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Interleukin-8 predicts 60-day mortality in premature infants with necrotizing enterocolitis☆

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ABSTRACT

Objective: The purpose of this study was to evaluate the predictiveness of circulating interleukin (IL)-8 for 60-day mortality in premature infants with necrotizing enterocolitis (NEC).
Background: NEC affects up to 5% of premature infants and remains a leading cause of mortality among neonates.
Methods: A total of 113 infants with surgically ($n = 50$) or medically ($n = 63$) treated NEC were retrospectively analyzed. Laboratory parameters including serum IL-8 were assessed at the diagnosis of NEC and during the preoperative workup.
Results: The 60-day mortality was 19% (22/113), 10% (6/63) in medical and 33% (16/50) in surgical NEC. IL-8 levels significantly correlated with 60-day mortality (odds ratio: 1.38; CI 1.14–1.67; $p = 0.001$). Median IL-8 levels at diagnosis were significantly higher in neonates who were later treated surgically (median = 2625 pg/ml; range: 27–7500) compared with those treated medically (median = 156 pg/ml; range: 5–7500; $p < 0.001$). The AUC to discriminate between medical and surgical NEC was 0.82 (CI, 0.74–0.90), and an exploratory IL-8 cutoff point could be established at 1783 pg/ml (sensitivity of 90.5%; specificity of 59.2%).
Conclusions: Our findings that serum IL-8 (i) correlates directly with 60-day mortality and (ii) differs significantly between medically and surgically treated infants may change the process of therapeutic decision making in NEC.

Necrotizing enterocolitis (NEC) remains one of the leading causes of morbidity and mortality in neonatal intensive care units, affecting up to 5% of premature infants [1]. Over the past few decades outcomes following the management of prematurity have continued to improve. Nevertheless, the mortality rates among infants with NEC remain unchanged, ranging between 15% and 30%, with more deaths occurring in surgically rather than medically treated infants [2–4]. Almost half of all infants with NEC require surgical treatment, and mortality can be as high as 60% in these patients [5,6].

Our understanding of NEC is limited. The uncertainties surrounding the precise etiology and outcomes of this condition make any prognostic assessment a major challenge. The initial clinical manifestations are nonspecific and indistinguishable from other gastrointestinal disorders [1]. Traditional systemic markers of inflammation [7] have not been found to be particularly helpful in the past and the same is true of clinical appearance [8–10]. The diagnosis is further complicated by the limited diagnostic accuracy of currently used diagnostic imaging modalities [11]. Early radiologic features of dilated bowel loops, paucity of intestinal gas, and pneumatosis intestinalis on plain abdominal radiographs have a low sensitivity for diagnosing NEC and are further hampered by interobserver variability [12]. In view of the high mortality after bowel perforation it is important to note that pneumoperitoneum as a definite sign for surgical intervention may be absent on plain radiographs in 50%–60% of cases [13,14]. Early identification of the most severely affected infants in need of surgical intervention could guide both neonatologists and pediatric surgeons in the management of these delicate patients.

Interleukin (IL)-8 is a proinflammatory chemokine that has been previously implicated in the pathogenesis of NEC. There is direct evidence to suggest that the amount of secreted IL-8 might reflect cellular activity at the site of inflammation not only in chronic bowel disease [15,16], but also in NEC [17–19]. Our study group [20] has recently demonstrated that serum IL-8 levels significantly correlated with disease extent in infants with NEC prior to surgery.

Given the finding that circulating IL-8 levels correlate with the degree of intestinal inflammation, we considered it mandatory to

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assess their prognostic value in NEC more closely, including not only surgical cases but also cases managed by medically. A retrospective study was designed to analyze serum IL-8 at the time of diagnosis of NEC as a predictor of 60-day mortality in premature infants. Secondary aims were to analyze IL-8 levels according to treatment modality and to evaluate the ability of IL-8 to distinguish, at diagnosis of NEC, between medical and surgical NEC.

1. Materials and methods

1.1. Study design and population

The study was conducted at the Medical University of Vienna following approval by the Ethics Committee of the Medical University of Vienna (EC 1187/2011). As it is a retrospective chart analysis study, with no therapeutic implications, the Ethics Committee deemed it unnecessary to obtain a written consent. Patient data from the period January 2003 through December 2010 were reviewed for inclusion of all premature infants who had been diagnosed with NEC and had undergone either surgical or medical treatment. The primary outcome measure was mortality after 60 days. NEC stages I to III were established based on clinical manifestations and radiographic findings using Bell's staging criteria [21] as modified by Walsh and Kliegman [22].

1.2. Treatment groups

All infants assigned to the medical treatment group had been managed in a strictly nonsurgical fashion. 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 [23]. Decision to perform surgery was made independently and was not influenced by this study whatsoever.

1.3. Demographic and clinical parameters

Demographic parameters that were reviewed 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 also reviewed, including the presence of a patent ductus arteriosus, administration of ibuprofen, corticosteroids and antibiotics, use of mechanical ventilation, and the administration of vasopressors prior to the diagnosis of NEC.

1.4. Laboratory parameters

Evaluation of laboratory parameters included serum IL-8 and C-reactive protein (CRP) levels, as well as platelet and leukocyte counts. These were recorded at the time of diagnosis before the initiation of treatment. Blood cultures obtained within 24 h prior to a diagnosis of NEC were also considered. To compare laboratory parameters at the time of NEC diagnosis with preoperative laboratory parameters in surgically treated NEC, we additionally recorded preoperative laboratory parameters in surgical NEC, which were obtained within 6 h prior to surgery.

1.5. Assessment of serum IL-8

A chemiluminescent sequential immunometric assay was used with the threshold set to 70 pg/ml as recommended by the manufacturer (Immulite; DPC, Los Angeles, CA). Serum levels were routinely evaluated for sepsis surveillance under a diagnostic workup protocol used whenever infection, sepsis or NEC was suspected in infants. No other cytokines were included in this protocol. 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 [24]. The IL-8 assay was calibrated up to 7500 pg/ml. At this level, the parameter exceeded its normal level by a factor of >100, so that patient sera reaching this point were not diluted further to measure the exact amount of IL-8.

1.6. Statistical analysis

Continuous variables were described using medians and range (min–max) because of nonnormal distributions. Categorical variables were described using absolute and relative frequencies. The effect of various variables on 60-day mortality was estimated using logistic regression models and was quantified by crude and adjusted odds ratios and 95% confidence intervals. Owing to the limited number of events only one variable could be used in order to adjust the effect of IL-8 on mortality for other variables. In a stepwise selection procedure platelet count was selected. Areas under ROC curves (AUC) were given with 95% Wald confidence intervals and compared for different independent variables as implemented in proc logistic of SAS. Cutoff points for ROC curves were sought to have at least 90% sensitivity and maximal specificity. Since the reported cutoff is not further used in the models applied to our data no adjustment such as cross-validation is needed. All variables with a right skewed distribution were transformed using the binary log such that the corresponding odds ratios gave the effect of doubling the respective variable. Log-transformed IL-8 levels were compared between medical and surgical NEC using an independent-samples *t*-test. Within surgical NEC, IL-8 levels were compared between time of diagnosis and time of operation using the sign test because of a markedly unsymmetrical distribution of the intraindividual differences. Since IL-8 was measured with an upper limit of detection at 7500 pg/ml all parametric analyses incorporating IL-8 were performed on 100 bootstrap samples and averaged using Rubin's rules (SAS proc mianalyze). In each of the bootstrap samples IL-8 levels above 7500 were imputed under the assumption of a truncated log-normal distribution.

p-Values were generated as a result of two-tailed statistical tests. *p*-Values ≤ 0.05 were considered statistically significant. All computations were performed using SAS software Version 9.3 (SAS Institute Inc., Cary, NC, 2010).

2. Results

2.1. Patients and 60-day mortality

Of 113 infants identified with a diagnosis of NEC, a total of 22 infants (19%) died within the first 60 days (Fig. 1). Baseline perinatal characteristics are summarized in Table 1. Surgical treatment was performed in 50 cases (44%), and medical treatment was carried out in 63 cases (56%). The median time from the diagnosis of NEC to surgery was 1 day (range: 0–8 days). Of those who received medical intervention 10% (6/63 infants) had a 60-day mortality compared to 33% (16/50 infants) in the surgical group. In the medical group three deaths were caused by multiorgan dysfunction syndrome (MODS), one by fulminant progressive NEC, one by cardiac arrest and one by sepsis. Within the surgical group eight deaths were caused by progressive NEC and MODS, six by sepsis, one by cardiac arrest and one by progressive intraventricular hemorrhage.

2.2. Serum IL-8 and 60-day mortality

IL-8 levels were found to correlate significantly with 60-day mortality (odds ratio: 1.38; CI 1.14–1.67; $p = 0.001$) (Fig. 2). The median IL-8 levels at the time of diagnosis of NEC are shown in Table 2.