Factors related to the biliary atresia prognosis post-portoenterostomy

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Abstract

Objective: this study considered the presence of congenital anomalies, ductal plate malformation, area of fibrosis and, mainly, the patient’s age in cases of biliary atresia submitted to surgery. The present study verified the influence of these factors on the follow-up of a biliary atresia sample.

Methods: a sample of 47 patients with biliary atresia was evaluated in a cross-sectional study. Their histologic specimens were stained for antibody anticytokeratin 19 and CAM 5.2 through immunohistochemistry in order to study biliary structures, and for picrosirius red to evaluate the area of fibrosis. The study of biliary structures was performed by two pathologists and the first author of the study. They were “blind” with regard to the clinical follow-up. The area of fibrosis was quantitatively evaluated. Data on the patients with regard to age, death and occurrence of liver transplantation were searched on the patients records.

Results: age at portoenterostomy varied between 24 and 251 days of life (90.4 ±44.8 days) and follow-up was available in 32 cases (72%). The nine cases (19%) with extrahepatic congenital anomalies associated to biliary atresia did not present different prognosis from the remaining patients. Age at portoenterostomy influenced the prognosis ($P=0.016$). The area of fibrosis was different on patients aged less than 60 days and those aged more than 90 days at portoenterostomy ($P=0.023$), but did not influence the prognosis. The presence of ductal plate malformation, as well, did not influence the follow-up.

Conclusions: age at portoenterostomy was the only factor that influenced prognosis on this sample of biliary atresia. It is necessary to increase the biliary atresia sample to check the influence of congenital extrahepatic anomalies on the follow-up post-portoenterostomy.


Introduction

Biliary atresia (BA) is a disorder unique to the neonatal period, which consists of partial or total obstruction of extrahepatic bile ducts. There are two forms of BA: congenital and acquired.¹ Congenital biliary atresia accounts for approximately 35% of the cases which, concomitantly with bile duct disorders, present associated congenital extrahepatic anomalies (ACEA), early onset of neonatal cholestasis and absence of bile duct remnants at the hepatoduodenal ligament. The presence of ACEA, at least the one related to BA-associated asplenia syndrome, has been correlated with poorer prognosis after portoenterostomy.² On the other hand, ductal plate malformation (DPM) in approximately 25% of BA cases³ was associated with an early and severe form of the disease. Fibrosis has been considered the histological...
expression of the severity level of BA and knowing its extension, in these cases, can help assess the prognosis. However, among the different variables related to postoperative outcome, age at portoenterostomy is still the most important factor for predicting the prognosis.4

The present study aimed at assessing the following prognostic implications, in BA cases, of: (1) histological findings, such as presence of DPM and extension of fibrosis; and (2) clinical findings, including age of patients at portoenterostomy and presence of ACEA.

Methods

The present study assessed 47 patients (males and females) with BA that were submitted to the investigation protocol of neonatal cholestasis in the Clinical Department of Gastroenterology of the Division of Pediatrics, Hospital de Clínicas de Porto Alegre, between 1987 and 1997. All patients were followed up by the same medical team from hospital admission to hospital discharge. Part of this sample was later attended to at the outpatient clinic. The diagnosis was made during exploratory laparotomy, which introduces Kasai procedure. The study was conducted cross-sectionally, with retrospective and prospective evaluations of the material surgically obtained from a wedge biopsy of the liver, fixed in formalin, and then embedded on paraffin. The retrospective study included patients who had already been submitted to biopsy at the beginning of the study; the prospective study included patients with the same characteristics, who were prospectively treated from the start of the study.

As for the assessment of ACEA in retrospectively studied patients, the information was collected from medical histories, since this assessment is regularly used for the diagnostic investigation of neonatal cholestasis. Physical and radiological examination data were analyzed. The exams included chest x-ray, echography of the abdomen and of specific systems, in addition to the description of clinical, surgical and autopsy findings, whenever possible. In the prospective study, ACEA was evaluated in each of the investigated patients, according to the same protocol. Only relevant congenital anomalies that affect the viability or fertility of patients were taken into consideration.

The histological examination of biliary structures was carried out by two pathologists and by the first author of the present study, who were blinded to clinical outcome. The findings were evaluated as to the presence of DPM, presence and distribution of mature bile ducts and ductular proliferation. The extension of fibrosis was quantitatively assessed. The prognosis was analyzed by considering the evolution of those cases followed up at the outpatient clinic, after portoenterostomy. The evaluation of the prognosis was based on the death of patients and on liver transplantation (Tx) in the follow-up period.

The assessed histological variables are described next.

**Ductal plate malformation:** characterized by the presence of biliary structures marked with CAM 5.2 or anticytokeratin 19 (CK 19) antibodies, with irregular, dilated morphology, forming a ring around the mesenchymal tissue, with or without portal vein branch at the center.7

**Mature bile ducts:** tubular structures, circular when cross-sectioned and long when longitudinally sectioned, with regular morphology, marked with CK 19 and CAM 5.2, located inside the portal spaces. Their presence in up to three portal spaces was defined as focal.

**Ductular proliferation:** defined as the presence of bile ducts in large numbers, located along the limiting plate or inside the mesenchyme. Its presence in up to three portal spaces was considered focal.

**Collagen density:** defined as the mean values of collagen percentage, quantified by the morphometric method, present in ten images of the histological material stained with picrosirius.

For the histological and immunohistochemical preparation of each tissue specimen collected from patients with BA on the day of portoenterostomy, four sections of 5µm, two of which were used for the reaction with CAM 5.2 (Becton-Dickinson) primary monoclonal antibodies and human monoclonal anticytokeratin antibodies (DAKO), were made, both for the study of biliary structures. The immunohistochemical technique consisted of ABC peroxidase, according to the method standardized by Hsu et al.5 In addition to marking with these antibodies, picrosirius staining was used to locate the collagen.6

The morphometric analysis of collagen density, used by Chevallier et al., was employed to assess the fibrotic area from the histological material stained with picrosirius, in ten images captured in a physical device in TIFF (tag image format). This method, conducted on a computerized imaging system, allowed for the automatic quantification of the fibrotic area present in a surface field. The analysis of the image was made with Image-Pro™Plus version 4.1 software (Media Cybernetics).

Statistically speaking, the assessment of differences between the quantitative variables was made through Student t-test or through one-way ANOVA. Duncan’s multiple range tests were used to determine post-ANOVA differences. Nonparametric Mann-Whitney U-test and Kruskal-Wallis ANOVA were used in cases of asymmetry. The determination of differences by Kruskal-Wallis ANOVA was carried out by Dunn’s multiple comparison test. In case of qualitative variables, the chi-square test and, if necessary, Fisher’s exact test was used. Nonparametric Spearman's correlation coefficient was used to assess the associations between ordinal and continuous variables. The significance level was α=0.05, and the statistical tests whose values were 0.05<P<0.1 were considered to have a threshold significance. The data were processed and analyzed in Excel version 97 and SPSS version 10.0.
The present study was assessed and approved by the Ethics and Research Committee of the Graduate Research Program of Hospital de Clínicas de Porto Alegre.

Results

The age of the cases evaluated in this study on the day of portoenterostomy ranged from 24 and 251 days of life, mean of 90.9 ± 44.8 days.

Nine cases (19%) presented ACEA. No statistical difference was found between the groups with and without ACEA as to age at portoenterostomy (P=0.390) (unpublished data). Table 1 shows the prognosis of cases with and without ACEA.

Among the cases in which outcome could be followed up after portoenterostomy (n=34; 72%), no significant difference was found between the groups. However, only one case with ACEA is still alive and has not been submitted to transplantation, whereas among the cases without ACEA, the distribution was more homogenous: approximately half of the cases are still alive and have not been submitted to transplantation (Tx).

Table 2 shows the age at portoenterostomy in relation to prognosis.

Nineteen cases (40% of the total cases, 56% at follow-up) died or were submitted to transplantation, whereas 15 (32% of the total, 44% at follow-up) are still alive and have not been submitted to transplantation. The age at portoenterostomy significantly affected the prognosis (P=0.016). The worst prognosis occurred in children aged between 69 days and those older than 90 days at the time of surgery.

Table 3 shows the association of age (according to age groups) with the collagen density of the biopsy material.

Collagen density in BA cases, regardless of age, comprised 6.6%±3.9% of the histological field surface. There was a positive association between older age and the levels of collagen density in the sample, with statistically significant difference (P=0.023) between the groups less than or equal to 60 days and greater than 90 days of life. The maximum value for collagen density in the group whose age was less than 60 days was 6.3%, whereas the value in the group aged more than 90 days was 23% of the total slide surface. The lowest collagen density occurred in one case belonging to the group aged between 61 and 90 days of life (1.7%). In the latter group, the maximum value for collagen density was 14%. There was a moderate positive correlation (r=0.37, P=0.011) between collagen density and age considered as continuous variable (unpublished data).

Table 4 shows the association between prognosis and collagen density in patients followed up after portoenterostomy.

No significant association was observed between collagen density and prognosis (P=0.166). A variation in collagen density was observed in ten images randomly captured in each case, which translates in wide standard deviations despite a reasonable number of patients involved in this comparison.

Table 5 shows the association of histological findings in biliary structures with the prognosis of the followed up cases.

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**Table 1 -** Postoperative evolution of patients with biliary atresia, according to groups presenting or not associated congenital extrahepatic anomalies

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Biliary atresia</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without</td>
<td>With</td>
</tr>
<tr>
<td></td>
<td>extrahepatic</td>
<td>extrahepatic</td>
</tr>
<tr>
<td></td>
<td>anomalies (n = 27)</td>
<td>anomalies (n = 7)</td>
</tr>
<tr>
<td>Dead or submitted to transplantation</td>
<td>13 (48)</td>
<td>6 (86)</td>
</tr>
<tr>
<td>Alive and not submitted to transplantation</td>
<td>14 (52)</td>
<td>1 (14)</td>
</tr>
</tbody>
</table>

Percentage between parenthesis
Statistical method: Fischer’s exact test

**Table 2 -** Relation between prognosis and age in patients submitted to portoenterostomy

<table>
<thead>
<tr>
<th>Prognosis</th>
<th>n</th>
<th>Age (survival period-days)</th>
<th>Difference</th>
<th>CI95%</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dead patients or submitted to transplantation</td>
<td>19</td>
<td>97.3 ± 35.9</td>
<td>27.3</td>
<td>5.3 to 49</td>
<td>0.016</td>
</tr>
<tr>
<td>Alive patients not submitted to transplantation</td>
<td>15</td>
<td>70.00 ± 23.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical method: Student t test
No significant difference could be found in terms of prognosis between the cases with and without DPM. Other histological findings in biliary structures have not influenced the prognosis. No correlation was observed between the presence of ductal plate malformation and age groups ($P=0.503$) (unpublished data).

### Table 3 - Relation between prognosis and colagen density in patients with biliary atresia

<table>
<thead>
<tr>
<th>Age group (survival period-days)</th>
<th>n</th>
<th>Colagen density (%)</th>
<th>CI 95%</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 60 &lt; 60</td>
<td>11</td>
<td>4.3±1.3³</td>
<td>3.5 a 5.2³</td>
<td></td>
</tr>
<tr>
<td>61 - 90</td>
<td>16</td>
<td>6.1±3.1</td>
<td>4.4 a 7.8</td>
<td>0.023</td>
</tr>
<tr>
<td>&gt; 90</td>
<td>20</td>
<td>8.1±4.7³</td>
<td>5.9 a 10.3³</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>6.6±3.9</td>
<td>5.4 a 7.7</td>
<td></td>
</tr>
</tbody>
</table>

Different index-letters represent statistically significant differences
Statistical method: ANOVA

### Table 4 - Relation between prognosis and colagen density in patients with biliary atresia

<table>
<thead>
<tr>
<th>Evolution</th>
<th>Colagen density (%)</th>
<th>Difference</th>
<th>CI95%</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dead or submitted to transplantation (n = 19)</td>
<td>7.6±4.5</td>
<td>1.87</td>
<td>– 0.8 to 4.6</td>
<td>0.166</td>
</tr>
<tr>
<td>Alive and not submitted to transplantation (n = 15)</td>
<td>5.7±2.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical method: Student t-test

### Discussion

The data presented in Table 1, although they did not show any significant difference between the groups with BA and those with and without ACEA, allowed finding that the distribution of patients without ACEA was homogenous in terms of prognosis: approximately 50% of the patients died or were submitted to transplantation, against 86% in the cases with ACEA. The fact that there is no relation between the presence of ACEA and worse prognosis in this study may be due to the small number of cases. This relation was detected by some authors in cases with laterality sequence anomalies. For Silveira et al. the most relevant prognostic factor is age at portoenterostomy. Table 2 shows that among 34 cases followed up at the outpatient clinic, 56% died or were submitted to transplantation during the study; most of the patients with a worse prognosis were submitted to surgery when they were older. A permanent bile flow, progressively lower as age advances, has been observed after Kasai procedure.

However, among the patients that had a satisfactory outcome in the present study, there were cases with more than 60 days of life and over 90 days (mean = 70.0; SD= 23.7), which seemingly justifies the use of portoenterostomy as an initial procedure in cases of BA at our center. Chardot et al. have confirmed a better survival rate in five and ten years after Kasai procedure in early operated patients, however, approximately 25% of their patients operated on after ninety days of life survived without liver transplantation for five years, and approximately 22% for ten years. According to these authors, this finding justifies the use of this procedure even in patients older than three months. In the present study, approximately 32% of the patients submitted to Kasai procedure (44% of those on outpatient follow-up), regardless of the age they were operated on, are still alive and have not been submitted to transplantation.

### Table 5 - Relation between histological findings in the biliary structures and prognosis in cases of biliary atresia followed in outpatients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dead or submitted to transplantation (n = 19)</th>
<th>Prognosis</th>
<th>Alive and not submitted to transplantation (n = 15)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ductal plate malformation</td>
<td>12 (60)</td>
<td>8 (40)</td>
<td>[Ductal plate malformation]</td>
<td>0.563</td>
</tr>
<tr>
<td>Present</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mature bile ducts</td>
<td>17 (90)</td>
<td>14 (93)</td>
<td>[Mature bile ducts]</td>
<td>0.693</td>
</tr>
<tr>
<td>Present</td>
<td>17 (90)</td>
<td>14 (93)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focused</td>
<td>4 (23)</td>
<td>4 (29)</td>
<td></td>
<td>0.750</td>
</tr>
<tr>
<td>Diffuse</td>
<td>13 (77)</td>
<td>10 (71)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ductular proliferation</td>
<td>11 (58)</td>
<td>5 (33)</td>
<td>[Ductular proliferation]</td>
<td>0.154</td>
</tr>
<tr>
<td>Focused</td>
<td>11 (58)</td>
<td>5 (33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffused</td>
<td>8 (42)</td>
<td>10 (67)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical method: chi-square test
The international literature reports a frequency of 42% in five years. Grosfeld et al. have found that age at portoenterostomy and presence of bile flow in the postoperative period influence the survival rate. They also reinforced that portoenterostomy should be maintained as an initial procedure in cases of BA, in order to prevent the morbidity associated with transplantation. Ohya et al. observed that survival ten years after portoenterostomy was associated with the resolution of jaundice in the postoperative period, age at surgery, extension of fibrosis, and size of bile ducts in the porta hepatis. Nevertheless, Tan et al. have not found any correlation between age at portoenterostomy and presence of patent bile ducts in the porta hepatis. They have affirmed that age is only important to the cases with less than 60 days of life at surgery. Other authors have not found any association between age and post-Kasai prognosis. One should take into consideration that the association between age and post-portoenterostomy prognosis is not linear and that the effect of a delayed surgery is only evident after some time limit. Groups of patients with age below this limit will not show an association between age and outcome.

As to the extension of fibrosis, there was a progressive increase with age, with a significant difference between the groups aged up to 60 days of life and the group aged more than 90 days (Table 3). Apparently, the over-90-day-old group was associated with a threshold for the extension of fibrosis. The nonexistence of a correlation between prognosis and superficial collagen density (Table 4) is in agreement with the findings of Tan et al.: fibrosis in the porta hepatis in all cases and absence of jaundice in approximately 27%. Up to the sixteenth week of life, no association between age and bile flow was found in the postoperative period; after this time, bile flow decreased, although it was still present in almost half of the patients.

As shown in Table 5, no association was observed between the presence of DPM and a worse prognosis in the postoperative period, which goes against Desmet’s statement that patients with this anomaly represent an early and severe form of the disease.

BA is correlated with the progressive destruction of interlobular bile ducts after the second month of life. In most children with BA, liver disease progresses regardless of bile duct patency, perhaps due to the recurrent episodes of cholangitis that aggravate fibrogenesis and/or due to the existence of a continuous morbid process that maintains functional and histological degradation of the liver. There is an abnormal pattern of the biliary tree in BA, which could be better understood after some consideration about the presence of DPM in the disease. Another relevant data regarding poor prognosis of several patients after Kasai procedure is the early presence of portal hypertension.

Most patients after Kasai procedure develop progressive hepatic fibrosis. Approximately one third of the cases present liver failure and require transplantation 12-14 months after surgery, while another one third needs transplantation during adolescence. The remaining patients survive, but present some liver disease. In the present study, only age at portoenterostomy influenced postoperative prognosis. The extension of fibrosis and histological alterations of biliary structures, including DPM, in the hepatic peripheral region, were not associated with a poorer prognosis. A greater number of cases should be studied before establishing an association of ACEA with poorer prognosis after portoenterostomy. The mean age at portoenterostomy is still high at our center in comparison with the previous assessment, showing that the referral of patients with BA is still late. Therefore, BA should be regarded as a medical emergency by pediatricians in our community.

References

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