Comparison of urinary neutrophil gelatinase-associated lipocalin, C-reactive protein and procalcitonin in the diagnosis of late onset sepsis in preterm newborns

Sabahattin Ertuğrul¹, Ali Annagur², Sevil Kurban³, Huseyin Altunhan⁴ & Rahmi Ors⁵

¹Sanlıurfa Maternity Hospital, Neonatal Intensive Care Unit, Ipekyolu Caddesi Baglarbasi, Sanlıurfa, 63050 Turkey, ²Selcuk University Selçuklu Medical Faculty, Division of Neonatology, Konya, Turkey, ³Department of Biochemistry, Necmettin Erbakan University Meram Medical Faculty, Konya, Turkey, ⁴Abant Izzet Baysal University Medical Faculty, Division of Neonatology, Bolu, Turkey, and ⁵Necmettin Erbakan University Meram Medical Faculty, Division of Neonatology, Konya, Turkey

Keywords: Sepsis, infant, premature, biomarkers, NGAL, human

Introduction

In spite of the developments in the diagnosis and treatment of neonatal sepsis, neonatal sepsis continues to be the most important cause of mortality and morbidity for newborn babies in the world [1,2]. There are no specific signs or symptoms in neonatal sepsis. It is difficult to distinguish the clinical findings of neonatal sepsis, especially during the initial period of sepsis from noninfectious causes. Therefore, biomarkers are of vital importance in the early diagnosis of neonatal sepsis. Since obtaining a positive blood culture, which is considered a definitive diagnostic tool in sepsis, is a time-consuming procedure, additional laboratory tests play a crucial role in confirming or ruling out sepsis and in the initiation and cessation of antibiotic therapy [3].

Neutrophil gelatinase-associated lipocalin (NGAL) is a protein from the lipocalin family weighing 25 kDa. In experimental animal studies, as signals related to cellular injury reached epithelial cells at the early periods of ischemic injury, this protein increased in the blood and urine [4]. In human, NGAL has been seen to be increased in cardiopulmonary by-pass, renal transplantation, renal injury following application of contrast agents, hemolytic syndrome, lupus nephritis, chronic renal insufficiency and sepsis [5]. During sepsis, it has been shown to increase in blood and urine independent from blood levels [6]. In children and adults, urine NGAL (uNGAL) has been shown to be a sensitive and an early marker in acute renal injury [7]. Also in clinical trials, it has been reported that serum and urine NGAL levels are significantly increased in bacterial infections in children and adults [6]. Unlike other markers, urine NGAL which requires no blood sample appears to be an ideal marker. A small amount of urine obtained under nonsterile conditions is enough for measurement.

To the best of our knowledge, in the only study that used uNGAL in the diagnosis of late sepsis in premature babies [6], there was no comparison with other biomarkers. In the present study, it was aimed to determine the value of uNGAL level in preterm newborns for the early diagnosis of neonatal late sepsis and to compare it with C-reactive protein (CRP) and procalcitonin (PCT).

Methods

The study was performed on premature patients at 7–28 days of age, hospitalized with a diagnosis of late sepsis in Necmettin Erbakan University Meram Medical Faculty Newborn Intensive Care Unit (NICU) between February and May 2011. Twenty-four premature babies with sepsis and 20 healthy premature controls were included in the study. The study was approved by the Clinical Investigations Ethics Committee. Informed consents were obtained from the parents.

Demographic and clinical characteristics, and the laboratory findings of each patient included in the study were recorded. Babies diagnosed by clinical and laboratory findings as late neonatal sepsis at the late neonatal period (7–28 days) and those under 36th gestational weeks were included in the study. Babies having acute renal injury, dialysis, abnormal karyotype, suspected congenital metabolic disease, cyanotic congenital heart disease, serious congenital malformation, perinatal asphyxia, maternal diabetes, intracranial hemorrhage, suspected and diagnosed necrotizing enterocolitis, those treated with indomethacin, ibuprofen and amphotericin B, history of or a requirement for surgical intervention, and patent ductus arteriosus (PDA) were excluded from the study. The control group was composed of babies born from a healthy mother (less than 36 weeks of gestational age), having no antenatally or postnatally detected renal pathology, having received no antibiotic treatment, having no history of perinatal asphyxia, with no observed congenital anomalies, and having no postpartum disorders.

The Töllner sepsis scoring was performed on all babies included in the study. In the sepsis group, CBC, CRP, PCT and blood culture samples were obtained from the blood and uNGAL samples were obtained from the urine at the onset and on the seventh day of treatment for each patient. For the control group, blood and urine samples were obtained once during the routine investigations.

Serum CRP measurements were performed in the Beckman Coulter instrument using the DDS commercial kit by immunoturbidimetric method and values over 10 mg/L were accepted as significant. Serum PCT measurement was performed using the chemiluminescence method with the Kryptor instrument with Sensitive-PCT kit (BRAHMS Diagnostica, Berlin, Germany), and values over 0.5 ng/ml were accepted as...
significant. Urine NGAL measurement was performed utilizing the immunoblot method with the BioVendor Human Lipocalin-2/NGAL Elisa commercial kit (Cat. No: RD191102200R). The results were given as ng/ml.

**Statistical analysis**

Statistical analyses were performed using the SPSS (Statistical Package for Social Sciences) software (version 17.0, SPSS Inc, Chicago IL, 60606, http://www.spss.com) for Windows 17.0 program. The Spearman's p correlation test was used for the analysis of the relationship between parameters. A p value of <0.05 was accepted as significant. For urine uNGAL, the cutoff values with ROC (receiver–operator curves) curve were determined.

**Results**

There were no statistical differences with regard to demographic characteristics between the groups (p > 0.05) (Table I).

There were differences but not statistically important between the sepsis and control groups with respect to white blood cell count, total neutrophil count and thrombocyte count (p > 0.05). There were statistical differences between C - reactive protein, PCT and uNGAL values (p < 0.05) (Table II).

The mean value of the Töllner sepsis scoring in the sepsis group was 9.17 ± 3.38.

When the first and the seventh day values were compared in the sepsis group, significant decreases in CRP, PCT and uNGAL levels were determined (p < 0.05).

When the babies were evaluated according to their birth weight (≤1499, 1500–2499, and ≥2500 g), the CRP, PCT and uNGAL values showed no significant differences (p > 0.05).

There was no significant correlation between the serum PCT and uNGAL values (p < 0.05, r: 0.426). No significant correlations were found for the other parameters. (p > 0.05).

The sensitivity, specificity, positive and negative predictive values of CRP, PCT and uNGAL values have been presented in Table III. The ROC curve was made for the urine NGAL (Figure 1), and the cutoff value was determined as 8.56ng/ml, and the sensitivity, specificity and positive and negative predictive values were determined.

**Table I. Demographic characteristics of babies included in the study.**

<table>
<thead>
<tr>
<th>Sepsis group</th>
<th>Control group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>1792 ± 516.49</td>
<td>1805 ± 506.39</td>
</tr>
<tr>
<td>Gestational weeks</td>
<td>32.88 ± 1.45</td>
<td>33 ± 1.49</td>
</tr>
<tr>
<td>Age (postnatal days)</td>
<td>12.54 ± 5.06</td>
<td>10.35 ± 2.54</td>
</tr>
</tbody>
</table>

**Table II. Laboratory findings in the sepsis and the control groups.**

<table>
<thead>
<tr>
<th>Laboratory findings</th>
<th>Sepsis group</th>
<th>Control group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell count (/mm³)</td>
<td>15206.25 ± 8888.28</td>
<td>10691.5 ± 2193.33</td>
<td>0.128</td>
</tr>
<tr>
<td>Total neutrophil count (/mm³)</td>
<td>6152.08 ± 5297.76</td>
<td>4237.5 ± 1548.59</td>
<td>0.487</td>
</tr>
<tr>
<td>Thrombocyte count (/mm³)</td>
<td>206833.3 ± 128711.56</td>
<td>275650 ± 94978.82</td>
<td>0.090</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>25.087a (11.9)b</td>
<td>7.05a (6.15)b</td>
<td>0.001</td>
</tr>
<tr>
<td>PCT (ng/mL)</td>
<td>17.11a (5.35)b</td>
<td>0.43a (0.25)b</td>
<td>0.000</td>
</tr>
<tr>
<td>uNGAL (ng/mL)</td>
<td>45.69 ± 18.37</td>
<td>5.78 ± 1.608</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**Discussion**

Most of the babies having suspected sepsis are treated with empiric broad- spectrum antibiotics, since the clinical findings were not specific to neonatal sepsis, and due to the difficulties in the diagnosis and treatment and the high mortality rate of up to 5–20% [2]. Early diagnosis and treatment is of vital importance in sepsis in order to decrease mortality. Because of the low sensitivity of diagnostic laboratory tests, to decide whether to initiate antibiotics or not, and also to cease antibiotics are difficult. New treatment modalities have been studied in sepsis in order to shorten the length of antibiotic treatment and hospital stay, and to provide the correct diagnosis and treatment in the fastest manner [8].

C-reactive protein is frequently used in the diagnosis of neonatal sepsis. In order to confirm neonatal infections, serial CRP measurements have been reported to be beneficial [9–11]. When the results of many investigations are considered, they show that CRP does not have adequate sensitivity (37–70%) or specificity (60–90%) at the onset of infection [12–14]. The low sensitivity levels of CRP found in studies prevent it from being an adequate diagnostic test alone in the early periods of sepsis.

There was a significant difference between the sepsis and the control groups with respect to CRP values. In our study similar to these, the sensitivity, the specificity the positive predictive value and the negative predictive value were 58.3, 80, 77.8, and 61.5%, respectively. Therefore, the delayed CRP elevation at the onset of infection showed that it should not be used alone in the diagnosis of sepsis and in deciding to begin antimicrobial therapy; serial measurements may be more useful in evaluating the efficacy of treatment, detecting complications and excluding infections.

Procalcitonin may also be used to monitor the activity and prognosis of severe bacterial infections. In studies performed, with PCT values at 2 ng/ml and higher, the sensitivity and specificity were 92 and 97%, respectively [13]. In the study performed by Chiesa et al. [15], they found the sensitivity of PCT as 92.6% and the specificity as 97.5%. In the study performed by Sakha et al. [16], the role of PCT in the diagnosis of neonatal sepsis and its relation with CRP were evaluated, and certain and suspect sepsis cases were included in the study. Although PCT is not valuable in the diagnosis of suspected sepsis, negative PCT may be useful to eliminate infection. There was a statistically significant difference for PCT levels between the sepsis and the control groups. However, PCT cannot ascertain or distinguish sepsis by 100%. When used together with other markers of sepsis, it is thought that more favorable results may be achieved for the definitive diagnosis of sepsis.

The production of NGAL increases with signals reaching the epithelial cells related to cellular injury such as ischemia-reperfusion injury, cytokine production and sepsis. In these conditions, its levels increase in the urine and blood [4,6]. uNGAL...
representing the renal pool, is not the filtrated NGAL; it is NGAL produced in the renal tubule [17].

It has been reported that uNGAL is an early marker of acute renal injury in children and adults [7]. In studies on acute renal insufficiency, uNGAL was found to have increased 10 to 100 times compared to the control group. In acute renal insufficiency, a rise in the creatinin levels begins 1–3 days later, whereas a rise in the uNGAL begins in 2 h [18]. In the study of Zappitelli et al. [19], on critical children patients, a rise in uNGAL concentrations appeared 48 h prior to the acute renal injury (ARI), and uNGAL was reported to be a good diagnostic marker for ARI. Furthermore, it has been shown that children with sepsis have higher uNGAL concentrations compared those without sepsis.

In a study by Fjaertoft et al. [20], when children with acute infection and suspected of having a viral infection were compared, NGAL and CRP were significantly elevated in those with acute bacterial infection. It has been shown that NGAL is a better marker for distinguishing bacterial and viral acute infections. In the study of Björkqvist et al. [21], NGAL levels were higher in patients with sepsis and NGAL reached maximum levels 24 h before the CRP elevation. Therefore, it has been stated that NGAL may be helpful as an early marker of nosocomial infections in newborns. Moreover, in the same study, NGAL levels at birth and on the third and fifth days in the control group were similar to normal adult levels.

The study of Lavery et al. on premature infants less than 1500 g [17] showed that uNGAL may be detected following birth. uNGAL was reported to be higher in those with low birth weight. However, these studies were performed without excluding factors like respiratory distress syndrome, PDA, indomethacin use and sepsis which may affect uNGAL levels. Huynh et al. [5], reported that the reference values of uNGAL were the same as the values obtained in children and adults. Furthermore, no significant differences were detected between uNGAL levels with respect to gender. We found no significant differences between CRP, PCT and uNGAL levels in the subgroups of sepsis divided as VLBW (very low birth weight), LBW (low birth weight), and those ≥2500 g. Also, we found no relationship between the gestational age of the baby and uNGAL level.

In the study of Parravicini et al. [22], on very low birth weight newborns, uNGAL values were found to be between 10 and 75 ng/mL, and the median value was 10 ng/mL. It was also shown that there is no relationship between postnatal and gestational age and uNGAL. In another study performed by Parravicini et al. [6], uNGAL levels were found as 179 ng/mL in those with very low birth weight suspected with sepsis but without complications and culture positive sepsis, and 6.5 ng/mL in the control group. They found uNGAL levels in infants with sepsis to be 30 times higher than that in healthy infants. There were no significant differences between sex, gestational age and postnatal age and uNGAL levels. On the fifth day of sepsis, uNGAL levels were found to decrease. In newborns with negative culture or suspected contamination, uNGAL levels were low. This study concluded that uNGAL may be useful as a negative predictive marker in the diagnosis of sepsis. In our study, we found significant differences in CRP, PCT and uNGAL levels between the sepsis group weighing ≥1500 g and LBW and the control group. However, the comparison of VLBW babies with the control group showed no significant differences between CRP levels, while there was a significant difference between PCT and uNGAL levels. In the diagnosis of sepsis in babies with VLBW and early gestational age, we observed that PCT and uNGAL were better markers than CRP.

In the meta-analysis of Vouloumanou et al. [23], in neonatal sepsis for serum PCT levels, the sensitivity and specificity values for PCT were 81 and 79%, respectively. In our study, the sensitivity and specificity values for PCT were 91.7 and 75%, respectively. Although there was a difference between the two groups with regard to sensitivity, the specificity values were similar. In the present study, the sensitivity and specificity values of uNGAL were 91.7 and 100%, respectively. The specificity value of urine NGAL was higher than PCT. This shows that uNGAL has a higher probability to rule out sepsis in patients without sepsis compared to PCT.

CRP and PCT begin to be released 4–6 h following the onset of infection; PCT peaks at the 6th-8th h, while CRP peaks at 36–48 h. The biological half-life is 19 h for CRP and 22.5 h for PCT. The procalcitonin level remains at the same level for about 24 h and then drops to normal in the next two days. C-reactive protein decreases by 50% daily following the acute phase response. After the onset of treatment, CRP levels begin to decrease. Urine NGAL rises rapidly in 2 h, earlier than CRP and PCT [24]. When it is considered that the condition of newborn sepsis patients may deteriorate in hours, an early rise in infection markers and their detection will be very important in the diagnosis.

In the first tests of the sepsis group in this study, CRP level was found to be higher than the cutoff value in 14 of 24 cases, while CRP persisted over the cutoff values in five cases after the seventh day. Serum procalcitonin was first found to be higher than the cutoff value in 14 of 24 patients. As seen in the present study, the number of patients with PCT and uNGAL over the cutoff values in sepsis were higher than that of CRP. Dependentely, the specificity of uNGAL is 91.7%, the sensitivity is 100%, the positive predictive value is 100%, and the negative predictive value is

| Table III. Statistical analysis of serum CRP, PCT and uNGAL values. |
|--------------------------|--------------------------|--------------------------|--------------------------|
|                          | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
| CRP                      | 58.3%       | 80%         | 77.8%                    | 61.5%                    |
| PCT                      | 91.7%       | 75%         | 81.5%                    | 88.2%                    |
| uNGAL                    | 91.7%       | 100%        | 100%                     | 90.9%                    |
90.9%; these values are better than CRP and PCT. The results of our study show that uNGAL may be a reliable marker in the early diagnosis of sepsis.

In conclusion, we found that uNGAL has higher sensitivity, specificity, and positive and negative predictive values than CRP and PCT in the diagnosis of late sepsis in premature newborns. Furthermore, contrary to other blood biomarkers, urine NGAL prevents unnecessary phlebotomies; it may be an ideal biomarker in neonatal sepsis, permitting it being studied in small amount of urine samples in noninvasive and nonsterile conditions.

However, in order to better understand the impact of uNGAL in the diagnosis of neonatal sepsis, larger studies are needed to confirm our findings.

Declaration of Interest: The authors report no conflicts of interest.

References
