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

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A R T I C L E   I N F O

Article history:
Received 14 May 2013
Received in revised form 18 May 2013
Accepted 31 May 2013

Key words:
Necrotizing enterocolitis
NEC
Outcome
Surgery
Interleukin-8
IL-8
Mortality
Neonates

A B S T R A C T

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 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). 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.

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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 intestina lis 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 pseudoperitonemias 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
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 66 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 32% (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.
2.3. Serum IL-8 and treatment modality

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; \( p < 0.001 \)).

Among infants treated surgically no difference in median IL-8 values was observed between the time of diagnosis of NEC and the time of operation (quartiles 0; 542). IL-8 values tended to remain constant or to slightly decrease between the time of NEC diagnosis and the time of surgical intervention. However, the comparison of IL-8 levels at disease onset with preoperative IL-8 values did not reach a statistical difference (\( p = 0.052 \)).

An explorative ROC analysis was performed to discriminate surgical NEC from medical NEC based on the IL-8 level at the time of diagnosis of NEC. The AUC was 0.82 (CI, 0.74–0.90), and an exploratory IL-8 cutoff point could be established at 1783 pg/ml. At this value IL-8 reached a sensitivity of 90.5% and a specificity of 59.2%.

2.4. Serum IL-8 and platelet counts

The median platelet counts are illustrated in Table 2. We were able to show that platelet count at disease onset (odds ratio: 0.44; \( p < 0.001 \)) was another significant predictor of 60-day mortality in addition to IL-8 levels. An explorative ROC analysis was performed with respect to predicting 60-day mortality at the diagnosis of NEC. To discriminate survivors from nonsurvivors at 60 days the AUC was 0.75 (CI, 0.64–0.87) for platelet count and 0.76 (CI, 0.66–0.86) for IL-8 (\( p = 0.969 \)). Furthermore, a multivariable model was established in which the effect of IL-8 was adjusted for platelet count. This revealed that the established association of IL-8 with 60-day mortality was independent of the effect of platelet count, as the effect estimate for IL-8 remained virtually unchanged (odds ratio: 1.36; CI, 1.11–1.67; \( p = 0.004 \)).

2.5. Platelet counts and treatment modality

Median platelet counts at diagnosis in medical NEC (238 G/l; range: 17–737) did not differ significantly from infants with surgical NEC (182 G/l; range: 13–695; \( p > 0.154 \)). To discriminate surgical from medical NEC based on platelet count at the time of diagnosis the AUC was 0.585 (CI, 0.47–0.70). The comparison of the AUC values of IL-8 and platelet counts to predict the need for surgical treatment showed a significant difference (\( p < 0.001 \)).

2.6. Other parameters and 60-day mortality

The use of vasopressors at the time of NEC diagnosis was found to correlate significantly with the 60-day mortality (odds ratio: 3.72; CI, 1.03–13.43; \( p = 0.044 \)) (Table 3). Leukocyte counts, CRP levels and blood cultures at the time of diagnosis did not significantly predict 60-day mortality (Table 3). Neither were the demographic and clinical variables of gestational age, birth weight, age at diagnosis, presence of

![Fig. 1. Kaplan-Meier survival curves. Primary end point of 60-day mortality according to medical and surgical NEC.](image1)

![Fig. 2. Box-whiskers plots. Distribution of IL-8 values by 60-day mortality of surgically versus medically treated infants with NEC.](image2)
now succeeded in demonstrating a direct correlation between IL-8 and NEC. In this study, which was conducted to verify these observations, we have found that the observed high levels of IL-8 on 60-day mortality significantly correlate with disease extent in infants with NEC. This observation is consistent with previous reports showing a significant association between IL-8 and mortality, thus supporting the observations made by Souza et al. [31] and Thyoka et al. [34]. An explanation for this might be related to our strategy of assessing 60-day mortality rather than overall survival.

3. Discussion

Little has previously been known about the prognostic value of circulating IL-8 in infants with NEC. There has only been one report on a potential correlation between plasma IL-8 and mortality in infants with bacterial sepsis [25]. This study also showed that IL-8 levels were significantly higher in infants with NEC than in infants with sepsis, but a correlation between serum IL-8 and mortality could not be assessed because of the 100% patient survival rate. The same limitation applies to the study by Edelson et al. [26], who also correlated plasma IL-8 levels with disease severity but not with patient outcome.

Our study group has previously reported that preoperative IL-8 levels significantly correlate with disease extent in infants with advanced NEC [20]. In addition, IL-8 evaluation is routinely implemented in our preoperative workup of NEC cases and we have long recognized that patients with high preoperative IL-8 levels might have a worse outcome. In this study, we were able to verify these observations, we have now successfully demonstrated a direct correlation between IL-8 levels and 60-day mortality in infants diagnosed with NEC.

These results are in contrast to Nadler et al. [18], who reported that intestinal IL-8 expression did not correlate with short-term mortality in 21 NEC patients. Discrepancies between IL-8 levels measured directly in affected tissue versus indirectly in serum might account for this disagreement. The former approach (as taken by Nadler et al.) potentially involves a sampling bias, as samples collected from the margin of necrotic tissue may fail to reflect the true degree of intestinal compromise. Given the mosaic-style pattern of NEC, especially in advanced cases, the levels of IL-8 present in peripheral blood might reflect the actual extent of the disease more accurately.

Another important finding within this study is that the platelet count may be a predictor of 60-day mortality following a diagnosis of NEC. This observation is consistent with previous findings of thrombocytopenia within ≤3 days of diagnosing the disease [27,28]. Both of these study groups observed positive correlations of thrombocytopenia with subsequent mortality and bowel necrosis. Although our study did not include serial evaluation of platelet counts, we do share the view that severe thrombocytopenia may be helpful in establishing the need for laparotomy in conjunction with radiographic signs and other laboratory parameters (highly elevated IL-8 levels being the most important).

Given the increased dependency of vasopressors, infants with surgical NEC seemed to be less well when compared to infants on medical treatment. Our study is in accordance with previous data, wherein the use of vasopressors at the time of disease onset was associated with an increased likelihood of a 60-day mortality [29]. The use of vasopressors showed only a weak univariate correlation with 60-day mortality in comparison to the clear laboratory parameters. Therefore, we did not select vasopressors in the stepwise selection procedure applied for adjusting the impact of IL-8 on 60-day mortality.

Birth weight and gestational age were not significantly associated with 60-day mortality in the present study. Several research efforts looking into both parameters as potential modifiers of mortality in NEC have yielded inconclusive results [30–34]. Our own findings did not show that birth weight or gestational age was significantly associated with mortality, thus supporting the observations made by Souza et al. [31] and Thyoka et al. [34]. An explanation for this might be related to our strategy of assessing 60-day mortality rather than overall survival.

Future research should further explore the potential of IL-8 to discriminate between cases of NEC requiring medical or surgical treatment, since any diagnostic approach exclusively relying on clinical and radiographic findings has its limitations. As numerous past surgical approaches have failed to improve the mortality of NEC, a key question addressing not the type but the timing of surgery, remains to be answered. Although not unanimously accepted, some clinicians suggest that outcomes could be improved by early removal of necrotic intestine or creation of a proximal enterostomy before intestinal perforation occurs.

Neu and Walker [35] recently called for the development of a more reliable staging approach that would allow for aggressive preventive measures, including biomarkers capable of accurately predicting the full expression of NEC. The ideal biomarker of NEC should substantially increase in concentration in the bloodstream at the onset of NEC, and its magnitude of increase should be proportional to the severity of intestinal injury. At the time of diagnosis of NEC, infants with surgical NEC showed significantly higher IL-8 concentrations compared to those with medical NEC. In addition, we were able to demonstrate that preoperative IL-8 levels did not differ from IL-8 levels detected at disease onset in surgical NEC. The detected serum IL-8 values in NEC, as indicated by previous studies, seem to stem from the diseased intestine, reflecting the exaggerated activation of the proinflammatory cytokine cascade. Interleukin-8 is the most potent chemoattractant to recruit neutrophils and phagocytes to the affected tissue. The observed high levels of IL-8 probably lead to an accumulation of such cells in the compromised intestine, which further boost the local inflammatory process and delay tissue regeneration. However, the conservative management might not be able to sufficiently down-regulate the

### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Surgical NEC</th>
<th>Medical NEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsurvivors</td>
<td>Survivors</td>
<td>Nonsurvivors</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>(n = 16)</td>
<td>(n = 34)</td>
</tr>
<tr>
<td>(218–7500)</td>
<td>(26.8–7500)</td>
<td>(127–7500)</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>12.22</td>
<td>8.07</td>
</tr>
<tr>
<td>(k/mm³)b</td>
<td>(1.79–23.80)</td>
<td>(1.80–36.70)</td>
</tr>
<tr>
<td>Platelets</td>
<td>132.0</td>
<td>230.5</td>
</tr>
<tr>
<td>(k/mm³)b</td>
<td>(13.0–332.0)</td>
<td>(34.0–695.0)</td>
</tr>
<tr>
<td>C-reactive protein (mg/dl)</td>
<td>4.26</td>
<td>5.05</td>
</tr>
<tr>
<td>(0.33–9.21)</td>
<td>(0.20–23.20)</td>
<td>(1.10–9.21)</td>
</tr>
<tr>
<td>Positive blood cultures</td>
<td>5/15 (33%)</td>
<td>6/29 (21%)</td>
</tr>
</tbody>
</table>

a Median values (min–max).  
b Upper detection limit (see the Materials and Methods section).

### Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OR</th>
<th>95% CI</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight</td>
<td>0.63</td>
<td>0.25–1.57</td>
<td>0.324</td>
</tr>
<tr>
<td>Gestational week</td>
<td>0.92</td>
<td>0.77–1.10</td>
<td>0.354</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>0.76</td>
<td>0.51–1.14</td>
<td>0.186</td>
</tr>
<tr>
<td>Medical treatment of PDA</td>
<td>1.43</td>
<td>0.51–3.97</td>
<td>0.495</td>
</tr>
<tr>
<td>Use of antibiotics</td>
<td>0.95</td>
<td>0.35–2.58</td>
<td>0.914</td>
</tr>
<tr>
<td>Mechanical ventilationb</td>
<td>3.40</td>
<td>0.92–12.55</td>
<td>0.066</td>
</tr>
<tr>
<td>Infant inflamb</td>
<td>1.49</td>
<td>0.43–5.17</td>
<td>0.532</td>
</tr>
<tr>
<td>Use of vasopressors</td>
<td>3.72</td>
<td>1.03–13.43</td>
<td>0.044</td>
</tr>
<tr>
<td>IL-8</td>
<td>1.38</td>
<td>1.14–1.67</td>
<td>0.001</td>
</tr>
<tr>
<td>Platelet counts</td>
<td>0.64</td>
<td>0.28–0.69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leukocyte counts</td>
<td>0.93</td>
<td>0.61–1.41</td>
<td>0.719</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>1.25</td>
<td>0.97–1.61</td>
<td>0.088</td>
</tr>
<tr>
<td>Positive blood cultures</td>
<td>1.22</td>
<td>0.41–3.60</td>
<td>0.723</td>
</tr>
</tbody>
</table>

PDA = patent ductus arteriosus; OR = odds ratio; CI = confidence interval.

a OR quantifies effect for each doubling of the considered risk factor owing to log transformation.

b Versus no ventilation.
proinflammatory response to allow recovery of the intestine in these infants. We assume that IL-8 levels obtained at the time when NEC is first diagnosed might be capable of distinguishing infants in need of surgery from prospective responders to medical treatment.

Despite the retrospective design of our study and the small number of deaths in the medical treatment group preventing any specific treatment recommendations, our findings still demonstrate that IL-8 offers additional information. IL-8 may prove especially useful in critical neonate cases requiring difficult clinical decision. Further studies involving a larger patient cohort are needed before serum IL-8 levels can be established as an unequivocally reliable parameter in clinical practice.

References