Diagnostic Value of IL-6 in Neonatal Sepsis

Neeraj Kumar¹, Manoj Kumar Singh¹*, Rajeshwar Dayal¹, Shikha Gupta¹, Ruchika Garg²

¹Department of Pediatrics, S.N. Medical College, Agra, UP, India.
²Department of Obstetrics and Gynecology, S.N. Medical College. Agra, UP India

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ABSTRACT

Background: Neonatal sepsis is a serious life-threatening condition with high mortality. The early and accurate diagnosis of sepsis is one of main challenge for prevention of mortality due to sepsis in NICUs. A great effort to reduce the neonatal mortality rate is put into looking for new reliable biomarkers. Among various biomarkers, IL-6 could be promising and reliable biomarker for early diagnosis of neonatal sepsis. Objective: To evaluate the diagnostic value of IL-6 in Neonatal Sepsis. Design: Prospective, observational study. Methodology: By ELISA method the level of serum IL-6 were assessed in 41 neonates with suspected sepsis and 42 healthy neonates with no clinical and laboratory data of infection. Result: The AUC for IL-6 and CRP were 0.87, 0.80 respectively. The cut off value for IL-6 was 181 pg/ml at which the sensitivity, specificity, PPV and NPV were 80.1%, 85.7%, 84.6%, 81.8% respectively. The cut off value for CRP is 3.78 mg/dl at which the sensitivity, specificity, PPV and NPV are 61%, 90.5%, 86.2%, 70.3% respectively. In culture positive patients sensitivity of IL-6 and CRP was 90% and 80%, respectively. In culture negative patients sensitivity of IL-6 and CRP was 71.4% and 42.8%, respectively. In EOS sensitivity of IL-6 and CRP was 86.3% and 50%, respectively. In LOS sensitivity of IL-6 and CRP was 73.6% and 72.6%, respectively. Conclusion: IL-6 is a novel biomarker with high sensitivity and good specificity for sepsis. This has better diagnostic value than CRP, especially in Blood culture negative and EOS.

*Corresponding author:
Dr. Manoj Kumar Singh, Department of Pediatrics, Assistant Professor, S.N. Medical College, Agra, India, 282002.
E-mail: drmanuped@yahoo.com

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Introduction
Neonatal sepsis is a global problem and is a significant contributor to morbidity & mortality. Approximately one million deaths a year occurring in the neonatal period (0-28 days) are caused by infection, accounting for over 25% of global neonatal deaths and 10% of all mortality in infants. The prognosis and outcome of neonatal sepsis depend on early diagnosis and on-time and efficient antibiotic therapy. The accurate and timely diagnosis of septicemia in the neonatal population is challenging and problematic because of nonspecific clinical presentation and poor diagnostic yield (sensitivity of 50% or less) and delay of the standard blood culture. As such, there is much interest in developing rapid and sensitive diagnostic assays for diagnosis of the infected neonate. An approach that has gained particular attention is detection of IL-6 level which is a cytokine and produced by monocytes, endothelial cells and fibroblasts. Preliminary studies suggest that this is a novel, early and reliable biomarker for the diagnosis of sepsis. IL-6 is currently under investigation in clinical practice as a reliable marker of neonatal sepsis.

Materials and Methods
After taking the ethical clearance from ethical committee, the study was carried out during the period from March 2014 to May 2015 on 83 neonates divided into:-

- **Patient group**: 41 cases with suspected sepsis admitted to NICU in S.N Medical college & Hospital AGRA.
- **Control group**: 42 healthy neonates with no clinical and laboratory data of infection.

An informed consent was taken from parents before enrollment in study. Suspicion of sepsis by the caring neonatologists was based on:

a) The presence of one or more clinical signs/symptoms of sepsis.

b) Presence of two or more risk factors (PROM>24 hs, Low birth weight (<2500 grams) or prematurity, Maternal fever, Foul smelling and/or meconium stained liquor, Single unclean or > 3 sterile vaginal examination(s) during labor, Prolonged labor (sum of 1st and 2nd stage of labor >24 hrs), Perinatal asphyxia (Apgar score <4 at 1 minute).

**Blood samples collection and storage**
Around 3 ml blood sample was collected by standard techniques. The sample divided into 3 parts:

- 0.5-1 ml injected directly into blood culture bottle.
- 1 ml placed into plane tube and centrifuged for obtaining serum sample and then stored at -80°C for IL-6 measurement.

**Blood culture**
0.5-1 ml of blood was injected into the Bactec culture vial under complete aseptic conditions. Positive vials were Gram stained and sub cultured and incubated in appropriate temperature and atmospheres according to established methods. Full identification of organisms was done with standard bacteriological and biochemical methods.

**Statistical analysis**
The collected data were tabulated and analyzed using SPSS version 16 software. ROC curve was used to determine cutoff values of IL-6 with optimum sensitivity and specificity in early diagnosis of sepsis. The accepted level of significance in this work was stated at P <0.05.

**Result**
This study was carried out on 83 neonates divided into two main groups:-

- **Patient group**: included 41 neonates with suspected sepsis. There were 17 females & 24 males; 30 LBW & 11 ≥2.5 birth weight; 19 preterm & 22 term; 22 EOS & 19 LOS.
- **Control group**: included 42 healthy neonates. There were 22 males & 20 females; 28 LBW & 14 ≥2.5 birth weight; 20 preterm & 22 term.

According to the results of blood culture, patient group was subdivided into two subgroups:

- **Proven sepsis group**: included 20 patients who are positive on blood culture.
- **Probable sepsis group**: included 21 patients who are negative on blood culture.

Blood culture was positive in 48.8% of our cases. Staphylococcus aureus was the most common causative organism of sepsis followed by Klebsiella and E. coli (12.2%, 7.3% and 7.3% respectively) (Table 1). Area under the receiver operating characteristics (ROC) curve (AUC) for CRP was 0.80 with sensitivity and specificity of 61% and 90.5%, respectively at the cut off value of 3.7mg/dl. Its PPV and NPV was 86.2% and 70.3%, respectively. The sensitivity of CRP among Blood culture positive, Blood culture negative, EOS and LOS patients were 80%, 42.8%, 50% and 72.6%, respectively. (Table 2)
Area under the receiver operating characteristics (ROC) curve (AUC) for IL-6 was 0.87 with sensitivity and specificity of 80.1% and 85.7%, respectively at the cut off value of 181pg/ml. Its PPV and NPV was 84.6% and 81.8%, respectively. The sensitivity of IL-6 among Blood culture positive, Blood culture negative, EOS and LOS patients were 90%, 71.4%, 86.3% and 73.6%, respectively.(Table-3)

**Discussion**

Infections are responsible for significant mortality and long-term morbidity for infants in the neonatal intensive care units. Early diagnosis of neonatal sepsis is essential prerequisite to improve survival and to improve therapeutic outcome. As such, there is much interest in developing rapid and sensitive diagnostic assays that can effectively predict and identify patients who are at risk of infection.

Among new biomarker, IL-6 could be one of the most interesting and reliable candidates for sepsis management, specifically for early diagnosis. So, our study was aimed to evaluate the diagnostic value of IL-6 in neonatal sepsis which would help us in early and accurate diagnosis of sepsis. The current study is carried out on 41 neonates with suspected sepsis and 42 healthy controls. According to culture result, the cases were classified into proven sepsis

<table>
<thead>
<tr>
<th>Blood culture result</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>5</td>
<td>12.2</td>
</tr>
<tr>
<td>Pseudomonas aerogenosa</td>
<td>1</td>
<td>2.4</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>3</td>
<td>7.3</td>
</tr>
<tr>
<td>E.Coli</td>
<td>3</td>
<td>7.3</td>
</tr>
<tr>
<td>Candida</td>
<td>2</td>
<td>4.9</td>
</tr>
<tr>
<td>Acenatobacter</td>
<td>2</td>
<td>4.9</td>
</tr>
<tr>
<td>Burkodelia</td>
<td>2</td>
<td>4.9</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>2</td>
<td>4.9</td>
</tr>
<tr>
<td>No growth</td>
<td>21</td>
<td>51.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>41</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 1: Frequency distribution of patient group according to result of blood culture.**

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>CRP</th>
<th>χ²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>41</td>
<td>25</td>
<td>24.16</td>
<td>&lt;0.001 (S)</td>
</tr>
<tr>
<td>Controls</td>
<td>42</td>
<td>4</td>
<td>4</td>
<td>38</td>
</tr>
<tr>
<td>Proven sepsis group</td>
<td>20</td>
<td>15</td>
<td>5</td>
<td>2.25</td>
</tr>
<tr>
<td>Probable sepsis group</td>
<td>21</td>
<td>10</td>
<td>11</td>
<td>1.04</td>
</tr>
<tr>
<td>EOS group</td>
<td>22</td>
<td>19</td>
<td>3</td>
<td>1.04</td>
</tr>
<tr>
<td>LOS group</td>
<td>19</td>
<td>14</td>
<td>5</td>
<td>1.04</td>
</tr>
</tbody>
</table>

**Table 2: Result of CRP in Study Population**

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>IL-6</th>
<th>χ²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>41</td>
<td>33</td>
<td>36</td>
<td>36.5</td>
</tr>
<tr>
<td>Controls</td>
<td>42</td>
<td>8</td>
<td>6</td>
<td>2.25</td>
</tr>
<tr>
<td>Proven sepsis group</td>
<td>20</td>
<td>18</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Probable sepsis group</td>
<td>21</td>
<td>15</td>
<td>6</td>
<td>2.25</td>
</tr>
<tr>
<td>EOS group</td>
<td>22</td>
<td>19</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>LOS group</td>
<td>19</td>
<td>14</td>
<td>5</td>
<td>1.04</td>
</tr>
</tbody>
</table>

**Table 3: Result of IL-6 in Study Population**
group (+ve blood culture) and probable sepsis group (-ve blood culture).

Although blood culture is the gold standard method for diagnosis but have limitation of sensitivity and delayed result. Hsu et al found 48.8% positive blood culture in his study. In another study done by Chacko and Sohi found that culture proven sepsis occurred in (41.6%) of cases with sepsis. The common organism isolates are Staphylococcus, Klebsiella and E. coli which was similar to study by Kumhar et al in a tertiary care hospital in New Delhi, India. This comes in disagreement with the study of Dzwonek et al, in which nearly half of the positive blood cultures grew Klebsiella pneumonia, also in the study of De Benedetti et al, the isolated pathogens included Klebsiella pneumonia (47.5%) most common. This variation may be due to differences in the environment, the microbial etiology, type of blood culture and supportive care practice between centers.

In the current study AUC for CRP was 0.80 with sensitivity and specificity of 61% and 90.5%, respectively. Its PPV and NPV was 86.2% and 70.3%, respectively. Banac et al compared the levels of CRP, PCT and IL-6 in diagnosis of neonatal sepsis in 58 infants. They reported that the sensitivity, specificity, NPV and PPV of CRP at time of diagnosis was 36%, 92%, 43% and 89% respectively using a cut off value of 14 mg/l.

In recent years, chemokines and pro-inflammatory cytokines such as tissue necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), IL-8 and pro-calcitonin (PCT) have been introduced as early markers in infected infants. IL-6 is produced by monocytes, endothelial cells and fibroblasts. The concentration of IL-6 rises rapidly after the onset of bacteremia, but its half life is short. Previous studies showed that IL-6 may be a valid non-invasive and rapid method for diagnosis of neonatal sepsis. The sensitivity of IL-6 assay ranged from 0.60 to 0.96. Martin et al reported the specificity of IL-6 as 0.69, while it was as high as 0.95 in another study by Ng et al. These differences in sensitivity, specificity and DOR of IL-6 for predicting NS may be due to various factors. Method of study, method of IL-6 assay, cut-off levels for IL-6 assay, neonates characteristics (e.g. birth weight) may be some possible explanations for the discrepancy. The discrepancy in some studies seemed to be due to low sample size. The pooled values of sensitivity (0.79) and specificity (0.84) showed favorable accuracy of IL-6 for predicting NS. In the current study, AUC for IL-6 was 0.87 with sensitivity and specificity of 80.1% and 81%, respectively. Its PPV and NPV was 80.5% and 81%, respectively. The result are comparable with the study by Buck et al who reported sensitivity, specificity and PPV of IL-6 to be 72.7%, 77.8% and 84% respectively and with the study conducted by Kuster et al reported sensitivity and specificity of IL-6 to be 85.7% and 85% respectively.

In the current work, we studied the variations in the Sensitivity of IL-6 and CRP among Blood culture positive, Blood culture negative, EOS and LOS patients. In blood culture positive patients sensitivity of IL-6 and CRP was 90% and 80%, respectively. In blood culture negative patients sensitivity of IL-6 and CRP was 71.4% and 42.8%, respectively. In EOS sensitivity of IL-6 and CRP was 86.3% and 50%, respectively. In LOS sensitivity of IL-6 and CRP was 73.6% and 72.6%, respectively. The explanation of this result may need further studying, as there are no available data about other works that study these relations to support or deny our result or to explain it.

According to several studies, the authors tend to confirm that IL-6 is a promising biomarker for early diagnosis of sepsis.

**Conclusion**

In light of the result of the present study, IL-6 is a novel biomarker with high sensitivity and good specificity for sepsis. This has better diagnostic value than CRP, especially in Blood culture negative and EOS cases and consequently, we can conclude that measurement of IL-6 can be useful for early diagnosis of neonatal sepsis, especially in Blood culture negative and EOS cases.

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**Competing Interests**

None declared

**Reference**


