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Serum levels of interleukin-8 and gut-associated biomarkers in diagnosing necrotizing enterocolitis in preterm infants ☆

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ABSTRACT

Background: In recent years several potential biochemical markers have been evaluated to facilitate a reliable diagnosis of necrotizing enterocolitis (NEC), but none have made progress to clinical routine. We performed a comparative assessment in premature infants to evaluate the diagnostic value of the routinely available cytokine interleukin (IL)-8, and two promising experimental biomarkers, the gut barrier proteins liver fatty acid binding protein (L-FABP) and intestinal fatty acid binding protein (I-FABP), respectively, for the diagnosis of NEC.
Methods: IL-8, L-FABP, and I-FABP concentrations were analyzed in the serum of 15 infants with NEC and compared with 14 gestational age-matched infants serving as a control group.
Results: Serum concentrations of I-FABP, L-FABP and IL-8 were significantly higher in infants with NEC compared with controls. IL-8 showed the highest diagnostic value with an area under the curve of 0.99, followed by L-FABP and I-FABP. In addition we found a significant correlation between IL-8 and both FABPs in infants with NEC.
Conclusion: Our results further advocate the possible role of IL-8 as a specific marker for NEC. The diagnostic value of IL-8 seems to be superior to I-FABP, and similar to L-FABP. The routinely availability facilitates IL-8 as a possible candidate for further clinical investigations.

Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in premature infants, and remains one of the leading causes for morbidity and mortality [1]. Surviving patients of NEC are facing severe sequelae including feeding problems, failure to thrive, dependence on parenteral nutrition, short bowel syndrome and neurodevelopmental impairment [2].

The exact pathogenesis remains to be elucidated, but one unifying hypothesis states a deregulated inflammatory response by the neonatal intestine to luminal bacteria [3]. In addition, intestinal epithelial injury is caused by different initiating events including intestinal ischemia, formula feeding, and colonization by opportunistic pathogens, leading to activation of the mucosal innate immune system and further damage of the epithelial barrier [4].

Against this background, several serologic biomarkers for the diagnosis of NEC have been investigated in the last decade, including markers of epithelial damage and gut barrier function, inflammation and pathogen invasion [5].

Interleukin (L)-8 seems to play a pivotal role in the activation of the proinflammatory cascade in NEC [6,7]. Our group has recently demonstrated that IL-8 may serve as a potential predictive marker in diagnosing NEC. We were able to show that IL-8 detected in the serum of infants with NEC was significantly correlated with disease extent [8] and that IL-8 was able to significantly differentiate between infants with surgical and medical NEC in a large patient collective [9].

Fatty acid binding proteins (FABP) comprise a group of cytoplasmatic small molecular mass proteins (~15 kDa) with high organ sensitivity. Elevated levels of intestinal fatty acid binding proteins (I-FABP) were shown in the plasma [10–13] and in the urine [14–16] of premature infants with NEC. Liver fatty acid binding protein (L-FABP) seems to be elevated in NEC compared with healthy control infants and septicemic patients [11,13]. Preliminary data on the diagnostic properties of fatty acid binding proteins for the early diagnosis of NEC are very promising, but remain to be determined. One drawback is the limited availability of these novel biomarkers in a daily routine clinical laboratory.

To the best of our knowledge there are no data available to show the association between a proinflammatory cytokine and markers of gut wall integrity. The aim of the study is to evaluate the diagnostic value of the routinely available cytokine IL-8 compared with the two experimental gut-associated biochemical markers I-FABP and L-FABP.

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Table 1
Demographic data of study patients according to groups.

	Control ($n = 14$)	NEC ($n = 15$)	p
Gestational age, median 25%–75% quartile (weeks)	29 (27–30)	28 (25–30)	0.941
Birth weight, median 25%–75% quartile (g)	1128 (790–1548)	1156 (660–1550)	0.975
Age at diagnosis, median 25%–75% quartile (days)	32 (8–63)	26 (17–54)	0.827
1-min APGAR, median 25%–75% quartile	8 (7–8)	8 (7–8)	0.618
5-min APGAR, median 25%–75% quartile	9 (9–9)	9 (8–9)	0.334
Male sex, n (% of total)	8 (57%)	10 (67%)	0.710
Twins, n (% of total)	4 (29%)	5 (33%)	1.00

1. Materials and methods

1.1. Study design and population

This prospective study was conducted at the Department of Pediatric Surgery and Pediatrics and Adolescent Medicine, Medical University of Vienna, and with the approval of the local ethics committee. Informed written consent was obtained from parents of all study subjects. Infants with NEC were recruited from January 2010 until July 2013. Each of the NEC blood samples was obtained from neonates exhibiting clinical signs and radiographic findings of NEC, according to modified Bell's staging criteria [17]. Blood samples were collected at the onset of clinical presentation of NEC. IL-8 was analyzed immediately after blood collection. Plasma from blood samples for I-FABP and L-FABP analysis was separated by centrifugation (3500 $\times g$ for 5 min) at 4 °C and stored at −80 °C until pooled analysis could be performed.

Control blood samples were collected from infants with a birth weight of less than 2000 g, and of younger than 6 months of age. Exclusion criteria were: any history of surgical intervention in the medical history, culture-proven or clinically suspected infection, chronic inflammatory diseases or congenital malformations. Signs for clinically suspected infection were at least one of the following symptoms: respiratory distress, feeding intolerance, abdominal distension, lethargy, irritability, or temperature instability.

1.2. Treatment groups

Infants with NEC were divided into two different treatment groups, medical NEC and surgical NEC. All infants assigned to the medical treatment group had been managed conservatively, and did not receive any surgical intervention. In the surgical treatment group, all diagnoses of NEC were confirmed intraoperatively. Indications for surgical intervention included evidence of intestinal perforation and/or clinical deterioration despite maximum conservative treatment [18]. The decision to perform surgery was made by the attending pediatric surgeon and was not influenced by this study whatsoever.

1.3. Demographic and clinical parameters

Demographic parameters recorded and evaluated for all patients included birth weight, gestational age, 1 min Apgar score, 5-min Apgar score, and age at diagnosis of NEC. Important clinical data pertaining to NEC were: frequencies of intraventricular hemorrhage, persistent ductus arteriosus, use of medication (steroids, antibiotics and vasopressors) and mechanical ventilation as well as hematologic indices and acute-phase markers like white blood cell count, platelet count and C-reactive protein.

1.4. Determination of IL-8

A sequential chemiluminescent immunoassay was used with the threshold set to 70 pg/ml as recommended by the manufacturer (Siemens Immulite; DPC, Los Angeles, CA). Median serum IL-8 levels of 27 (20–1213) and 29 (20–778) pg/ml are documented in the literature for a total of 351 healthy neonates over two consecutive time spans [19].

1.5. Immunoassays for I-FABP and L-FABP

Serum concentrations of I-FABP and L-FABP were determined by using commercially available ELISA kits (Hycult Biotech, Uden, the Netherlands). Sample workup was done according to the manufacturer's recommendation. Plasma samples were diluted 1:20 and 1:100 for I-FABP and L-FABP, respectively. Absorption was determined on a microplate reader (Statfax 3200, Awareness Technology Inc., Palm City, FL) at 450 nm.

1.6. Statistical analysis

Values of patient groups are presented as median values. Continuous variables were tested for normal distribution by applying the Kolmogorow–Smirnow test. When test failed (significant result $p < 0.05$) Mann–Whitney U test was used for testing significance otherwise Student's t-test was used. Spearman rank-order correlation was used to evaluate correlation between parameters. Correlation coefficients (r_{rho}) of 0.7–1.0 or −0.7 to −1.0 were considered as strong correlations 0.5 to 0.7 or −0.5 to −0.7 as moderate correlations. Variables with lower correlation coefficients were not considered even when they were significant. The reported p-values are the results of two-sided tests. A p-value <0.05 was considered statistically significant. Statistical calculations were done using SPSS 17.0 software (IBM; Armonk, 22 NY). SigmaPlot 11.0 (Systat Software GmbH., Erkrath, Germany) software was used to perform the ROC analysis. The method of DeLong et al. [20] implemented to the SigmaPlot software ROC option was used to compare AUCs. The report shows results for all pairs of data sets. The difference of each area pair, its standard error, 95% confidence interval, and chi-square statistic and its associated p-value for the area comparison were calculated. The heat map was drawn by using Excel 2007 software (Microsoft, Redmond, WA).

2. Results

2.1. Characterization of the study groups

During the study period a total of 15 infants with NEC were included and compared with 14 gestational age, birth weight and age at diagnosis-matched infants serving as a control group (details are shown in Table 1). Clinical characteristics including laboratory parameters of NEC and control infants are summarized in Table 2. No significant differences were found for the following parameters: 1-min and 5-min APGAR score, frequency of male sex and the frequency of twin pregnancies.

In the NEC group comprising 15 infants, 10 received surgical and 5 received medical treatment, respectively.

2.2. Significant increase of IL-8 and gut barrier markers in serum of NEC patients

Serum concentrations of IL-8, I-FABP and L-FABP were significantly higher in patients with NEC when compared to the control group (IL-8, 46 pg/ml vs 1562 pg/ml, $p < 0.001$; I-FABP, 1.4 ng/ml vs 2.8 ng/ml, $p = 0.010$; L-FABP, 35.0 ng/ml vs 122.4 ng/ml, $p < 0.001$). Fig. 1a illustrates the concentrations of all the three serum