

Prognostic Value of Liver Histology at the Time of Kasai Procedure in Children with Biliary Atresia

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Abstract

Objectives: Biliary atresia is a chronic cholestatic disease resulting from irreversible extrahepatic bile duct occlusions, which leads to liver transplantation in childhood. Solid prognostic values are lacking.

Methods: We investigated the prognostic validity of the PELD score and other laboratory parameters that concern the transplantation-free survival of children with biliary atresia. We correlated the degree of liver fibrosis and neoproliferation of bile ducts at the time of Kasai hepatic portoenterostomy (KPE) and liver transplantation with serological and clinical parameters. Liver biopsies were obtained at the time of KPE in 26 children and directly before the liver transplantation (LTx) of 22 children. The ISHAK score was used to calculate liver fibrosis. Neo-proliferation of the biliary tract was shown by expression of cytokeratin 7.

Results: The survival rate with their own liver was significantly high in children who underwent KPE in less than 28 days of age ($p < 0.01$, cut off 30 days, sensitivity 95.5%, specificity 100%, negative predictive value 80%). Patients with liver enzyme values AST < 83 U/l before surgery also pointed to a significantly better result than patients with higher values ($p < 0.01$, sensitivity 100%, specificity 100%). The bilirubin clearance in children in the group without LTx three months after KPE was significantly better (0.6 (0.1-1.2) vs. 10.18 (0.3-21.9) mg/dl, $p < 0.05$). PELD score and histology had no predictive value.

Conclusions: This study confirms that histological findings do not correlate with serological markers at KPE. However, age and serological markers at the time of KPE appear to be a predictive factor for a positive outcome.

Keywords: ISHAK score, Liver fibrosis, Prognostic markers, Pediatric liver transplantation, PELD score

Introduction

Biliary atresia is the most common cause of LTx in children [1,2]. Although several causes have been hypothesized — including genetic, embryological, infectious, and immunological factors [3-6] the etiology of this infantile obstructive cholangiopathy remains unknown. An untreated extrahepatic obstruction of the bile ducts leads to progressive inflammatory destruction of the extrahepatic and intrahepatic bile duct system, to cholestasis and secondary liver damage with consecutive fibrosis, cirrhosis, and portal hypertension and subsequently to death [6,7].

The focal point of treatment is to restore bile flow to the intestine, which can be achieved with KPE. In most cases, this involves a bridging therapy to gain time for a later LTx, which is necessary in about 80% of cases [8,9]. One of the major risks in managing this disease is the timing of diagnosis as delayed referral may lead to a late diagnosis which, in turn, may result in an urgent need for LTx [10]. Abnormal prognostic parameters at the time of diagnosis indicate the progress of the disease and consequently, the consideration of adequate treatment.

The correlation of KPE timing and survival with the patient's own liver has been widely discussed in various literature and the cut-off has been reduced from 12 weeks to 30 days, as reported by Nio et al. 2003 [11-16]. Bilirubin clearance after KPE also appears to have an influence on transplantation-free survival [17-20]: children with normal bilirubin serum levels three to six months after KPE had significantly higher survival rates without LTx. In addition to bilirubin, Goda et al. found a clear correlation between AST and ALT levels and transplantation-free survival [21].

There are various theories in literature on the correlation between fibrosis at the time of KPE and the clinical course of the children with the disease. Certain studies showed no correlation exists between the two [22], while others showed a remarkable link between the degree of fibrosis and long-term outcome [23,24]. In this study, fibrosis was measured using the Pico-Sirius Red Staining, which is a specific method for identifying collagen I, collagen II, and collagen III. It was used in renal fibrosis measurements and has shown informative correlations between function and histology [25-27]. In the 1990s, this method was used by Moragas et al. to identify liver fibrosis in children who will undergo LTx. Another change in liver histology in children with BA is neoproliferation of the bile ducts, which can be measured through cytokeratin-7 staining, indicating an increased presence of neo-bile ducts as a reaction of the liver to cholestasis and as an indicator of liver damage [28,29].

In this study, we have thus investigated whether the histological findings at the time of KPE and LTx correlated with the clinical course measured by biochemical variables in order to be used as prognostic parameters. Therefore, we also used the pediatric end-stage liver disease

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score (PELD) as an objective tool to prioritize children waiting for LTx. A higher PELD score in children is associated with increased mortality before LTx [30,31].

Patients and Methods

51 patients in a period of 6 years with suspected biliary atresia were included in this study. They all underwent the same diagnostic procedure at the KUNO University Children's Hospital, Regensburg. They were first subjected to endoscopic retrograde cholangiopancreatography (ERCP) and, if the bile duct was present, a biopsy was performed for further diagnosis. In the absence of a biliary tract, KPE was performed. In this case, the diagnosis was confirmed by the operative findings (biopsy, cholangiography). Patients' regular follow-ups in our pediatric clinic were done by ultrasound and routine laboratory tests. For this study, the clinical course from KPE for up to 3 months post-surgery was used to assess prognosis markers. The median observation period is 5.1 months (0.9-80.6). The indication for liver transplantation was synthetic liver dysfunction including portal hypertension.

Histopathology

Needle biopsies were taken from the explanted livers during the KPE and directly before liver transplantation. They were immediately fixed in formalin, alcohol, and acetic acid before being embedded in paraffin wax. Fibrosis was measured by a single senior pathologist and evaluated semi-quantitatively according to the ISHAK Fibrosis Score [17,32]. To measure the neoproliferation of the bile ducts, we used cytokeratin-7 immunohistochemistry.

Pico-Sirius Red (PSR) staining

The PSR staining was performed according to standard procedures [17]. Fibrosis was then assessed using the ISHAK score [32].

CK7 staining

The CK 7 staining was performed automatically by Roche's BenchMark ULTRA IHC/ISH staining module. The histological scoring was adjusted according to Yabushita et al. [26,27].

PELD score

The PELD score was evaluated immediately before and after the KPE [31]. Influencing factors are age, bilirubin levels, INR, albumin, and growth failure.

Statistical analysis and ethical tuning

Statistical analyses were performed with SPSS, Version 23.0 (SPSS, Inc., Chicago, IL). The data are displayed with a median and range. Overall patient survival was analyzed using Kaplan-Meier analysis, wherein $p < 0.05$ was considered statistically significant. To correlate the different parameters, we used Spearman's rank correlation coefficient. For the central tendencies between the transplanted and the non-transplant group, we used the Mann-Whitney U-test. To compare the values between the groups, we used the Wilcoxon rank-sum test. The study was approved by the Ethics Committee of the University of Regensburg (16-101-0168).

Results

For this study, 51 patients with neonatal cholestasis and suspected biliary atresia were included (Table 1). The patients were diagnosed by ERCP to visualize the extra- and intrahepatic bile ducts. 18 patients had successful visualization of the bile ducts and underwent liver

biopsy for differential diagnosis, while 33 children showed no contrast enhancement of the bile system up to the left and right hepatic duct. 7 patients showed an advanced stage of the disease with evidence of synthetic liver dysfunction (including portal hypertension) and were listed for LTx without KPE. 26 children underwent Kasai surgery (13 male and 13 female patients; mean age: 52 days, range: 17-91 days). At the end of the observation period 4 of them survived with their own liver while 22 had to undergo liver transplantation. Reason for transplantation were synthetic liver dysfunction including portal hypertension. The median age of the non-transplant patients was 22 days (range: 17-28 days) for the KPE, while the transplant patients were older (median age: 57 days; range: 31-91 days). The median age during liver transplantation was 189 days (range 103-389 days). 3 patients died after the transplantation due to sepsis (n=2) or organ failure (n=1). Patients who survived with their own liver had an observation period of at least two years.

Table 1: Demographic data of study patients

Demographic data	N
Patients	51
Female/Male	20/31
Biliary tract present/absent in ERCP	18/33
Performed Kasai procedure	26
Female/male KPE patients	13/13
Other diagnosis	18
Primary LTX	7
W/o LTX	4
With LTX	22
LTX and deceased	3
Survival (2 years) KPE group	88.50%
	Median (Range)
Age at KPE (days) all patients	52 (17-91)
Age at LTX after KPE	189 (103-389)
Age at KPE (w/o LTX)	22 (17-28)
Age at KPE (with LTX)	57 (31-91)
Time (months) until LTX	5.1 (0.9-80.6)

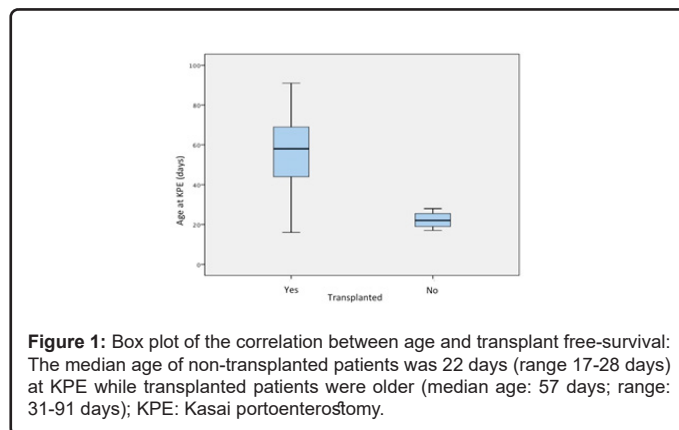
Non-transplant patients underwent KPE significantly earlier than transplant patients (exact Mann-Whitney-U-Test: $z = -2.8$, $p = 0.002$). BA patients who underwent KPE after 30 days of age have a negative predictive value of 0.80 (sensitivity 95.5%, specificity 100%; Figure 1). Liver enzyme levels were also different between the two groups at KPE: AST and ALT levels were significantly lower in patients who survived the observation period without LTx ($p = 0.000^{**}$; Table 2). The LTx cut-off in our study population was >83 U/l for AST and >48 U/l for ALT with a sensitivity level of 100% and specificity of 100%. Bilirubin levels in both groups showed no significant differences before KPE.

Table 2: Transplanted and non-transplanted patients: serum levels and histological findings

	w/o LTX (n= 4)	With LTX (n=22)	P value
Age at KPE (days)	22 (17-28)	57 (16-91)	$p = 0.002^{**}$
Age at LTX (days)	-	189 (103-389)	-
AST level at KPE (U/l)	69(48-83)	168 (68-604)	$p = 0.000^{**}$
AST after 3 months (U/l)	42 (35-109)	298 (62-553)	$p < 0.05^*$
AST level at LTX (U/l)	-	187.5 (69-697)	-

ALT level at KPE (U/l)	40 (26-48)	117 (53-401)	p=0.000**
ALT after 3 months (U/l)	39.5 (34-122)	170.5 (30-436)	P<0.05*
ALT level at LTX (U/l)	-	101 (21-417)	-
Bilirubin at KPE (mg/dl)	7.55 (3.9-12.3)	7.6 (4.3-9.8)	n.s.
Bilirubin after 3 months (mg/dl)	0.6 (0.1-1.2)	10.18 (0.3-21.9)	p<0.05*
Bilirubin at LTX	-	14.85 (0.3-24.9)	-
DeRitis (AST/AALT) at KPE	1.89 (1.35-1.97)	1.52 (1.04-2.45)	n.s.
DeRitis (AST/ALT) after 3 months	0.96 (0.83-1.35)	1.64 (0.68-2.2)	P<0.05*
DeRitis (AST/ALT) at LTX	-	1,83 (1.12-3.29)	-
PELD at KPE	5 (3-5)	7 (4-28)	n.s.
PELD after 3 months	-4 (-6 - -3)	11 (0-25)	P=0.005**
PELD at LTX	-	17 (-10-26)	-
ISHAK at KPE	3.25 (1-5)	3.52 (2-6)	n.s.
IHSAK at LTX	-	5.62 (4-6)	-
CK7 at KPE	1.0 (0-2)	1.0 (0-2)	n.s.
CK7 at LTX	-	2.0 (2-3)	-

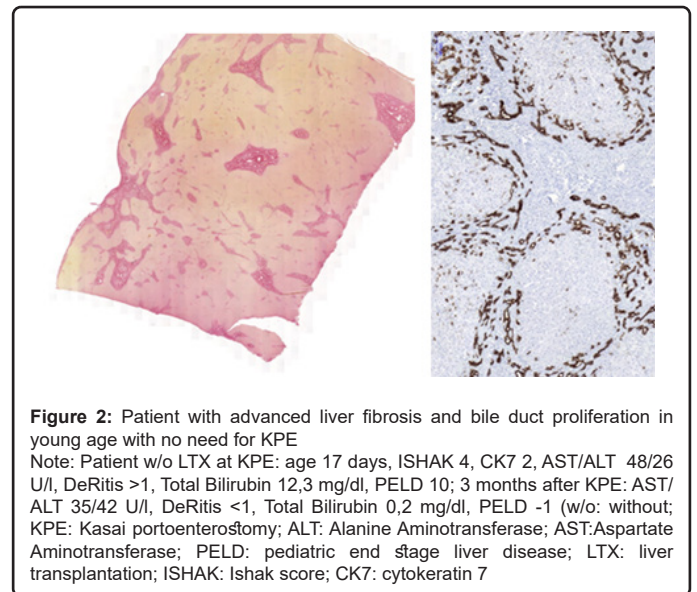
(ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; CK7: Cytokeratin 7; ERCP: Endoscopic retrograde cholangiopancreatography; KPE: Kasai Hepatic Portoenterostomy; LTX: Liver transplantation; PELD: Pediatric end-stage liver disease)



Three months after KPE, patients who survived with their own liver showed significantly lower AST and ALT levels than patients who needed LTx (p<0.05). Bilirubin clearance was also significantly better (without LTx: 0.6 (0.1-1.2); with LTx: 10.1 (0.3-21.9); p<0.05). The deRitis quotient was significantly higher in the LTx group (0.96 vs. 1.64, p<0.05).

The PELD score at the time of KPE was significantly lower in the non-transplant group (without LTx: 5 (3-5) vs. LTx: 7 (4-28); p<0.05). This was confirmed after three months when the non-transplant group showed a significantly lower PELD score (without LTx -4 (-6 - -3); with LTx 11 (0-25); p=0.005).

The histological findings involving the ISHAK fibrosis score and the CK 7 staining did not show a clear correlation between the degree of fibrosis or neoproliferation of the bile ducts in both groups at the time of KPE and even three months after surgery (Table 2). From the histological findings, the clinical course of the patients could be inferred (Table 2, Figure 2). No correlation between histology and PELD score could be established (p>0.05).



Discussion

Prognostic value of fibrosis and neoproliferation of the bile ducts at the time of KPE for transplantation-free survival in biliary atresia and correlation of histological findings with clinical parameters could not be found in this study. However, serum levels of liver enzymes and bilirubin levels after three months are of significant predictive value for transplantation-free survival. Furthermore, the PELD score was clearly correlated with the clinical course but is not suitable as a prognostic method for determining transplantation-free survival. In addition, the time of KPE had a clear influence on survival with the patient's own liver.

Prognostic parameters are needed to predict the clinical course of children with BA. Different aspects, such as serum parameters, age at the time of KPE, and postoperative steroid therapy have been investigated [11-21,33-37] in this study, we were able to evaluate the histological findings at the time of KPE, after LTx and the clinical course in comparison to patients without LTx. We were thus able to confirm the findings of Pape et al. [17] that liver fibrosis as measured by the PicoSirius Red staining, evaluated by the ISHAK Fibrosis Score, shows no correlation with the outcome in children with BA. In addition, the evaluation of neoproliferation of bile products by CK7 staining showed no correlation with disease progression in either group at KPE or LTx. A correlation between both staining methods and serum parameters of liver enzymes and therefore to the PELD score was also not found (p>0.05).

However, the serum values of the liver enzymes as well as the bilirubin values allowed prognostic conclusions to predict a clinical course. We found a cut-off value for transplantation-free survival at the time of KPE for AST levels <83 U/l and for ALT <48 U/l with a sensitivity level of 100% and a specificity of 100%. These observations were confirmed three months after KPE with an equally significant difference in both groups. We have similar results to those published by Goda et al. in 2013 [21], which showed a prognostic AST value two months after KPE as a reliable indicator of long-term outcomes. Moreover, this was confirmed by the fact that the DeRits-quotient had improved significantly in the non-transplant group.

As for the development of bilirubin levels, we have identical findings to those found in existing literature [17-20]: Children without LTx had significantly lower bilirubin levels (0.6 mg/dl vs. 10.18 mg/dl)

three months after KPE. We were thus able to confirm bilirubin as a practical prognostic parameter for long-term outcomes.

A frequently discussed issue in children with BA is the time of diagnosis and the subsequent start of treatment. Once again, we were able to show that early KPE is an important prognostic factor since all patients for whom LTx was not necessary were operated on before 30 days of age. This cut-off is the same as that published by Matsui and Kudo in 2006 from the Japanese biliary atresia registry and much earlier than indicated in other studies, e.g. by Lin et al., who set a value of 60 days of age [16,38]. Patients undergoing LTx had a median age at KPE of 52 days, and survival with their own liver was 6 months on average. A conceivable explanation for this finding that contrasts those in existing literature [10] is the high patient selection filtered through ERCP diagnosis to exclude other reasons for cholestasis with generally better outcomes [39]. This procedure leads to a very select patient population where of the 51 patients suspected to have biliary atresia, 18 could be excluded by ERCP. Of the remaining 26 patients with confirmed biliary atresia who underwent KPE in our facility, 15% still had their own liver at the end of the observation period. This contradicts the results of other study groups in Europe [40,41] with better results. In our opinion, this is due to the highly specific diagnostics leading to the highly select patient population. In this manner, other underlying diseases with similar appearance but with better outcomes (such as Alagille syndrome) are excluded.

Conclusion

The histological findings at the time of KPE had no predictive value for the long-term outcome of patients with KPE. We also found no correlation of histology with clinical course. However, serological markers like AST, ALT, and bilirubin, as well as the time of KPE, do appear to have predictive value. The PELD score is a useful marker for visualizing the clinical progress of the disease.

Limitations

This study is limited because of the small number of patients available, which is something that is attributed to the rarity of the disease in question. However, there are clear trends that are deemed worthy of being looked into in larger studies. In addition, the possibility of making a sampling error when the area of biopsy is not representative of the degree of fibrosis is reported. The Ishak Score was also originally developed for the classification and staging of chronic hepatitis B and not for fibrosis resulting from BA.

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