Differential diagnosis of neonatal cholestasis: clinical and laboratory parameters

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Abstract

Objective: To evaluate if clinical and laboratory parameters could assist in the differential diagnosis of intra and extra-hepatic neonatal cholestasis (NC).

Methods: Retrospective study of NC patients admitted at the Pediatric Hepatology Outpatient Clinic of the teaching hospital of Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil, between December 1980 and March 2005. The approach to the diagnosis of NC was standardized. According to diagnosis, patients were classified into two groups: I (intra-hepatic neonatal cholestasis) and II (extra-hepatic neonatal cholestasis). In order to verify if there was association with the categorical variable, the chi-square and Mann-Whitney tests were used, with corrections for age for the covariance analysis (ANCOVA). The determination of accuracy of the clinical and laboratory variables for differentiation of the groups was made using the analysis of the ROC curve.

Results: One hundred and sixty-eight patients were evaluated (group I = 54.8% and group II = 45.2%). In the patients with less than 60 days of life there was predominance of intra-hepatic causes, whereas, in those older than 60 days, there was predominance of extra-hepatic etiology (p < 0.001). Median birth weight was lower in group I (p = 0.003), as well as length at birth (p = 0.007). Median values of direct bilirubin were higher in group II (p = 0.006). Values of gamma-glutamyltransferase (GGT) (10 times higher than the limit of normality) presented sensitivity of 56.3%, specificity of 91.5%, and accuracy of 75.7% for the diagnosis of extra-hepatic cholestasis.

Conclusion: In the present study, extra-hepatic NC presented greater weight and length at birth, fecal hypocholia/acholia, choluria, hepatomegaly, increase in GGT (10.8 times higher than the limit of normality), and a delay for investigation in the tertiary center.

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Introduction

Neonatal cholestasis (NC) is a syndrome that poses a major diagnostic challenge to the clinician from an anatomical standpoint. A rapid investigation is always considered to be pathologically necessary.[1-3]

The frequency of NC in the population is difficult to evaluate. Incidences of 1/2,500,[4] 1/5,000,[5] and 1/9,000[6] live births have been recorded in the literature. The focus of the initial approach is to differentiate between intrahepatic

(IHC) and extrahepatic (EHC) cholestasis. Differentiation between IHC and EHC may have a success rate of 90 to 95% when various diagnostic methods are used.[2]

IHC represents about 2/3 of all cases of NC.[4,7-9] Once established that cholestasis is intrahepatic, the new challenge is to determine the etiological diagnosis. In a classification proposed by Dellert & Balistreri,[8] there are around 40 possible causes of IHC. Despite this fact, part of the cases is

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of unknown etiology. The causes of EHC are less numerous and include biliary atresia (BA), choledochal cyst, biliary sludge, and biliary duct stricture.[1,10]

According to the guideline of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN), any infant noted to be jaundiced at 2 weeks of age (or 3 weeks of age in those who are breastfed) should be investigated for cholestasis by measuring serum bilirubin levels.[2]

Treatable disorders, such as sepsis, hypothyroidism, panhypopituitarism, and inborn errors of metabolism (for example, galactosemia), must be recognized and treated promptly to prevent significant progression of the disease. In BA, urgency in diagnosis is associated with the performance of the Kasai porto-jejunostomy before the infant is 60 days of age and is directly related to successful treatment.[2,9,12,13]

The most important findings in BA are jaundice and acholic stools starting between ages 4 and 8 weeks in infants who are otherwise apparently in good health.[14]

For early identification of BA cases, the use of a stool color card,[3,15] ultrasonography for the presence of the triangular cord sign,[16,17] and also correlation of clinical, laboratory and histopathological findings have been proposed.[3,14,18-20]

This study is aimed at evaluating whether clinical and laboratory parameters may help to establish differentiation between IHC and EHC.

Methods

A retrospective study was conducted from December 1980 to March 2005 to review the medical charts of patients referred to a tertiary care center [Pediatric Hepatology Outpatient Facility at Hospital de Clínicas of Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil] for investigation of NC. Inclusion criteria were: cholestatic jaundice starting within the first 3 months of life and performance of liver biopsy during the first year of life.

The protocol for data acquisition contained the following information: fetal distress, breastfeeding, gender, age, birth weight, length at birth, dark urine, a history of jaundice at birth or after 1 month of age, pale or acholic stools, vomiting, edema, ascites, hepatomegaly, and splenomegaly.

Laboratory test results were obtained in absolute numbers and number of times the upper limit of normal [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma-glutamyl-transferase (GGT)], in addition to albumin, International Normalized Ratio (INR) and direct bilirubin (DB). To determine the etiology, the following tests were reviewed: liver biopsy, serological test results for cytomegalovirus (CMV), human immunodeficiency virus (HIV), Epstein-Barr virus (EBV),

rubella, toxoplasmosis, and syphilis. Whenever necessary, data were also collected from other tests performed, such as measurement of alpha-1-antitrypsin level, measurement of sweat sodium and chloride concentrations, study of inborn errors of metabolism, hormone levels, study of polymerase chain reaction for CMV (PCR-CMV) in the blood, and antigenemia.

Patients were divided into two groups: group I with IHC and group II with EHC.

Ethical aspects

The study was approved by the Research Ethics Committee of the School of Medicine of UNICAMP.

Statistical analysis

To compare categorical variables between groups of patients with cholestasis, the chi-square test or Fisher's exact test was used, whenever necessary. To compare numerical variables between groups, the Mann-Whitney test was used. Due to the age difference between the groups, comparisons were repeated and corrected for age, using analysis of covariance (ANCOVA).

Receiver operating characteristic curve (ROC) analysis was carried out to obtain cutoff values for GGT (absolute values and number of times the upper limit of normal) to distinguish between IHC and EHC, using the final diagnosis as the gold standard.

Results

One hundred and sixty-eight patients were evaluated (group I = 54.8% and group II = 45.2%). A significant difference was observed between the groups regarding the following variables: gender, age, birth weight, height at birth, dark urine, acholic stools, hepatomegaly, direct bilirubin, absolute value and number of times the upper limit of normal for GGT.

The predominant gender was male (62.64%) in group I and female in group II (55.26%) with p = 0.026.

In infants investigated before 60 days of age, intrahepatic causes predominated, whereas in those investigated after 60 days of age, extrahepatic causes predominated (p < 0.001).

Weight and length at birth were lower in group I. Number of patients with birth weight lower than 2,500 g was higher in group I. Hepatomegaly, pale/acholic stools, and dark urine predominated in cases of EHC. Results are shown in Table 1. The median serum level of direct bilirubin was higher in group II (p = 0.006). The same occurred with GGT level which was shown as the number of times the upper limit of normal (p < 0.001) (Table 2).

For the diagnosis of EHC, serum levels of gamma-glutamyl-transferase ≥ 429.5 U/L or > 10.8 times the upper limit of normal had a sensitivity of 56.3%, a specificity of 91.5%, and an accuracy of 75.7% (Figures 1 and 2).

There were no statistical differences between groups I and II concerning history of fetal distress, breastfeeding, history of jaundice at birth or after the first month of life, vomiting, splenomegaly, presence of edema, and ascites.

Discussion

In the investigation of neonatal cholestasis, the main aim is to make an early diagnosis and promptly implement the most appropriate therapy. For example, cases of sepsis, hypothyroidism, and metabolic disease require early diagnosis and treatment to prevent progression of the disease and its consequent worsening. BA must be diagnosed promptly because early performance of the Kasai procedure (before 2 months of age) is directly related to

successful therapy.[2] Liver biopsy is an excellent diagnostic test, as long as it contains five to seven portal tracts and is performed by an experienced pathologist with expertise in pediatric blood pathology. However, the gold standard of diagnosis is exploratory laparotomy.[2,11,21]

Despite their help in differentiating intrahepatic from extrahepatic cholestasis, the clinical and laboratory parameters evaluated failed to indicate the cause of cholestasis. The most promising approaches for the early diagnosis seem to be the use of stool color identification cards and careful attention to the presence of acholic stools associated with serum bilirubin levels.[3,15] There have been various attempts to combine clinical and laboratory parameters for the differential diagnosis of IHC and EHC. More recently, Dehghani et al.[21] studied 62 infants with NC and found a prevalence of acholic stools in 94.7% of patients with BA and 56.5% of those with IHC. Results from the study by Dehghani et al. were comparable to those found by Mowat et al.,[18] which were 83 and 52%, respectively.
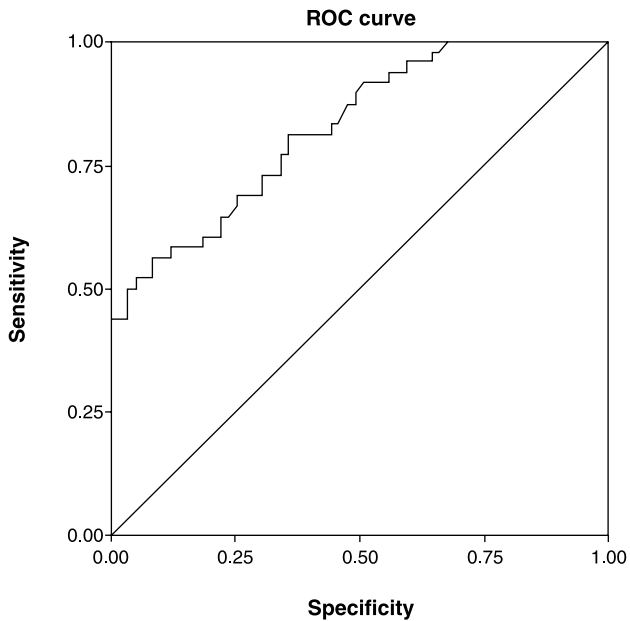
Table 1 - Patient clinical characteristic in the first examination, according to group

Variable	n	Group I (n = 92)	Group II (n = 76)	p
Gender	168			0.026
Male		57	34	
Female		35	42	
Age (days)	168	56±46	77±36	< 0.001
Birth weight (g)	165	2,630±775.18	2,975±432.81	0.003
Number of patients with birth weight < 2,500 g	165	31 (n = 90)	5 (n = 75)	< 0.001
Length at birth	122	47±4 (n = 60)	48±1.85 (n = 62)	0.007
Pale/acholic stools	167	64 (n = 91)	68	0.002
Dark urine	168	64	67	0.004
Hepatomegaly	157	37 (n = 86)	50 (n = 71)	< 0.001

Table 2 - Laboratory test results performed at the beginning of investigation, expressed as the median (interquartile range)

Variable	n	Group I (n = 92)	Group II (n = 76)	p
ALT	159	2.58 (0.10-18.25)	3.63 (0.43-19.45) (n = 67)	0.090
AST	159	4.69 (0.17-44.00)	5.37 (0.02-17.86) (n = 67)	0.546
AP	121	1.16 (0.13-12.10) (n = 77)	1.53 (0.13-10.50) (n = 56)	0.346
GGT	107	3.91 (0.49-8.49) (n = 59)	12.68 (2.10-6.63) (n = 48)	< 0.001
Albumin (g/dL)	89	3.77 (1.68-4,84) (n = 51)	3.70 (1.19-5.60) (n = 38)	0.684
INR	59	1.04 (1.00-2.77) (n = 15)	1.14 (0.87-2.08) (n = 44)	0.649
DB (mg/dL)	159	6.35 (1.10-19.60) (n = 90)	8.30 (1.68-17.00) (n = 69)	0.006

ALT = alanine aminotransferase; AP = alkaline phosphatase; AST = aspartate aminotransferase; DB = direct bilirubin; GGT = gamma-glutamyl-transferase, albumin; INR = International Normalized Ratio.
The median level of hepatic enzymes is shown as the number of times the upper limit of normal.

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ROC curve

GGT = gamma-glutamyl-transferase; ROC = receiver operating characteristic.
Number of times the upper limit of gamma-gt: sensitivity = 58.3%; specificity = 91.5%; accuracy = 76.6%; n = 107.
Cutoff: number of times GGT $\geq$ 10.80.

Figure 1 - Results of ROC curve analysis for number of times the upper limit GGT values
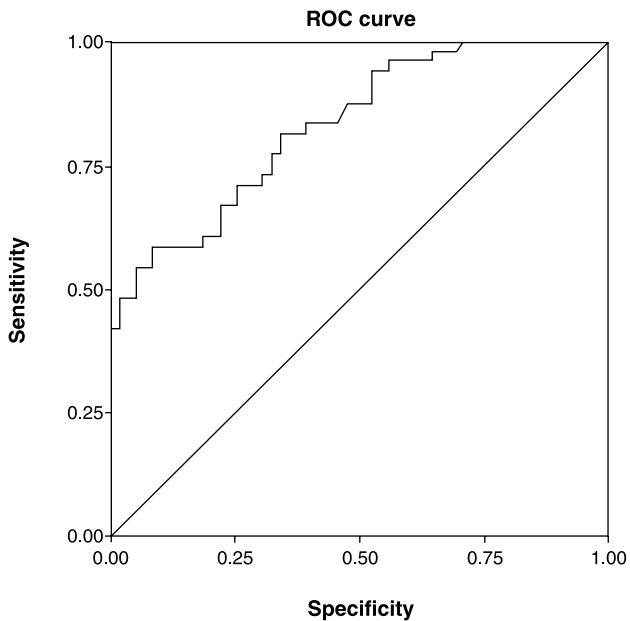
ROC curve

GGT = gamma-glutamyl-transferase; ROC = receiver operating characteristic.
Absolute GGT values area = 0.825; 95%CI 0.749-0.901; p < 0.001; sensitivity = 56.3%; specificity = 91.5%; accuracy = 75.7%; n = 107.
Cutoff value: GGT $\geq$ 429.5.

Figure 2 - Results of ROC curve analysis for absolute GGT values

No statistically significant differences were found in serum levels of AST, ALT and AP, similar to results obtained in the present study.

Weight and length at birth were lower in the IHC group, possibly reflecting prematurity, failure to thrive, neonatal complications, metabolic disorders, and congenital infections.[8,14,19,22,23]

The median age of infants at the time of referral is a cause for concern. In the EHC group, age at referral was over 60 days (77 days) of life and higher than that in the IHC group. Data published in Brazil show that in the majority of cases (76 to 94.7%), the diagnosis of BA is made after 8 weeks of life.[10,24-27]

It is likely that infants in the IHC group were more promptly investigated, due to a more severe systemic illness and arrived earlier at a referral center. In the EHC group, referral was delayed, resulting in the performance of the Kasai portoenterostomy at a later date. Perhaps this may have contributed to a more frequent finding of hepatomegaly.

Dark urine and pale/acholic stools predominated in infants with EHC, and the median serum levels of direct bilirubin was higher, reflecting a more marked clinical picture of cholestasis.

GGT is an enzyme that transfers the gamma-glutamyl group from glutathione and other peptides. It is present in the biliary epithelium and hepatocytes. GGT levels are elevated in various liver diseases. GGT is also present in the kidney, breast, intestine, brain, pancreas, and spleen. Normal values vary with age, gender, and diagnostic methods. That is why emphasis is placed on results expressed as the number of times the upper limit of normal.[28] It is recognized as a useful test for differentiating familial cholestatic syndromes.[8]

The use of GGT to differentiate IHC from EHC was not recommended by Moyer et al.,[2] who classified it as level C evidence, because a wide variability of tests makes the interpretation of results more difficult.

Wright & Christie[29] reported that GGT > 300 UI/L could be suggestive of EHC. Lai et al.[17] found GGT values > 300 UI/L with a specificity of 82.8% and accuracy of 72% for the diagnosis of EHC. Wang et al.[30] concluded that normal or low levels of serum GGT may indicate a more severe case of idiopathic IHC, albeit reversible.

In the present study, the GGT value expressed as the number of times the upper limit of normal was also higher in cases of EHC.

Using a GGT cutoff value > 203.5 UI/L or 5.8 times the upper limit of normal, specificity was 81.3% and accuracy was approximately 72%. When we used a cutoff value > 429.5 UI/L or 10.8 times the upper limit of normal for GGT, specificity was 91.5% and accuracy was approximately 76%.

In patients with NC who presented with a good weight and height at birth, pale/acholic stools, dark urine, hepatomegaly, and a marked elevation in serum GGT (> 10.8 times the upper limit of normal), the most likely diagnosis was EHC. This should be remembered, since there was a delay in referring these patients to tertiary care centers, which decreases their chance of benefitting from the Kasai procedure.

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