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Histological evolution of fibrosis in patients with biliary atresia

Evolução histológica da fibrose em pacientes com atresia biliar

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ABSTRACT

Objective: To evaluate the evolution of histological findings of patients with biliary atresia (BA), emphasizing the progression of fibrosis by comparing the diagnostic liver biopsy to the surgical liver biopsy, performed during Kasai portoenterostomy. **Methods:** Retrospective study with 51 patients with BA submitted to portoenterostomy, and both diagnostic (DLB) and surgical liver biopsies (SLB). The samples were blindly reviewed by two pathologists. **Results:** Median age at DLB and at SLB was 69 and 77 days, respectively. The median time between biopsies was eight days. Cirrhosis was more frequent in SLB than in DLB, both according to the Metavir score ($p = 0.006$) and the Ishak score ($p = 0.016$). The Metavir score increased one or more points in 29/51 (56.9%), with evidence of progression to liver cirrhosis in 11/29 (37.9%) of those who had progression of fibrosis. Median age at surgery of those who had a progression of fibrosis was 77 days, while in those 11 who progressed to cirrhosis, this median was 92 days. Cirrhosis was seen in 12/51 (23.5%) SLB patients. The clinical variable age at surgery had a statistically significant difference regarding the presence or absence of cirrhosis in SLB ($p = 0.024$). Cirrhosis was not related to survival with native liver or biliary drainage. **Conclusion:** Most infants with BA have liver fibrosis at diagnosis and it progresses rapidly. The presence of cirrhosis is correlated with the age at surgery, which suggests the importance of this clinical variable in the evolution of fibrosis.

Key words: biliary atresia; biopsy; liver; liver cirrhosis.

RESUMO

Objetivo: *Avaliar a evolução dos achados histológicos de pacientes com atresia biliar (AB), enfatizando a progressão da fibrose e comparando a biópsia hepática diagnóstica (BHD) com a biópsia hepática cirúrgica (BHC), realizada durante a portoenterostomia de Kasai.* ***Método:*** *Estudo retrospectivo com 51 pacientes portadores de AB submetidos a portoenterostomia, BHD e BHC.* ***Resultados:*** *A idade mediana para BHD e BHC foi de 69 e 77 dias de idade, respectivamente. O tempo mediano entre as biópsias foi de oito dias. A cirrose foi mais frequente na BHC do que na BHD, tanto de acordo com o escore de Metavir (*p = *0,006) quanto com o escore de Ishak (*p = *0,016). O escore de Metavir aumentou um ou mais pontos em 29/51 (56,9%) pacientes, com evidências de progressão para cirrose hepática em 11/29 (37,9%), naqueles com progressão da fibrose. A idade mediana para cirurgia dos que tiveram progressão da fibrose foi de 77 dias; nos 11 que evoluíram para cirrose, essa mediana foi de 92 dias. A variável clínica da idade no momento da cirurgia apresentou diferença estatisticamente significativa em relação à presença ou à ausência de cirrose (*p = *0,024). A cirrose não foi associada à sobrevida com fígado nativo ou drenagem biliar.* ***Conclusão:*** *A maioria das crianças com AB tem fibrose hepática no momento do diagnóstico, e a doença progride rapidamente. A presença de cirrose está correlacionada com a idade à cirurgia, o que sugere a importância dessa variável clínica na evolução da fibrose.*

Unitermos: *atresia biliar; biópsia; fígado; cirrose hepática.*

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RESUMEN

Objetivo: *Analizar la evolución de los hallazgos histológicos de pacientes con atresia de vías biliares (AVB), resaltando la progresión de la fibrosis y comparando la biopsia hepática diagnóstica (BHD) con la biopsia hepática quirúrgica (BHQ) mediante la hepatoportoenterostomía de Kasai.* ***Método:*** *Estudio retrospectivo con 51 pacientes con AB sometidos a hepatoportoenterostomía, BHD y BHQ.* ***Resultados:*** *La edad mediana para BHD y BHQ fue 69 días y 77 días, respectivamente. El tiempo mediano entre las biopsias fue ocho días. La cirrosis fue más frecuente en la BHQ de lo que en la BHD, tanto de acuerdo con el escore de Metavir (*p = *0,006) como con el escore de Ishak (*p = *0,016). El escore de Metavir aumentó un punto o más en 29/51 (59,9%) pacientes, con evidencias de progresión hacia cirrosis hepática en 11/29 (37,9%), en los pacientes con progresión de la fibrosis. La edad mediana para cirugía de los que tuvieron progresión de la fibrosis fue 77 días; en los 11 que evolucionaron hacia cirrosis, esa mediana fue 92 días. La variable clínica de edad en el momento de la cirugía presentó diferencia estadísticamente significante con respecto a la presencia o ausencia de cirrosis (*p = *0,024). La cirrosis no fue asociada a la sobrevida con hígado nativo o drenaje biliar.* ***Conclusión:*** *La mayor parte de los niños con AVB tiene fibrosis hepática en el momento del diagnóstico, y la enfermedad avanza rápidamente. La presencia de cirrosis está relacionada con la edad al momento de la cirugía, lo que sugiere la importancia de esa variable clínica en la evolución de la fibrosis.*

Palabras clave: *atresia biliar; biopsia; hígado; cirrosis hepática.*

INTRODUCTION

Biliary atresia (BA) is a progressive fibro-obliterative disease of the biliary tract, being the most common cause of severe chronic liver disease in children[1]. Its incidence varies from 1:3.125 to 1:18.000 live births[2, 3], with a little prevalence in females (1.2:1)[4, 5]. It is the main cause of cholestatic jaundice in the first months of life (25%-40%)[6] and of liver transplant in infants, accounting for around 50% of referrals[7, 8] and for 10% of transplants at any age[9].

Its diagnosis is based mainly on clinical, laboratory, ultrasound and histological evaluations. Liver biopsy is the most accurate test for diagnosis, with accuracy varying from 90% to 95%[10]. Duct proliferation, portal fibrosis and absence of sinusoidal fibrosis are histological characteristics that better predict BA[11].

Kasai portoenterostomy is an alternative therapy to restore biliary flow in patients with BA[8]. Nevertheless, fibrosis progresses rapidly before surgery and, even after biliary drainage, it may continue to progress, leading to portal hypertension and cirrhosis[12]. The causes of liver fibrosis progression remain unclear in BA. Although fibrosis is intuitively associated with cholestasis, the evolution of the former in patients who became anicteric after Kasai portoenterostomy suggests that there are non-cholestatic fibrogenic pathways. This might involve immune and non-immune mechanisms, such as oxidative stress and recurrent cholangitis[13].

In BA, the progression of liver fibrosis is rapid and aggressive[14]. The present study has the purpose of evaluating the evolution of histological findings in patients with BA, highlighting the histological progression of fibrosis in the period between diagnostic and surgical liver biopsies (at the moment of portoenterostomy).

METHODS

Patients and study design

This is a retrospective study of patients diagnosed with BA at Hospital das Clínicas da Universidade Federal de Minas Gerais (UFMG) from 1979 to 2016. Its non-probabilistic sample initially comprised 93 patients submitted to portoenterostomy at the hospital who had both diagnostic liver biopsy (DLB) and surgical liver biopsy (SLB). Out of these, 42 were excluded as they had preoperative biopsies at other services or because the material obtained was inappropriate for analysis. The final sample of the study was composed of 51 patients, and, consequently, 102 biopsies.

Variables and definitions

The following variables were evaluated:

- clinical variables – gender, age at DLB and age at surgery;
- laboratory variables – albumin, coagulogram [prothrombin activity (PA)], tissue enzymes [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] and canalicular enzymes [gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP)], admission exams, and bilirubin [total bilirubin (TB) and direct bilirubin (DB) on admission and in the sixth postoperative

month]. The presence of bile flow was defined as TB lower than 2 mg/dl six months after portoenterostomy[15];

• histopathological variable – the blinded histopathological analysis of liver biopsy slides was performed by two pathologists from the Pathological Anatomy Service of Hospital das Clínicas da UFMG, who did not know either the patients' data or when the exam was conducted. Each liver fragment was fixed in 10% saline formaldehyde and processed according to routine histological techniques until paraffin embedding. The paraffin block was submitted to microtomy, and five slides with histological sections measuring from 5 µm to 7 µm were obtained from each block and stained with hematoxylin and eosin (HE), Gomori or Masson trichrome, Picrosirius red and periodic acid-Schiff (PAS) with and without diastase.

The degrees of fibrosis and inflammatory activity were evaluated using Metavir[16] and Ishak *et al*. (1995)[17] scores. Liver lobule architecture was classified as preserved, partially subverted or subverted. Giant cell transformation and duct proliferation were evaluated for their presence or absence. Cholestasis was classified as low, moderate or high intensity and according to location (bile plugs). Needle biopsy and wedge biopsy were considered appropriate when they had at least eight portal tracts.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS®) program, version 20 (IBM, New York, USA) and MedCalc, version 16.8.4. Quantitative data were described by means and standard deviation, when they had a normal distribution verified by the Shapiro-Wilk test, or median (Q1; Q3), when the distribution was not normal. Qualitative variables were described by absolute frequencies and percentages. Non-normal distributions between two independent groups were compared using Mann-Whitney test. Means with normal distribution were compared using Student's *t* test, and categorical variables were compared using Pearson's chi-square test. Data of quantitative and qualitative variables from dependent groups were analyzed using Wilcoxon test (non-normal), McNemar test (two categories) and McNemar Bowker test (more than two categories), respectively.

Independent variables were: gender, TB and DB, tissue and canalicular liver enzymes, albumin and PA, age at surgery and histopathological findings.

Reliability analysis of histological data was performed using Cohen's Kappa coefficient for categorical variables and Bland Altman plot and intraclass correlation coefficient (ICC) for quantitative variables. Cohen's Kappa coefficient determined if there was an agreement among observers in the histological analysis of DLB and of SLB. For Cohen's Kappa coefficient, the Landis and Koch[18] scale was used(< 0: poor; 0-0.19: insignificant; 0.2-0.39: slight; 0.4-0.59: moderate; 0.6-0.79: high or 0.8-1: almost perfect). For the ICC correlation, the following classification was used[19] (< 0.4: poor; > 0.4 and ≤ 0.75: acceptable; or ≥ 0.75: excellent). Results with $p < 0.05$ were considered statistically significant.

Analysis of survival with native liver was performed using Kaplan-Meier curves and their comparisons, using the Wilcoxon test.

Ethical aspects

This research project was approved by the Research Ethics Committee of UFMG, no. 77.0.203000-09.

RESULTS

Fifty-one patients underwent Kasai portoenterostomy with a suitable histological material both for DLB and SLB. Out of these, 26/51 (51%) were males. Median ages at DLB and at SLB were 69 and 77 days of life, respectively. Median of TB in the sixth postoperative month was 6 mg/dl (Q1; Q3/2; 10) and 11/51 (21.6%) patients had bile flow. **Table 1** summarizes clinical and laboratory data.

Analysis of agreement between observers regarding histology

Comparisons of descriptive categorical variables between two pathologists regarding DLB were almost perfect concerning lobular architecture, giant cell transformation, intensity of cholestasis, duct proliferation, degree of fibrosis using both the Metavir and the Ishak scores and inflammatory activity using the Metavir score. The correlation was high for the location of cholestasis and inflammatory activity using Ishak. In the analysis of SLB, the agreement was almost perfect for lobular architecture, giant cell transformation and degree of fibrosis using Ishak. It was high for duct proliferation, intensity of cholestasis, and inflammatory activity and fibrosis using Metavir. It was moderate for the location of cholestasis and inflammatory activity using Ishak (**Table 2**). In the analysis of agreement between examiners for the evaluation of the number of portal spaces, an excellent correlation was observed in both biopsies, data that was confirmed by Bland Altman plots (Table 2).

TABLE 1 – Description of clinical and laboratory variables of patients diagnosed with BA

Variable	$n = 51$
Sex (male/female)	26/25
Age on admission (days)*	62 (48/88)
Age at DLB (days)*	69 (54/92)
Age at surgery or age at SLB (days)*	77 (62/105)
Interval between biopsies (days)*	8 (5/15)
TB on admission (mg/dl)**	10.2 ± 3.1
DB on admission (mg/dl)**	7 ± 2.7
AST (U/l) on admission*	183 (130/297.5)
ALT (U/l) on admission*	128 (79.5/183.5)
ALP (U/l) on admission*	513.5 (167.8/791.8)
GGT (U/l) on admission*	521 (224/936.8)
Albumin (g/dl) on admission*	4 (3/4)
Coagulogram: PA (%) on admission*	85.5 (75/96.8)
TB 3th month (mg/dl)*	7.1 (2.4/12.3)
DB 3th month (mg/dl)*	4.6 (1.9/7.8)
TB 6th month (mg/dl)*	6 (2/10)
DB 6th month (mg/dl)*	4 (1.1/7.4)

*BA: biliary atresia; DLB: diagnostic liver biopsy; SLB: surgical liver biopsy; TB: total bilirubin; DB: direct bilirubin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma glutamyltransferase; PA: prothrombin activity; *median (Q1; Q3); **mean ± standard deviation.*

TABLE 2 – Analysis of agreement among examiners referring to histology of 51 patients diagnosed with BA

Variables	DLB		SLB	
	Kappa (SE Kappa)	*p*	Kappa (SE Kappa)	*p*
Lobular architecture	0.932 (0.047)	< 0.0001	0.807 (0.075)	< 0.0001
Giant cell transformation	0.963 (0.037)	< 0.0001	0.932 (0.046)	< 0.0001
Location of cholestasis	0.775 (0.089)	< 0.0001	0.48 (0.12)	< 0.0001
Intensity of cholestasis	0.852 (0.07)	< 0.0001	0.769 (0.083)	< 0.0001
Duct proliferation	1 (0)	< 0.0001	0.697 (0.162)	< 0.0001
Metavir score				
Inflammatory activity	0.904 (0.055)*	< 0.05	0.695 (0.087)*	< 0.05
Fibrosis	0.968 (0.032)*	< 0.05	0.695 (0.087)*	< 0.05
Ishak score				
Inflammatory activity	0.714 (0.066)*	< 0.05	0.432 (0.102)*	< 0.05
Fibrosis	0.968 (0.024)*	< 0.05	0.841 (0.053)*	< 0.05
no. of portal space	0.991 (0.983/0.995)**	< 0.0001	0.991 (0.985/0.995)**	< 0.0001

*BA: biliary atresia; DLB: diagnostic liver biopsy; SLB: surgical liver biopsy; SE Kappa: standard error Kappa; *pondered Kappa; **intraclass correlation coefficient 95%.*

Comparison of diagnostic and surgical liver biopsies

The main histological findings for the BA diagnosis were as follows: duct proliferation, biliary plugs and portal fibrosis. Duct proliferation was verified in 86.3% of patients in DLB, biliary plugs in 78.4%, and portal fibrosis in 84.3%. When analyzing SLB, the same histological changes occurred in 86.3%, 78.4% and 92.2%, respectively.

Median time between biopsies was eight days. The number of portal tracts has shown a statistically significant difference ($p < 0.0001$) between exams, with a mean of eight for DLB and 17 for SLB. While DLBs were performed with needle, SLBs were obtained using wedge (60%) or needle (40%) techniques. The lobular architecture was preserved in 52.9% and subverted in 9.8% of DLB, whereas in SLB, these values were 23.5% and 25.5%, respectively ($p = 0.001$). Cirrhosis was more frequent in the surgical biopsy both using the Metavir ($p = 0.006$) and the Ishak scores ($p = 0.016$). No statistically significant difference was seen between biopsies as for histopathological variables regarding giant cell transformation, presence and intensity of cholestasis, biliary plugs, duct proliferation and inflammatory activity in both scores (**Table 3**).

TABLE 3 – Comparison of diagnostic and surgical liver biopsies of 51 patients diagnosed with BA

Variable	DLB	SLB	*p*
Age (days)	69 (54/92)	77 (62/105)	< 0.0001[1]
no. of portal tracts mean ± SD	8 (8/10)	17 (11/20)	< 0.0001[1]
Lobular architecture			
Preserved	27 (52.9%)	12 (23.5%)	0.001[3]
Partially subverted	19 (37.3%)	26 (51%)	
Subverted	5 (9.8%)	13 (25.5%)	
Cirrhosis Metavir			
Yes	2 (3.9%)	12 (23.5%)	0.006[2]
No	49 (96.1%)	39 (76.5%)	
Cirrhosis Ishak			
Yes	1 (2%)	8 (15.7%)	0.016[2]
No	50 (98%)	43 (84.3%)	
Giant cell transformation			
Yes	32 (62.7%)	30 (58.8%)	0.791[2]
No	19 (37.3%)	21 (41.2%)	
Cholestasis			
Yes	46 (90.2%)	48 (94.1)	0.625[2]
No	5 (9.8%)	3 (5.9)	
Cholestasis			
Low	18 (39.1%)	19 (39.6%)	0.135[3]
Moderate	22 (47.8%)	19 (39.6%)	
High	6 (13.1%)	10 (20.8%)	
Biliary plugs			
Yes	40 (78.4%)	40 (78.4%)	1[2]
No	11 (21.6%)	11 (21.6%)	
Duct proliferation			
Yes	44 (86.3)	44 (86.3)	1[2]
No	7 (13.7)	7 (13.7)	

Fibrosis Metavir			
0	8 (15.7)	4 (7.8)	
1	18 (35.3)	7 (13.7)	
2	13 (25.5)	19 (37.3)	0.038[3]
3	10 (19.6)	9 (17.6)	
4	2 (3.9)	12 (23.5)	
Inflammatory activity Metavir			
0	27 (52.9)	25 (49)	
1	21 (41.2)	19 (37.3)	0.254[3]
2	3 (5.9)	7 (13.7)	
Fibrosis Ishak			
0	8 (15.7)	4 (7.8)	
1	8 (15.7)	2 (3.9)	
2	8 (15.7)	5 (9.8)	
3	11 (21.6)	13 (25.5)	0.252[3]
4	11 (21.6)	12 (23.5)	
5	4 (7.8)	7 (13.7)	
6	1 (2)	8 (15.7)	
Inflammatory activity Ishak			
Median (Q1; Q3)	1 (1; 2)	1 (1; 2)	0.984[1]

BA: biliary atresia; DLB: diagnostic liver biopsy; SLB: surgical liver biopsy; SD: standard deviation; [1]Wilcoxon Test; [2]McNemar Test; [3]McNemar-Bowker Test.

Comparison of clinical and laboratory variables of patients who have developed fibrosis or not, according to Metavir

When comparing the Metavir score for fibrosis in biopsies (DLB × SLB) of each patient, it was seen that in 29/51 (56.9%) the score increased in one or two points in the scale and there was a progression to cirrhosis in 11/29 (37.9%) patients who had developed fibrosis. In 22 patients with no such progression, 16/51 (31.4%) maintained the degree of fibrosis and it decreased in 6/51 (11.8%) patients.

No statistically significant difference was found among patients who developed fibrosis or among those who did not develop it when compared to the variables studied (TB and DB on admission and in the sixth month of the postoperative period and the interval between biopsies). The median age at surgery of those who developed fibrosis was 77 days and 92 days in the 11 patients who progressed to cirrhosis.

When performing the same evaluation, but using the Ishak score, 31/51 (60.8%) patients increased their degree of fibrosis, 13 (25.5%) maintained it and seven (13.7%) decreased it.

According to the Metavir score, there was an increased inflammatory activity in 12/51 (23.5%) patients a decrease in six (11.8%) and maintenance in 33 (64.7%). Similar results were seen using the Ishak score, which has shown an increased inflammatory activity in 9/51 (17.7%) patients, a decreased inflammatory activity in 10 (19.6%) and stabilized in 32 (62.7%).

Comparison of only needle diagnostic and surgical liver biopsies

Sample size was reduced to 20 patients (40 biopsies) when comparing DLB and SLB obtained using only the needle technique and with at least 10 visible portal tracts. The median number of portal tracts observed was 10 for DLB and 11 for SLB. Cirrhosis was more frequently identified in SLB using both the Metavir ($p = 0.005$) and Ishak ($p = 0.02$) scores and the median time between biopsies was 11 days. Metavir score for fibrosis increased 1-2 points in 13/20 (65%) patients and five of them exhibited progression to cirrhosis. Median age at surgery was 74 days in the patients who developed fibrosis and 93 days in the five patients who progressed to cirrhosis.

Comparison between patients with and without cirrhosis in SLB, according to Metavir

Cirrhosis was identified in 12/51 (23.5%) surgical biopsies. The clinical variable age at surgery showed a statistically significant difference regarding the presence or absence of cirrhosis in SLB ($p = 0.024$), as cirrhotic patients were operated later, median age was 92 days. In non-cirrhotic patients the median age at surgery was 71 days. No statistically significant difference was observed between patients with and without cirrhosis regarding the variables studied (TB and DB in admission and in the sixth month of postoperative period and bile flow).

Survival

Survival with native liver was 64.7% in one year, 52.6% in two years, 36.4% in five years and 23.9% in 10 years.

Progression or no progression of fibrosis, evaluated according to the Metavir score, has not been related to survival with native liver (Wilcoxon $x^2 = 0.650, p = 0.420$) (**Figure 1A**). The presence or absence of cirrhosis at the moment of portoenterostomy has also not been associated with survival without the liver transplant (Wilcoxon $x^2 = 0.412, p = 0.521$) (**Figure 1B**).

DISCUSSION

Histological comparison at different moments that precede the portoenterostomy may contribute to the understanding of different surgical outcomes of this severe disease. The literature provides studies evaluating histological features with the best accuracy to diagnose BA, as well as the correlation among findings regarding surgical liver biopsy and survival. Considering that studies to

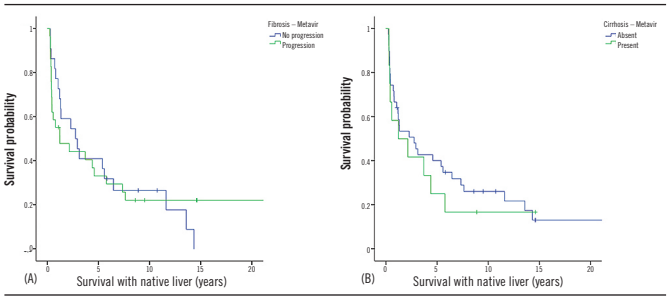

FIGURE 1 – *Progression or no progression of fibrosis, evaluated according to the Metavir score and presence or absence of cirrhosis at the moment of portoenterostomy*

evaluate the progression of liver fibrosis in patients with BA are scarce[20, 21], this work aims at contributing with more data on the evolution of fibrosis prior to portoenterostomy.

When comparing DLB and SLB of each patient, the progression of fibrosis was noted in 56.9% and in 60.8% of the patients using the Metavir and Ishak scores, respectively, in a median of interval of eight days. Moreover, cirrhosis was more frequent in SLB, both using the Metavir and the Ishak scores, highlighting the rapid histological progression of the condition. Lampela *et al*. (2014)[21], when comparing SLB to the histological material obtained from the native liver of infants, with a median age of 4.2 years, submitted to a successful Kasai portoenterostomy, discovered a rapid progression of fibrosis in patients with an adequate bile flow. Tanano *et al*. (2003)[20] established a parallel between SLB and the liver biopsy of 26 patients performed, on average, 22 months after Kasai portoenterostomy. Progression of fibrosis was seen in 16/19 (84.2%) patients who had bile flow and in all seven patients who did not have biliary drainage. In addition, the progression of fibrosis was more accentuated in the group of jaundiced patients. Even though absence of biliary flow, and consequently surgical failure, is a factor that contributes to the rapid evolution of fibrosis, the presence of biliary drainage evidently does not prevent fibrosis progression. The reason why fibrogenesis is so fast and intense in BA remains unclear. A better understanding of the developing process of liver fibrosis may be essential do elucidate the pathogenesis of BA.

In the present study, no statistically significant difference was seen among patients whose degree of fibrosis increased or not regarding the age at surgery. However, when only the 11 patients whose fibrosis progressed to cirrhosis were studied, the median age at surgery was 92 days of life, whereas in those whose fibrosis did not progress, it was 75 days. The presence or absence of cirrhosis in SLB had a statistically significant relationship with the age at surgery, which suggests the importance of this clinical variable in the evolution of fibrosis. Authors suggest a relationship between the age at surgery and the degree of liver damage or fibrosis[14, 22].

The degree of liver fibrosis is one of the most important evaluations for the diagnosis and the prognosis of chronic liver disease[23]. Shteyer *et al*. (2006)[22] verified that the degree of fibrosis at the moment of portoenterostomy was one of the determining factors in the survival with native liver. Weerasooriya *et al*. (2004)[14] classified fibrosis according to its severity in three types: mild, moderate and marked. For those authors, there was a correlation between the intensity of fibrosis and the lower survival with native liver. Nevertheless, other studies have not shown any significant association between these two variables[24, 25]. The controversial results regarding fibrosis may be explained by the different techniques and scoring systems used to quantify it[26]. Moreover, there are difficulties inherent to the analysis of its extension, involving the histological sample and the staining methods[23].

A "strong" or "almost perfect" agreement was observed for staging of fibrosis using Metavir or Ishak scoring systems, both in DLBs and SLBs. These data are similar to those found in the literature. Goodman *et al*. (2007)[27] report that most publications using Metavir and Ishak scores and Cohen's Kappa coefficient to evaluate scores of fibrosis in liver diseases have shown agreement levels varying from "moderate" to "almost perfect" (Kappa 0.5-0.9). Both the disagreements, among pathologists, during the interpretation of biopsies, and the scoring systems used are factors that may interfere with the histopathological evaluation[23]. These facts were not observed in this study.

The representativeness of the material obtained in the liver biopsy is important for histological analysis. When carefully studied, the biopsy has an accuracy of 95% if the sample has five to seven portal tracts[28]. In the present study, there was a difference as to the number of portal spaces between the two biopsies. This difference is believed to have influenced the results, considering that the DLB was obtained by puncture and the SLB was obtained by direct visualization puncture and/or wedge resection, which might ensure a greater number of portal tracts[20]. Pape *et al*. (2009)[26] reported that the degree of fibrosis was similar in liver biopsies with needle and with wedge in infants with BA. However, the fact that wedge biopsies may include capsular or subcapsular tissue, a region in which histological activity and fibrosis may be overestimated[23], constitutes a limitation of the study. Nevertheless, these issues do not invalidate the results, which suggest a rapid progression of the liver fibrosis as a common consequence of insults to the organ parenchyma still with no clear etiopathogeny in the literature. Similar results were obtained when only the patients who underwent biopsies using the needle technique were compared.

The study was retrospective, which made it difficult to recover material for the histological evaluation and, consequently, caused

the exclusion of a large number of patients in the final analysis. The reduced sample size, justified by the strict inclusion criteria (both liver biopsies performed in the service and the number of portal spaces in the samples) and by the rarity of the disease, is another limitation of the present study.

The comparison of liver biopsies at two distinct moments shows how the disease evolves in a short period of time. In some cases, intervals as short as eight days were enough to see the progression of fibrosis. Data from the present study confirm that most infants with BA already had liver fibrosis at diagnosis. The presence of cirrhosis is related to age at surgery, suggesting the importance of this variable in the evolution of fibrosis, although cirrhosis was not related to survival with native liver or to biliary drainage.

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