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Nonobstructive Neonatal Cholestasis: Clinical Outcome and Scoring of the Histopathological Changes in Liver Biopsies

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ABSTRACT

The clinical outcome of nonobstructive neonatal cholestasis (NC) cases varies greatly and the prognosis is generally unpredictable. In this study, we aimed to evaluate the prognostic benefits of qualitative analysis of histopathological changes in nonobstructive NC cases. A total of 28 nonobstructive NC cases (18 neonatal hepatitis; 10 intrahepatic bile duct paucity) were studied. We analyzed the relationship between histopathological and clinical parameters. Hepatic inflammation, bridging necrosis, pericellular fibrosis, giant cell transformation, and extramedullary hematopoiesis were evaluated and scored according to their absence or presence in each case. The sum of the histopathological scores was accepted as "total pathological injury score." The height percentiles, the presence and the degree of hepatomegaly and ascites, and serum alanine aminotransferase (ALT), albumin, and bilirubin levels and prothrombin time were also evaluated and scored. The patients were divided into 2 clinical course groups considered "good" or "bad" according to the total clinical scores. For statistical analysis, Pearson's chi-square test, Mann-Whitney *U*-test, and receiver operating characteristic curve were used. We found a statistically significant negative relation between the clinical course and total pathological injury score ($P = 0.042$) and pericellular fibrosis ($P = 0.016$). In conclusion, during the interpretation of liver biopsies of nonobstructive NC, scoring of histopathological changes should be done for assessing the clinical prognostic outcome.

Key words: neonatal cholestasis, prognosis, histopathological changes, scoring

INTRODUCTION

Neonatal cholestasis (NC) is a condition characterized by jaundice and dark urine with hypocholic or acholic stool persisting more than 14 days in the infantile period. The impairment in the formation or flow of bile can be at any point from the bile production site to its drainage to the duodenum. The population incidence of NC has great geographic variation, ranging between 1:500 and 1:5000 live births [1]. It encompasses a large spectrum of disease entities, some of them the reflection of obstructive and others of nonobstructive etiologies [1–4]. Liver biopsy is necessary to differentiate the obstructive pathologies such as

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biliary atresia (BA), which accounts for the majority of the obstructive diseases, from the nonobstructive pathologies. The obstructive NC cases can be treated with surgery, and the prognosis depends for the most part on the timing and success of the surgery. The nonobstructive NC entities include cholestasis with detectable etiological causes, such as various infections, toxic causes, and metabolic disorders, in addition to intrahepatic bile duct paucity (IBDP) and idiopathic neonatal hepatitis (NH), which are characterized by lack of detectable underlying etiological factors.

Idiopathic NH and IBDP comprise a great part of the nonobstructive idiopathic NC (INC) cases [1,3] and are generally treated with symptomatic therapies. Although their prognosis is generally good, the risk of poor clinical outcome has also been documented. In previous studies, although several clinical and laboratory parameters have been reported as prognostic indicators, there have been no accepted data about the relation between the histopathological changes and the prognosis in idiopathic NH and IBDP [3,5–8]. In this study, we aimed to determine the prognostic significance of some histopathological changes in the liver biopsies of nonobstructive INC cases consisting of idiopathic NH and IBDP.

METHODS

Forty-four unselected consecutive NC cases admitted between 1997 and 2002 were reviewed retrospectively. These patients were admitted to the hospital with the symptoms of persistent cholestasis with icterus, direct hyperbilirubinemia higher than 2.5 mg/mL, and increased serum levels of alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT), which began after 2 weeks' postpartum. They had no family history. Those with the symptoms and features of obstructive etiologies (11 cases of BA and 1 case of Alagille's syndrome) and those with detectable infection (1 case of Epstein-Barr virus, 1 case of hepatitis B virus infection), metabolic disease (1 case of alpha-1 antitrypsin deficiency), and toxic and nutritional (1 parenteral nutrition) etiologies were excluded. In total, 28 patients with nonobstructive NC who showed microscopic features of idiopathic NH and IBDP and who lacked evidence of any etiological cause on laboratory tests and clinical investigations were included in the study.

With the exception of 1 wedge biopsy, all of the 28 specimens were from percutaneous liver biopsies and had been taken before therapy. They were fixed in 10% formalin for 24 to 48 hours at room temperature and embedded in paraffin wax. The 5-μm-thick, hematoxylin and eosin- and Masson's trichrome-stained sections were prepared from paraffin blocks. Two pathologists separately evaluated the slides twice to study the intraobserver and interobserver consistency. The interval between the evaluations was 6 months. Intraobserver and interobserver consistency was analyzed with kappa value (Cohen's kappa). The kappa value is a measure of agreement adjusted for the chance expected agreement. The kappa value is 0 if agreement is no better than chance. If agreement is perfect, the coefficient is 1. According to Landis and Koch [9], we considered the strength of agreement as slight for values between 0 and 0.19; fair for values between 0.20 and 0.39; moderate for values between 0.40 and 0.59; substantial for values between 0.60 and 0.79; and almost perfect for values greater than 0.80.

In order to compare the clinical course of cases with histopathological features, some of the clinical follow-up parameters and histopathological findings were scored. Clinical follow-up period varied from patient to patient, with an average of 3.86 years (range, 1 to 6 years). For the good prognosis group, the mean follow-up period was 3.785 years (range, 1 to 6 years; median, 3.5 years). For the poor prognosis group, the mean follow-up period was 4.285 years (range, 2 to 6 years; median, 4 years). To score the clinical data, we used the parameters of the CHILDS method, which is used for standard follow-up procedure in chronic hepatitis. The clinical follow-up parameters that were evaluated and scored at the end of the follow-up period were height percentiles, the presence and degree of hepatomegaly and ascites, serum ALT, albumin, and bilirubin levels, and prothrombin time. These clinical follow-up parameters were scored as summarized in Table 1. A total clinical score (TCS) was obtained for each case by adding all the clinical and laboratory scores. Next, the cases were divided into two prognostic groups according to the TCS. The cases with TCS of 4 or higher were accepted as the "good clinical course" group, and the cases with TCS of less than 4 were

Table 1. Scoring of clinical and laboratory parameters

Clinical and laboratory parameters	Score 1	Score 0
Height percentile	In normal limits according to age	<3% of normal limits
Hepatomegaly	Absent or <2 cm	>2 cm
Ascites	Not detectable by physical examination	Detectable by physical examination
ALT level (U/L)[a]	≤60	>60
Albumin level (g/dL)	≥3.25	<3.25
Bilirubin level (g/dL)	≤2.5	>2.5
Prothrombin time (s)	≤16	>16

[a]ALT, alanine aminotransferase.

accepted as the "bad clinical course" group. All cases of idiopathic NH and IBDP were evaluated and scored according to the same parameters.

To score histopathological data, the presence of portal, periportal, or lobular inflammation; bridging necrosis with inflammation; bridging necrosis with fibrosis; pericellular, portal, and periportal fibrosis; giant cell transformation; and extramedullary hematopoiesis was evaluated blindly without knowledge of the clinical parameters and scores. They were scored as present (1) and absent (0), without grading severity for each case (Table 2). A total pathological score (TPS) for hepatic injury was obtained by adding all scores of the histopathological parameters for each case. As all the cases demonstrated bilirubin stasis and Kupffer cell activation, these 2 features were not included in the analysis. The results of the senior pathologist's first evaluation were used in statistical analysis. The relations between the histopathological parameters and TPS and prognosis were analyzed statistically with Pearson's chi-square test, Spearman's rho correlation, Mann-Whitney *U*-test, and receiver operating characteristic (ROC) curve.

Table 2. Histopathological parameters evaluated for assessing the total pathological hepatic injury score

Histopathological parameters	Score 1	Score 0
Portal inflammation	Moderate or severe	Absent or mild
Periportal inflammation	Moderate or severe	Absent or mild
Lobular inflammation	Moderate or severe	Absent or mild
Bridging necrosis with inflammation	Present	Absent
Bridging necrosis with fibrosis	Present	Absent
Portal fibrosis	Moderate or severe	Absent or mild
Periportal fibrosis	Moderate or severe	Absent or mild
Pericellular fibrosis	Present	Absent
Giant cell transformation	Present	Absent
Extramedullary hematopoiesis	Present	Absent

RESULTS

Table 3 summarizes all of the clinical and histopathological features of the patients. The age of the patients at the time of biopsy ranged from 4 months to 48 months (mean, 17.75 months). Thirteen (46.43%) were aged 2 months or less at the time of the biopsy; 10 were female and 18 were male. The follow-up period was 4 to 6 years for 18 patients, 3 years for 6 patients, 2 years for 3 patients, and only 1 year for 1 patient. Seventeen cases with TCS of 4 or higher comprised the "good clinical course" group, and 11 cases with TCS of less than 4 comprised the "bad clinical course" group. The liver biopsy diagnosis was NH in 18 cases and IBDP in 10 of the cases. All liver biopsies of the cases showed the features of cholestasis such as cytoplasmic feathery degeneration, formation of cholestatic rosettes, cholestatic plugs, and Kupffer cell activation. Those cases that demonstrated bile ducts fewer than 0.5 bile duct per portal area in addition to the cholestatic features were diagnosed as IBDP. The cases that showed predominantly parenchymal lobular inflammation with intense giant cell transformation in addition to the cholestatic features were diagnosed as idiopathic NH. The number of portal areas of each case is also shown in Table 3. The mean number of portal areas was 9.889 (SD = 3.817) in the liver biopsies of idiopathic NH cases and 8.400 (SD = 4.060) in the liver biopsies of the IBDP cases. The mean number of portal areas for all cases was 9.357 (SD = 3.889).

Table 4 shows the intraobserver and interobserver variability for the histopathological param-

Table 3. Clinical and histopathological features, total pathological hepatic injury scores (TPS) of hepatic injury and total clinical scores (TCS) of all cases

Case	Age	Sex	PI	pPI	LI	BI	BF	PF	pPF	pCF	GC	EMH	Diagnosis	TPS	TCS	PA *n*
1	2 mo	M	+	+	+	−	−	+	+	+	+	+	NH	8	4	12
2	1 y	F	+	+	+	−	−	+	+	+	+	+	NH	8	5	11
3	2 mo	F	+	+	+	−	−	+	+	+	−	+	NH	7	7	8
4	6.5 mo	M	+	+	+	+	+	+	+	+	+	−	NH	9	1	12
5	40 d	F	+	+	+	−	−	−	−	−	−	+	NH	4	7	6
6	4 mo	M	+	−	+	+	−	−	−	−	+	+	NH	4	3	7
7	3.5 mo	M	+	+	+	+	−	−	−	−	+	+	NH	6	7	5
8	2 mo	M	+	+	+	−	−	−	−	−	+	+	NH	5	7	5
9	1.5 mo	M	+	+	+	+	−	+	+	+	−	+	NH	8	2	12
10	5 mo	F	+	+	−	+	+	+	+	+	+	−	NH	8	3	14
11	3 mo	F	+	+	−	−	−	−	−	−	−	−	NH	2	7	15
12	3 mo	F	+	+	+	+	+	+	+	+	+	−	NH	9	2	15
13	2 mo	M	−	−	−	−	−	+	+	+	+	+	NH	5	6	5
14	8 mo	M	+	+	+	+	+	+	+	+	+	−	NH	9	0	10
15	40 d	M	+	+	−	−	−	−	−	−	+	+	NH	4	5	6
16	2 mo	F	−	−	+	−	−	−	−	−	+	−	NH	3	3	16
17	3 mo	F	+	+	-	−	−	−	−	+	+	−	NH	4	2	12
18	2 mo	M	+	+	+	−	−	+	−	+	+	+	NH	7	7	7
19	4 mo	M	+	+	+	−	−	−	−	−	+	+	IBDP	5	1	6
20	3 mo	M	+	+	+	−	+	+	+	+	+	−	IBDP	8	2	11
21	2 mo	M	+	+	−	−	−	−	−	−	+	+	IBDP	4	7	5
22	3 mo	M	+	+	−	+	+	+	+	−	−	−	IBDP	6	7	5
23	45 d	M	+	−	−	−	−	+	+	+	−	+	IBDP	5	3	8
24	1 y	M	+	−	−	−	−	+	−	−	+	+	IBDP	4	7	11
25	2 mo	M	+	+	+	−	−	−	−	+	+	+	IBDP	6	4	8
26	2 mo	M	+	−	−	−	+	+	+	−	−	−	IBDP	4	3	5
27	4 mo	M	+	+	+	+	−	−	−	−	−	+	IBDP	5	5	7
28	7.5 mo	F	+	+	+	−	−	−	−	−	−	−	IBDP	3	3	18

PI, portal inflammation; pPI, periportal inflammation; LI, lobular inflammation; BI, bridging necrosis with inflammation; BF, bridging necrosis with fibrosis; pPF, periportal fibrosis; pCF, pericellular fibrosis; GC, giant cell transformation; EMH, extramedullary hematopoiesis; PA *n*, total number of portal areas of the liver biopsy; M, male; NH, idiopathic neonatal hepatitis; F, female; IBDP, intrahepatic bile duct paucity.

Table 4. Kappa values for histopathological parameters

Histopathological parameters	Pathologist A1 versus A2 kappa value	Pathologist B1 versus B2 kappa value	Pathologist A2 versus B2 kappa value
Portal inflammation	1.000	1.000	1.000
Periportal inflammation	0.300	0.789	0.579
Lobular inflammation	0.462	0.239	0.472
Bridging necrosis with inflammation	0.916	0.916	1.000
Bridging necrosis with fibrosis	0.837	1.00	0.920
Portal fibrosis	0.571	1.00	0.674
Periportal fibrosis	0.653	0.856	0.710
Pericellular fibrosis	1.00	1.00	0.857
Giant cell transformation	0.747	0.851	0.761
Extramedullary hematopoiesis	0.764	0.924	0.837

A and B denote two independent pathologists; 1 and 2 denote first and second evaluation rounds.

eters of hepatic inflammation, bridging necrosis with fibrosis, pericellular fibrosis, giant cell transformation, and extramedullary hematopoiesis during the 1st and 2nd examination rounds by pathologists A and B. The strength of agreement was almost perfect or substantial for 8 parameters (portal inflammation, bridging necrosis with inflammation and fibrosis, portal and periportal fibrosis, giant cell transformation, pericellular fibrosis, and extramedullary hematopoiesis) of the

10 total histopathological parameters. Periportal and lobular inflammation demonstrated the lowest degree of agreement.

We compared the NH cases with the IBDP cases according to the histopathological parameters (hepatic inflammation, bridging necrosis with fibrosis, pericellular fibrosis, giant cell transformation, and extramedullary hematopoiesis), TPS, and TCS. Using the chi-square test, no statistically significant difference could be established between NH and IBDP with respect to the histopathological parameters, TPS, TCS, and clinical course. Thus, the statistical analyses of the prognostic effects of the histopathological parameters were studied together without dividing the cases according to their diagnosis. Ten of the 18 NH (55.55%) and 5 (50%) of the 10 IBDP cases were in the poor prognosis group. We analyzed the difference between the NH and IBDP cases according to the clinical course using the chi-square test, and there was no statistically significant prognostic difference between NH and IBDP ($P = 0.883$).

The statistical analysis of the relation between the prognostic groups and the histopathological parameters, including portal, periportal, lobular, and bridging inflammation; pericellular, portal, periportal, and bridging fibrosis; giant cell transformation; and extramedullary hematopoiesis, was done using the chi-square test, and the results are summarized in Table 5. Among the histopathological parameters, pericellular fibrosis demonstrated a statistically significant relationship with bad clinical course ($P = 0.016$). Moreover, the relations between prognosis and bridging necrosis with fibrosis ($P = 0.076$) and bridging necrosis with inflammation ($P = 0.095$) were very close to the statistically significant level.

As 13 of the 28 patients (46.43%) were 2 months of age or younger, we divided them into 2 groups: those 2 months of age or younger and those older than 2 months of age. We compared the TPS and TCS of the 2 groups using Mann-Whitney *U*-test. The mean TPS was 5.38 ± 1.66 (median = 5.0) for the patients 2 months or younger and 6.00 ± 2.36 (median = 6.0) for the patients older than 2 months. The mean TCS was 5.00 ± 1.91 (median = 5.0) for those 2 months or younger and 3.67 ± 2.47 (median = 3.0) for those older than 2 months. There was no significant difference between the TPS ($P = 0.44$) and TCS ($P = 0.130$) of these 2 age groups. We also investigated whether there was a difference between these 2 age groups according to the clinical course using the chi-square test, and the difference was statistically insignificant between the younger ($\leq$2 months) and older (>2 months) patients ($P = 0.122$).

The correlation between the TPS and TCS was analyzed using Spearman's rho correlation coefficient, and a statistically negative correlation between them was demonstrated ($r = -0.408$; $P = 0.031$). Also, the relation between the TPS of the good versus bad clinical course groups was compared using Mann-Whitney *U*-test. TPS median of the bad clinical course group was 8 and that of the good clinical course was 5, and the difference between these groups was statistically significant ($P = 0.04$). We also analyzed the prognostic value of the TPS using the ROC curve, and we demonstrated that TPS could be used as a prognostic denominator (area under the ROC curve = 0.74; $P = 0.043$).

DISCUSSION

In general, there have been remarkable efforts toward quantitative analysis of histopathological changes in order to obtain objective parameters for the clinical course in nontumoral liver pathologies. Many histopathological scoring systems have thus been created for nontumoral liver diseases in the recent studies. However, to date there has been no such accepted and published scoring system for NC.

Cholestasis in neonatal ages differs etiologically and pathologically from that in adults and is named NC. Approximately 80% of etiologies of NC are BA, NH, and IBDP. It is well known that BA differs from NH and IBDP, because the treatment is mainly surgical for BA and medical for the others [1,3,4]. The prognosis of BA greatly depends on the timing and success of the surgery [4]. But the outcome in NH and IHBD may demonstrate resolution, liver failure, or a chronic cholestatic course [1,2,4,5]. No histopathological parameter has yet to be accepted as related with prognosis. The presence of family history, degree and duration of jaundice, and peak serum bilirubin levels have been reported as clinical and laboratory indicators of poor prognosis in idiopathic NH [4–7]. Recent studies have described uncommon genetic disorders associated with bile acid synthesis or transport defects characterized by low

Table 5. Relation between histopathological parameters and clinical course groups using chi-square test

Histopathological parameters		Clinical course groups[a] Good ($n = 17$) (TCS ≥ 4)	Bad ($n = 11$) (TCS < 4)	*P* value
Portal inflammation	(+)	15	11	
	(−)	2	—	0.505
Periportal inflammation	(+)	12	9	
	(−)	5	2	0.668
Lobular inflammation	(+)	9	8	
	(−)	8	3	0.435
Bridging inflammation	(+)	3	6	
	(−)	14	5	0.095
Pericellular fibrosis	(+)	6	9	
	(−)	11	2	0.016
Portal fibrosis	(+)	9	7	
	(−)	8	4	0.705
Periportal fibrosis	(+)	6	7	
	(−)	11	4	0.246
Bridging fibrosis	(+)	2	5	
	(−)	15	6	0.076
Giant cell transformation	(+)	10	9	
	(−)	7	2	0.197
Extramedullary hematopoiesis	(+)	13	5	
	(−)	4	6	0.125

[a]TCS, total clinical score.

serum levels of gamma glutamyl transferase and family history [10–12]. These disorders are characterized by microscopic features of NH with giant cell transformation, and a few of them show periportal or lobular fibrosis. Some of the cases described as idiopathic NH with poor prognosis in the past might be such bile acid synthesis disorders. None of our cases had a family history of NC or low serum GGT levels. IBDP is an extremely heterogeneous disease group for which there is no clinical or histopathological prognostic indicator [5,7].

In this report, we studied nonobstructive idiopathic NC cases including NH and IBDP, which belong in the medically treated group of NC. We attempted to evaluate the relationship between histopathological features and clinical parameters. We looked for the prognostic difference between idiopathic NH and IBDP. As we did not detect any statistically significant prognostic difference between the NH and IBDP cases, we analyzed the relation between TPS, TCS, and prognostic groups without separating the cases according to histopathological diagnosis. Although we believe that the clinical outcome is in general similar between idiopathic NH and IBDP, we realize the number of our cases is statistically small for making distinct comments about prognostic differences of these diseases. In order to make an objective comparison between prognostic outcome and histopathology, we proposed a numerical scoring system for both the histopathological changes and the clinical parameters. TPS reflected the total histopathological hepatic injury in this study. The cases in the poor clinical course group according to the clinical parameters showed a statistically significantly higher TPS versus those of the good clinical course group.

We also analyzed the difference in TCS between the cases 2 months of age or younger and those older than 2 months. Not only the TCS but also the TPS showed statistically insignificant differences between these 2 age groups. But this statistically insignificant outcome might be the result of the small number of the cases. In the future, larger series with more standardized data might show prognostic difference according to age in idiopathic NH and IBDP cases.

Fibrosis in nontumoral liver diseases, especially in chronic hepatitis, is always an alarming feature for progression. The degree of fibrosis is the expression of the staging in liver diseases [13].

It is generally based on portal, periportal, and bridging fibrosis. In our NC cases, bridging necrosis with fibrosis and inflammation correlated with poor prognosis, close to the statistically significant level, but this was most probably caused by the numerical limitation of the patients. We believe that the relation between bridging fibrosis and inflammation with the clinical course would also be demonstrated at a statistically significant level in a larger series. Pericellular fibrosis is not accepted as a staging parameter in widely accepted grading and staging procedures. But as we noted a statistically significant relationship between pericellular fibrosis and poor prognosis in our cases, we propose that pericellular fibrosis should have been noted in the pathology reports separately.

The observer variability is a measure of the reliability and reproducibility of a diagnostic procedure, especially for the methods based on the scoring of some histopathological criteria. We analyzed the interobserver and intraobserver consistency in this study. The most important parameters correlated with prognosis, including pericellular fibrosis, bridging fibrosis and bridging inflammation, had kappa values greater than 0.85, indicating a perfect consistency. The parameters of periportal and lobular inflammation demonstrated the lowest degree of agreement. The extramedullary hematopoiesis sometimes simulates lobular and periportal inflammation and causes difficulties in their evaluation, especially in the biopsies with minimal inflammation. Furthermore, massive giant cell transformation causes misinterpretation of the periportal limiting membrane and thus of the periportal inflammation.

In this study, the histopathological parameters of portal and periportal inflammation and fibrosis and of bridging inflammation and fibrosis were evaluated in the portal areas. There are several studies in the literature about the ideal biopsy size and the number of portal areas in interpretation of liver biopsies [14–19]. In our study, the mean number of portal areas in our cases was 9.357 (SD = 3.889), which can be considered as acceptable. Generally, liver biopsies with a minimum of 5 portal areas or 1.5-cm-long liver biopsies were considered as acceptable size in most of those studies, especially for interpretation of chronic hepatitis [17–19]. Colloredo and colleagues [16] suggested indicating the number of complete portal areas in the pathology reports, which can be available also for the interpretation of the liver biopsies of NC cases. As none of these studies included NC cases, we considered the cases with a minimum of 5 portal areas as sufficient for evaluation.

As a result, we determined that the evaluation of the histopathological hepatic injury by scoring of the histopathological parameters—pericellular, portal, periportal, and bridging fibrosis; portal, periportal, lobular, and bridging inflammation; giant cell transformation; and extramedullary hematopoiesis—is a valuable prognostic indicator in nonobstructive idiopathic NC cases. We are aware that the categories of idiopathic NH and IBDP are diminishing with the advances in genetics and molecular pathology and that the cases considered as idiopathic NH today might be categorized differently in the future. In addition, we know that the liver shows a limited spectrum of histopathological changes for different etiopathological agents. Our cases showed that pericellular fibrosis in particular is an important histopathological finding predicting poor prognosis and that it is an important parameter that must be reported separately in pathology reports. In conclusion, during interpretation of nonobstructive NC cases, reporting the severity of histological changes by scoring will be very useful for predicting prognosis and obtaining objective and standard data for future studies.

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