Diagnostic and prognostic values of high sensitive C-Reactive Protein, Tumor Necrosis Factor and Interleukin-1 β in Neonatal Sepsis


1Child Health Department, National Research Centre
2Clinical pathology Department, National Research Centre
3Medical Physiology Department, National Research Centre

Abstract: Background: Sepsis and septic shock in newborn infants have a high risk of morbidity and mortality. Despite advances in medicine, diagnosis of neonatal sepsis remains as a major challenge. The aim of this study is to evaluate the value of high sensitive C-reactive protein (hs CRP), tumor necrosis factor (TNF) and interleukin -1β (IL-1β) in the diagnosis and follow-up of neonatal sepsis.

Method: 25 septic and 25 healthy newborns were included in the study. TNF-α, IL-1β, and hs CRP were serially measured on days 0 and 5 in the patients and once in the controls. Töllner's sepsis score (TSS) was calculated for each patient. Results: CRP and TNF-α levels in septic neonates at each study day were significantly higher than in the controls (P = 0.02) and (P<0.01) respectively. Also TNF-α levels were higher in culture positive group compared to culture negative one (p=0.03) and these levels declined significantly after 5 days of beginning treatment (p<0.001). IL-1β levels did not differ from healthy neonates. Conclusion: CRP and TNF-α are mediators of inflammation and can be used at the diagnosis and for evaluation of the therapeutic efficiency in neonatal sepsis.

Key words: hsCRP, TNF-α, IL-1 β, neonatal sepsis

INTRODUCTION

Sepsis remains one of the most common diseases of the neonatal period and is still a significant cause of mortality and morbidity (Klein and Marcy, 2005). Factors linked to its prevalence among newborns (NB), especially preterm infants, include the need for invasive procedures combined with immunological immaturity (Strunk et al., 2011).

Neonatal sepsis generally exhibits an insidious onset, with signs and symptoms the majority of which are highly nonspecific and easily confused with conditions to be expected based on the age and clinical progress of very low weight NB, which is sometimes unstable (Jackson et al., 2004). Blood cultures are considered the gold standard for diagnosis of neonatal sepsis. Nevertheless, their positivity varies widely (50 to 87%) and the results are not available rapidly for use in defining therapeutic management. For this reason, other, faster, laboratory tests are used. The tests used include the white blood cell count (WBC) and assays for markers of inflammatory reaction in serum, such as interleukin-8, C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and procalcitonin (Ng PC, 2004), (Kaufman and Fairchild, 2004).

The underdeveloped immune system predisposes preterm newborns to infection, which is a major cause of neonatal morbidity and mortality. Sepsis and endotoxin activate monocytes, macrophages, lymphocytes, fibroblasts, and endothelial cells that produce and secrete IL-1, TNF-α, α-interferon, IL-6, IL-8, and other proinflammatory cytokines (Kurt et al., 2007), (Lam and Ng PC, 2008).

The aim of this study is to determine serum IL-1β, hs CRP, and TNF-α levels in neonatal sepsis at the time of diagnosis and after therapy and to show the meaningful on the follow up.

Subjects and Methods:

This prospective study was performed on 25 newborns who were hospitalized for neonatal sepsis at NICU of El-helal Specialized Hospital of pediatrics. Another 25 healthy newborns who had normal clinical and laboratory findings were included as a control group. The later newborns were selected from the neonatal unit or well-baby outpatient clinic (healthy, noninfectious newborns). Inclusion criteria of septic group were positive clinical signs of sepsis and/or history of factors associated with increased risk for infection and parental informed consent. Exclusion criteria were congenital malformations, congenital infections associated with the TORCH complex, and refusal of parental consent. Diagnosis of sepsis was done according to the 2001 International Sepsis Definitions Conference criteria (Levy et al., 2003). Clinical signs of sepsis were defined as the presence of three or more of the following categories of clinical signs: apnea, tachypnea (>60/min), nasal flaring, retraction, cyanosis, respiratory distress-bradycardia (<100/min), tachycardia (>180/min), hypotonia, seizures-poor skin colour, capillary refilling time longer than two seconds, irritability, and lethargy. Historical factors associated with increased risk for infection included premature rupture of the membranes (in term...
infants > 18 hours), maternal fever during labour, intraamniotic infection, and chorioamnionitis. Two or more abnormal values of the sepsis screen (as white blood cell count < 4000 or > 10,000 mm$^3$, immature-to-total neutrophil ratio higher than 0.2 and CRP positivity) were considered as supportive for diagnosis of infection. The sepsis group was subdivided into culture-proven sepsis (positive blood culture), culture-negative sepsis (negative blood culture, but clinical signs of sepsis with positive sepsis screen and/or a history of risk factors, and antibiotic treatment longer than 7 days).

Töllner’s sepsis score (TSS) was calculated for each patient (Töllner, 1982). This scoring system consists of clinical (skin color, body temperature, muscle tone, breath rate, abdominal distension, imperfect microcirculation, and risk factors) and laboratory (leukocyte and thrombocyte counts, CRP, immature/total neutrophil ratio) parameters. A point was given for each parameter (0, 1, 2, or 3) in respect of their worsening. For example, 0 for normal muscle tonus, 1 for hypotonia, 2 for flask tonus, 0 for normal leukocyte count, 1 for leukocytosis, and 3 for leukopenia. According to this scoring system, patients who have > 10 points were determined as having sepsis.

Chest radiographs were routinely performed. Hematological and biochemical markers including a complete blood count, differential white cell count, and levels of CRP, TNF-α, IL-1β were serially measured. The initial (2 mL) blood samples were obtained from patients on day 0 (at the time of sepsis diagnosis). Another sample was obtained from each patient on days 5 for follow-up in the patient group after beginning of therapy. However, only one blood sample (2 mL) was obtained from each healthy control subject. Serum was separated by centrifugation at 4°C and stored in 200 μL aliquots at −70°C until analysis.

Serum hsCRP levels were determined with an enzyme-linked immunosorbent assay (ELISA) technique using commercial kits (BioCheck, Inc 323 Vintage Park Drive Foster City, CA 94404) and the sensitivity of detection level was 0.01 mg/dl. Serum concentrations of cytokines TNF-alpha were measured using commercially available ELISA kits (Bender MedSystems GmbH Campus Vienna Biocenter) and the sensitivity of detection for TNF was 2.3 pg/ml. Serum levels of IL-1β were measured using Enzyme immunoassay kit (Immunotech SAS, a Beckman coulter company- 130 av.de lattre Tassigny- B.P. 177-13276 Marseille Cedex 9 France) and the sensitivity of detection for IL-1β was 1.5 pg/ml in the protocol 0-250 pg/ml.

Analysis of the obtained data was done using Statistical Package for Social Science (SPSS) program version 14 (Dudley et al., 2004). Data was summarized as mean ± SD. Non parametric test (Mann Whitney U) was used for analysis of two independent quantitative data and Wilcoxon for two dependent quantitative data as data was not symmetrically distributed. Simple linear correlation (Spearman’s correlation) for quantitative data was also done. P-value is considered significant if < 0.05*.

**Results:**

In total, 50 newborns were included in the study: 25 sepsis patients (16 culture proven sepsis and 9 culture-negative sepsis), and 25 control. Table 1 shows the characteristics of the study group.

<table>
<thead>
<tr>
<th>Character</th>
<th>Sepsis group (n=25)</th>
<th>Controls (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender M/F</td>
<td>15/10</td>
<td>14/11</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>2230± 720</td>
<td>2354± 534</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>34.7± 2.1</td>
<td>35.7± 2.4</td>
</tr>
</tbody>
</table>

There were no significant differences between the two groups for gestational age, sex or birth weight.

Among gram-negative bacteria, Klebsiella pneumonia was clearly the most common isolate followed by Pseudomonas aeruginosa and Acinetobacter SPP. Staphylococcus aureus was the most common gram-positive isolate. Among non-bacterial etiology, Candida albicans was isolated in 3 cases. (Figure 1)
The mean CRP results were elevated at both measurement times in septic group, and with statistically significant difference with relation to the control group (p = 0.001). These levels decreased significantly during the time of therapy (p=0.02) (Table 2). On the other hand, the mean CRP results didn’t differ significantly between culture proven and culture negative sepsis (Table 3).

Serum levels of TNF-α were significantly higher in septic group compared to controls (p<0.001). Also these levels were significantly lower after 5 days of treatment compared to levels at diagnosis (p<0.001) (Table 2). Patients with a positive culture had significantly higher TNF levels than those from whom no pathogen was isolated (p=0.03) (Table 3). Among patients with a positive bacterial culture, those with a pure gram-negative infection had significantly higher TNF-α levels than patients with a pure gram-positive infection or fungal infection (p<0.01).

On the other hand, serum levels of IL-1β were not higher in the patient group on days 0 or day 5 than the control group. Also its levels didn’t differ significantly at diagnosis and after therapy.

TSS did not correlate with the serum levels of CRP, TNF-α, IL-1β, or hematological values including I/T ratio, leukocyte, and thrombocyte counts (P > 0.05)

Five patients (20%) died due to systemic inflammatory response syndrome. One of them had hydrops fetalis and two others had respiratory distress syndrome while the remaining two had fulminant late onset sepsis and also three of the five patients had disseminated intravascular coagulation (DIC). Interestingly, we find a significant increase in the levels of TNF-α in four of these patients in comparison to those who survived (p<0.01), while the levels of other biomarkers were comparable between those who died and survivors.

### Table 2: Serum biomarkers in Sepsis and control groups (mean ±SD)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Sepsis group</th>
<th>Control</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At day 0</td>
<td>At day 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>10.9±13.6</td>
<td>4.1±2.8</td>
<td>1±0.9</td>
<td>0.001*</td>
</tr>
<tr>
<td>TNF (pg/ml)</td>
<td>231.9±130.7</td>
<td>61.7±57.4</td>
<td>32±25.5</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>41.1±28.1</td>
<td>34.4±18.5</td>
<td>28±21.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

P1= group (a) Vs group (b)  
P2= group (a) Vs group (c)  
P < 0.05 = statistically significant, P < 0.01* = statistically highly significant

![Fig. 2: CRP levels at diagnosis, 5 days after therapy and controls](image)

![Fig. 3: TNF-α levels at diagnosis, 5 days after therapy and controls](image)
Table 3: Serum biomarkers in culture proven and culture negative groups (mean ±SD)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Culture positive group (n= 16)</th>
<th>Culture negative group (n=9)</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At day 0 (a)</td>
<td>At day 5 (b)</td>
<td>At day 0 (c)</td>
<td>At day 5 (d)</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>14.5±15.9</td>
<td>4.8±3</td>
<td>4.3±2</td>
<td>2.8±1.9</td>
</tr>
<tr>
<td>TNF (pg/ml)</td>
<td>274.3±142.7</td>
<td>68.6±60.7</td>
<td>156.5±55.2</td>
<td>49.5±51.5</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>41.7±24.2</td>
<td>31±15.9</td>
<td>40±35.6</td>
<td>40.4±22.9</td>
</tr>
</tbody>
</table>

P1= group (a) Vs group (c)                          P2= group (b) Vs group (d)

P < 0.05 = statistically significant, P < 0.01* = statistically highly significant

Fig. 4: TNF in culture proven and culture negative groups at diagnosis

Discussion:

In the present study, 64% of the patients had a microbiologically documented infection. Other studies on sepsis have reported similar results (Kumar and Rizvi, 2009). The gold standard for diagnosing neonatal sepsis remains blood culture, even though in many cases blood cultures are negative in the face of strong clinical indicators of septicemia, as well as in autopsy proven disseminated bacterial or fungal infection. Moreover, maternal antibiotics given in the majority of preterm deliveries may suppress the growth of bacteria in culture. Negative blood cultures in apparently septic neonates may also result from insufficient sample size (Paolucci et al., 2012).

The mean CRP results were elevated at both measurement times in septic group, with relation to the control group. Also its levels decrease significantly after five days of beginning treatment this come in coincidence with previous studies where CRP has been well-studied as an adjunctive test for the diagnosis of neonatal sepsis (Erdeve et al., 2011). Furthermore, normalization of CRP may be a good indicator of resolution of infection with treatment, and several authors have suggested that normalization of CRP can be used to determine duration of antibiotic therapy (Ng and Lam, 2010). Although CRP is generally considered a nonspecific biomarker, research has shown that it has high specificity for neonatal systemic infection because preterm infants have a narrow spectrum of disease compared with older patients. Noninfectious inflammatory conditions that can confound the diagnosis of sepsis in adult patients, such as rheumatoid arthritis, other connective tissue diseases, and inflammatory bowel disease, occur rarely in neonates (Pourcyrous et al., 2005).

It was also found that TNF-α levels were significantly higher in patients with microbiologically documented infections than in patients with only clinically documented infections, a finding in conformity with the reports of other studies (Kumar and Rizvi, 2005). (Shouman and Badr, 2010). In addition, a significant increase in the levels of TNF-α was found in patients who died in comparison to those who survived. Performing TNF-α test routinely in patients of sepsis can give rapid results in a day rather than the three to seven days required for bacterial culture and can act as surrogate markers of microbial infection. A positive microbiological report with a raised TNF-α thus carries a much greater significance and the patient immediately should be classified in a higher risk category and aggressive treatment started (Cesur et al., 2009). Furthermore, there was a significant difference in TNF levels between patients with gram-negative and gram-positive infections. This again stresses the differences in host responses between gram-negative and gram-positive infection, and this should be borne in mind while formulating supplementary or adjunctive therapeutic agents (Kumar and Rizvi, 2009).

Our data indicated that the serum concentrations of IL-1β in infected infants were not higher in patient group compared to control and IL-1β was a less satisfactory marker. It seems that the monocytes of newborn infants may be unable to secrete adequate IL-1β and prostaglandin E2 (fetal or maternal) and IL-6 may suppress IL-1β production in infections (Ucar et al., 2008). Therefore, we do not propose the use of IL-1β as an acute phase reactant in neonatal sepsis.
Also we found that there was not any correlation between TSS and the measured biomarkers or hematological parameters. In 2003, Caksen et al reported that there was not a significant difference for leukocyte counts, cytokine levels, and TSS between the blood culture-positive and -negative groups in septic newborns. We consider that Töllner sepsis score is itself not helpful in early diagnosis of neonatal bacterial infections.

As seen in this study, Serum levels of CRP and TNF-α are mediators of inflammation and can be used at the diagnosis and at the evaluation of the therapeautic efficiency in neonatal sepsis. Assessment of TNF-α levels can give an indirect evidence of bacterial invasion and whether the septicemia is due to gram-positive or gram-negative bacteria. This information will be invaluable in instituting timely appropriate treatment. The elevated TNF-α levels highlight the beneficial role of monoclonal anti-TNF-α antibody fragments in patients with microbiologically documented sepsis in general and in Gram-negative infection in particular.

REFERENCES


