early Kasai portoenterostomy (KPE) is known to improve outcomes, the optimal age threshold for intervention remains debated.

# Methods

Liver biopsies obtained during KPE from 24 patients with BA were retrospectively reviewed. Histopathological features, including portal fibrosis, bile duct proliferation, and associated cholestatic changes, were systematically evaluated. Clinical data, bilirubin levels, and native liver survival (NLS) were analyzed in relation to age at KPE, with particular focus on 45- and 60-day thresholds.

# Results

All cases demonstrated portal fibrosis and bile duct proliferation. Severe fibrosis (grades 3–4) and diffuse ductular proliferation were significantly more frequent in patients undergoing KPE after 45 days of age and correlated with increasing age at surgery. Patients operated before 45 days exhibited significantly lower preoperative bilirubin levels and less advanced histopathological changes. During follow-up, patients with NLS shorter than one year predominantly underwent KPE after 45 days. Elevated direct (> 1 mg/dL) and total (> 2 mg/dL) bilirubin levels at postoperative month three were strongly associated with reduced NLS, whereas a ≥ 30% reduction in total bilirubin predicted prolonged native liver survival. Kaplan–Meier analysis demonstrated a significant survival advantage for patients undergoing KPE within the first 45 days of life.

# Conclusion

Our findings indicate that a 45-day age threshold more accurately reflects histopathological progression, early biochemical response, and native liver survival in BA. These results support considering KPE within the first 45 days of life to optimize long-term outcomes.

## Introduction

Biliary atresia (BA) is an idiopathic progressive sclerosing inflammatory cholangiopathy involving intrahepatic and extrahepatic biliary trees and rapidly results in hepatic cirrhosis. It is one of the most feared scenarios in a child with jaundice as none of the medical treatments is curative for BA. Therefore it is still the leading indication for liver transplantation (LT) in childhood, despite its rarity [1].

BA accounts for approximately 25–30% of neonatal jaundice cases, being more prevalent in Asia and the Pacific region. It affects females slightly more frequently than males. Epidemiologically it resembles a multifactorial disease of unknown cause, affected by both genetic predisposition and environmental factors [2].

There are some hypotheses about cholestasis and the mechanisms leading to rapidly progressing fibrotic changes in BA. After an unknown damage to the biliary tree, a reactive remodelling process begins, and the locally increased activity of the Hedhehog (HH) signaling pathway seems to negatively affect the development of neonatal bile ducts and disrupts bile flow. HH-activated stromal cells and cholangiocytes may produce profibrotic agents that accelerate fibrosis [3, 4]. Some others indicate that TGF-beta produced by bile duct epithelial cells and hepatocytes induces periductular activated hepatic stellate cells which deposit excess Type 1 collagen and cause fibrosis [5, 6].

It was considered as an incurable, rapidly progressive disease until in 1950s Dr. Morio Kasai described Kasai portoenterostomy (KPE) which enabled maintaining biliary drainage and decelerating cirrhotic progression [1]. KPE takes place as a palliative surgical procedure and it mainly delays the time when the patient needs a liver transplant to varying degrees [7]. BA must be considered as an emergency as age at KPE is an independent risk factor for a worse native liver-survival (NLS) [8]. Unless the patient undergoes KPE, they die within 2 years after birth.

The gold standard tool for BA diagnosis is liver biopsy interpretation. Identifying etiological clues by examining the portal and lobular compartments searching for any direct or secondary reactive cholestatic changes, the presence of bile duct proliferation, identifying the compartment which inflammation is concentrated on, assessing the severity of hepatocyte damage, etc. is essential to make a correct diagnosis of BA other than many other neonatal liver diseases. In addition, morphological evaluation may also help determining the stage of the disease and predicting survival, mainly by assigning the stage of portal fibrosis [9].

This study aims to determine the diagnostic histopathological features and prognostic parameters for BA by evaluating clinical and pathological findings together. Liver biopsies obtained during the Kasai procedure in BA cases were examined histopathologically in detail to gather clues for differential diagnosis and investigate the validity of these parameters, which we anticipate may help predict prognosis. We searched for solid and practical diagnostic aids for this progressive disease that requires rapid diagnosis. The age range that positively affects NLS for KPE operation was also investigated.

# Methods

26 BA cases were identified in Ankara University Medical School Ibn-i Sina and Cebeci Hospitals' database between years 2010-2023. Liver biopsies taken during KPE operation were obtained from the archive of pathology department for re-evaluation. H&E, Masson-Trichrome and Reticulin stained slides were examined by an experienced liver pathologist and a 2-year pathology resident together. Clinical

information was searched from the database, then checked and improved by pediatric surgery department.

The day of the KPE, if present, the time and the number of cholangitis attacks during follow-up, the presence of portal venous thrombosis (PVT), oesophageal varices, postoperative hospitalization, hepatic function tests (bilirubin levels, ALT, AST, GGT, INR), NLS and the need of a LT, were noted clinically. It was accepted that NLS continued in patients who were able to continue their lives without experiencing major health problems and without being placed on the transplant list during routine check-ups.

It was determined that 25 of the cases underwent KPE operation. One case presented with severe life-threatening accompanying diseases and was excluded from the study. Additionally, for one case who was operated at the age of 110 days with lacking clinical information, only morphologic analyses were assessed.

Histopathologically; fibrosis score (Modified-Scheuer System), portal stromal oedema and expansion, bile duct proliferation severity and diffusiveness, presence of bile duct paucity, bile plugs, pseudorosette formation, giant cell transformation, hepatocyte damage / necrosis, extramedullary hematopoesis and presence of ductal plate malformation were assessed (Figures 1-3).

Fibrosis was evaluated on histochemical Trichrome and Reticulin-stained slides by using 4-tired Modified-Scheuer scoring system (Figure 1). Additionally, all cases were graded adopting a 3-tired scoring system applied to the following parameters: (I) ductal proliferation: A slight ductular proliferation noticed only at high power magnification (few bile ducts per portal tract) was interpreted as mild; prominent bile duct proliferation, which was noticeable at first glance from the low power magnification, was evaluated as moderate; proliferation that was severe and surrounded the portal tract was considered severe proliferation (Figure 2).

Ductular reaction observed in more than 50% of portal tracts was considered diffuse, while fewer were considered as focal. In biopsy samples containing at least 5 portal areas, the presence of bile duct loss in more than the half of portal tracts was evaluated as bile paucity [9]. Feathery or ballooning degenerations were considered as cholestatic hepatocellular injury [5].

Nonparametrical procedures including Chi-square tests, Mann Whitney U and Spearman Correlation tests were used for statistical analysis (SPSS 22.0). The results were evaluated within the 95% confidence interval, and the $p<0.05$ value was considered significant. Additionally, cases were grouped based on age at KPE as within the first 45 days and within the first 60 days and were compared in terms of morphological features and native liver survival outcomes. Kaplan-Meier curves were created in Graphpad-Prism for NLS.

# Results

a. **Patient Characteristics**

Twenty-four patients with biliary atresia were included in the analysis; 66.7% were female (n=16). Among the 23 patients who underwent KPE within the first 90 days, the mean age at KPE was 59.8 days (median: 58). Eight patients (33.3%) underwent KPE within the first 45 days of life, while the remaining 16 patients were operated after 45 days; half of the cohort (n=12) underwent KPE after 60 days.

b. **Histopathological Findings**

All cases demonstrated portal fibrosis, most commonly grade 2 (41.7%) or grade 3 (45.8%). When grouped as mild (grades 1–2) and severe fibrosis (grades 3–4), severe fibrosis was identified in 13 patients and was significantly associated with older age at KPE (Table 1). Patients with severe fibrosis were approximately 20 days older at surgery compared with those with mild fibrosis ($p<0.05$), and a moderate positive correlation was observed between age and fibrosis score ($r=0.498$, $p<0.05$). Notably, 92.3% of patients with severe fibrosis underwent KPE after 45 days of age.

Bile duct proliferation was present in all cases and increased in severity with advancing age at surgery ($r=0.466$, $p<0.05$). Moderate-to-severe ductular proliferation was observed in 83.3% of patients and was significantly more frequent in those undergoing KPE after 45 days ($p<0.01$) (Table 2). All patients with mild or focal ductular proliferation were younger than 45 days at the time of surgery, whereas diffuse ductular proliferation predominated in patients older than 45 days ($p<0.05$) (Table 3).

Portal stromal edema with expansion, bile plugs, and hepatocellular injury were universal findings. Pseudorosette formation and giant cell transformation were observed in 87.5% and 66.6% of cases, respectively. Bile duct paucity was not identified in any patient.

**c. Clinical Findings and Native Liver Survival (NLS)**

Preoperative direct bilirubin levels were significantly lower in patients who underwent KPE before 45 days of age ($p<0.01$). Total bilirubin levels were also lower in this group ($p<0.05$), supporting a clinically meaningful biochemical distinction at this threshold.

During follow-up, cholangitis occurred in 63.1% of patients and oesophageal varices in 52.9%, without significant associations with age or histopathological parameters.

Among 22 patients with available follow-up data, 11 did not develop a life-threatening need for liver transplantation. Of the eight patients with NLS shorter than one year, seven had undergone KPE after 45 days of age.

Postoperative third-month bilirubin levels were strongly associated with NLS. Among patients with NLS shorter than one year, the majority exhibited direct bilirubin levels >1 mg/dL and total bilirubin levels >2 mg/dL at postoperative month three. In contrast, a ≥30% reduction in total bilirubin at postoperative month three was observed in 10 of 11 patients with NLS longer than one year ($p<0.05$). All patients with insufficient or worsening bilirubin reduction at month three had undergone KPE after 45 days of age.

Kaplan–Meier survival analysis demonstrated significantly reduced native liver survival with increasing age at KPE when stratified by <45 days, 45–60 days, and >60 days (Figure 4), with the most pronounced survival advantage observed in patients operated within the first 45 days of life (Figure 4).

## Discussion

BA is a progressive, sclerosing inflammatory disease of the extrahepatic bile ducts that rapidly leads to biliary cirrhosis in the neonatal period. Timely diagnosis followed by early KPE remains the most critical determinant of NLS until definitive treatment with liver transplantation. The present study demonstrates that a 45-day age threshold, rather than the traditionally emphasized 60-day cut-off, more accurately reflects both histopathological disease progression and early biochemical response.

The prognosis of BA depends on a complex interplay of early diagnosis, surgical timing, and postoperative management. Histopathological evaluation provides important prognostic information, particularly regarding the extent of portal fibrosis, which has consistently been associated with inferior clinical outcomes and reduced NLS [2]. In this study, we aimed to characterize the histopathological spectrum of BA and to correlate these findings with surgical timing and clinically relevant biochemical parameters.

Previous studies have reported variable results regarding the histopathological features that best distinguish BA from other neonatal cholestatic disorders. While ductular proliferation and portal fibrosis are commonly emphasized as characteristic findings, features such as bile plugs, portal stromal edema, pseudorosette formation, giant cell transformation, and extramedullary hematopoiesis may also be observed in other obstructive cholangiopathies [9, 10]. Consistent with the literature, our cohort demonstrated widespread portal fibrosis, ductular proliferation, portal edema, bile plugs, and hepatocellular injury across nearly all cases, whereas bile duct paucity was absent, supporting its occurrence in later disease stages. Importantly, our findings highlight that portal fibrosis and bile duct proliferation are present early in the disease course and progress rapidly with increasing age at surgery. Severe fibrosis (grades 3–4) and diffuse ductular proliferation were significantly more frequent in patients undergoing KPE after 45 days of age, underscoring the narrow window during which histopathological damage remains potentially reversible.

The timing of KPE has long been recognized as a major determinant of outcome in BA. Although KPE performed before 60 days is generally considered acceptable, several large-scale and meta-analytic studies suggest superior outcomes when surgery is performed within the first 30–45 days of life [8, 11]. In line with these data, our survival analysis demonstrated significantly longer NLS in patients undergoing KPE before 45 days. While survival curves between the < 45-day and 45–60-day groups did not reach statistical significance, the < 45-day group consistently exhibited a more favorable trajectory. Notably, seven of the eight patients who required liver transplantation within the first year after KPE had undergone surgery after 45 days of age.

Beyond histopathology, early postoperative bilirubin dynamics emerged as a strong clinical correlate of outcome. Elevated direct (> 1 mg/dL) and total (> 2 mg/dL) bilirubin levels at postoperative month three were associated with reduced NLS, particularly within the first two years following KPE. Conversely, a ≥ 30% reduction in total bilirubin at month three was strongly associated with prolonged NLS [12, 13]. Strikingly, all patients demonstrating insufficient bilirubin reduction had undergone KPE after 45 days of age, further reinforcing the clinical relevance of this threshold.

# Declarations

### Ethics approval and consent to participate

This retrospective study was approved by the local institutional ethics committee. The requirement for informed consent was waived.

### Consent for publication

Not applicable.

### Conflict of interest

The authors declare that they have no conflict of interest.


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### Author contributions

ATB and BS conceived and designed the study. ATB, FS and PK collected the data. ATB and BS performed the histopathological evaluation. ATB performed the statistical analysis. All authors contributed to manuscript writing and approved the final version.

### Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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# Tables

Table 1: Fibrosis scores

| | **Fibrosis Score** | **Chi-square** | **1 – 2 (mild)** | **3 – 4 (severe)** |
|---|---|---|---|---|
| | | Group 1 (0-45 days) | n=7 | n=1 |
| | | Group 2 (46-90 days) | n=4 | n=12 |
| p value | | | | **p<0.01** |

Table 2: Bile Duct Proliferation Severity

| Bile Duct Proliferation Severity | Chi-square | 1 (mild) | 2 – 3 (moderate-severe) |
|---|---|---|---|
| | Group 1 (0-45 days) | n=4 | n=4 |
| | Group 2 (46-90 days) | n=0 | n=16 |
| p value | | | **p<0.01** |

Table 3: Bile Duct Proliferation Diffusiveness

| Bile Duct Proliferation Diffusiveness | Chi-square | Focal | Diffuse |
|---|---|---|---|
| | Group 1 (0-45 days) | n=3 | n=5 |
| | Group 2 (46-90 days) | n=0 | n=16 |
| p value | | | **p<0.05** |

# Figures

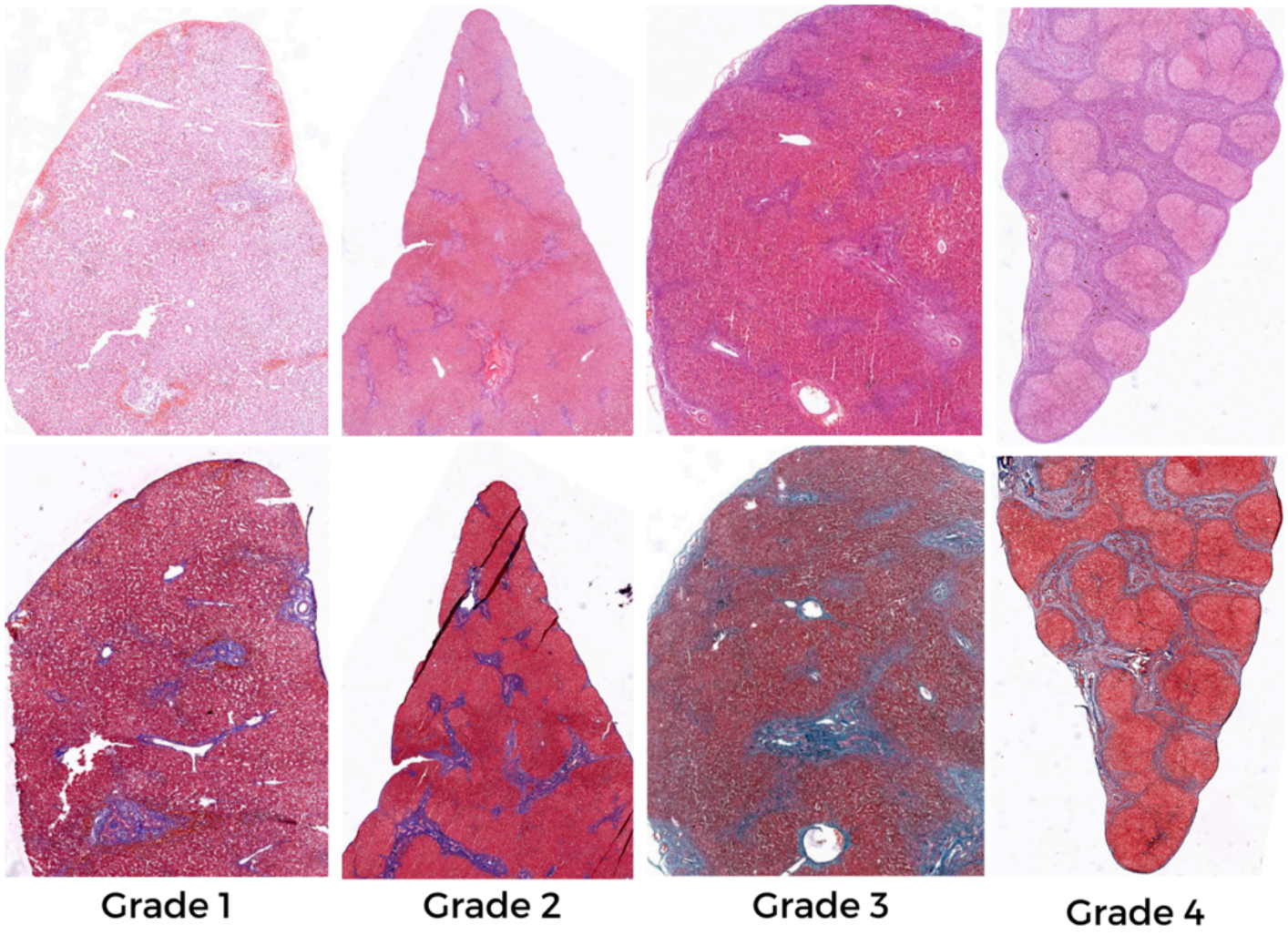



**Figure 1**

Samples from our cases evaluated according to Batts-Ludwig modified Scheuer fibrosis score.

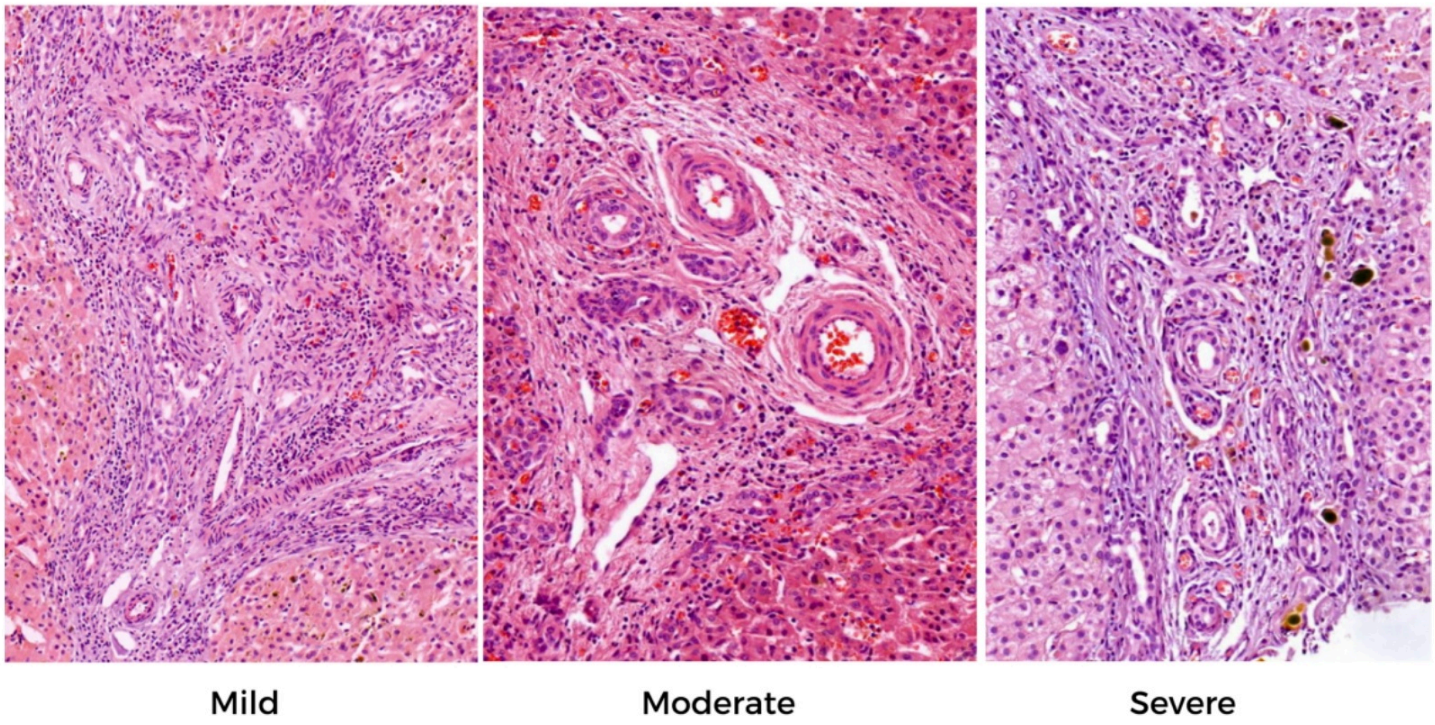



**Figure 2**

Evaluation of bile duct proliferation severity

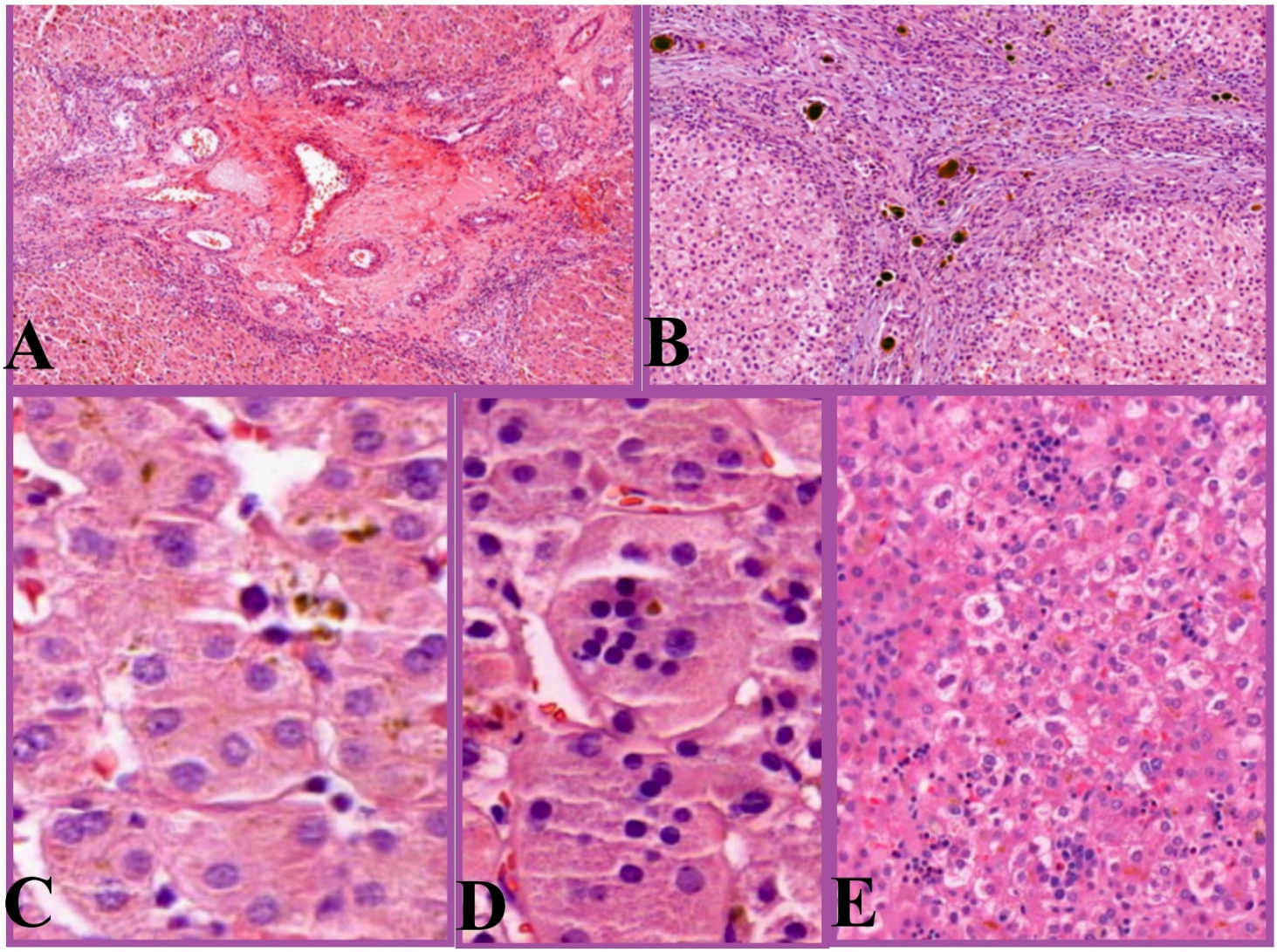



**Figure 3**

Histopathological features (H&E). **A.** Portal tract characterized by prominent expansion with edema. **B.** Bile plugs. **C.**Pseudorosette formation. **D.** Giant cell transformation. **E.**Extramedullary hematopoesis.

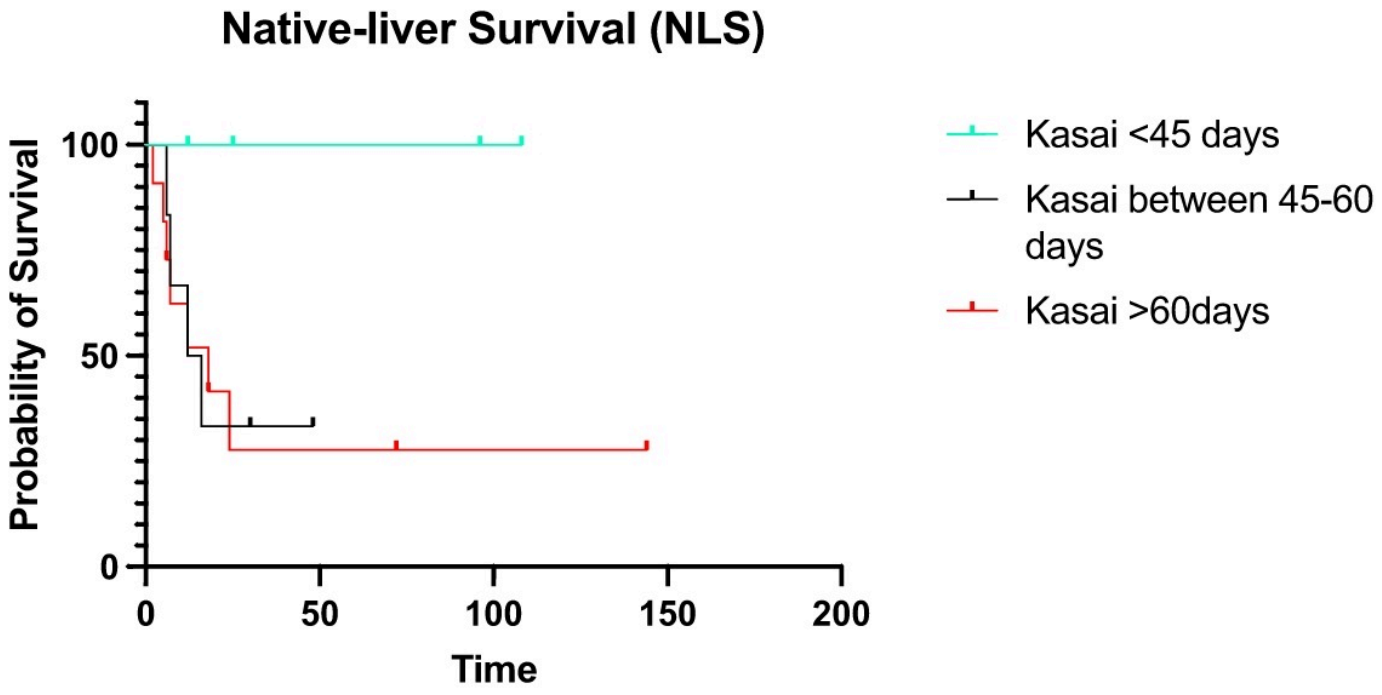



**Figure 4**

Native-liver survival for <45 days, 45-90 days and >90 days-ages ($p<0.05$).