Role of Serum Procalcitonin as a Marker of Neonatal Sepsis

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Abstract- Background: Despite the advances in perinatal and neonatal care and use of newer potent antibiotics, the incidence of neonatal sepsis remains high and the outcome is still severe.

Objective: To study the ROLE OF SERUM PROCALCITONIN AS A MARKER OF NEONATAL SEPSIS and To compare procalcitonin with CRP as a diagnostic marker for neonatal sepsis

Methodology: Hospital Based prospective observational study. 50 neonates (preterm & full term) with clinically suspected sepsis were studied during 1 year from Jan 2016 to Dec 2016 in Chaitanya Hospital Chandigarh. Conventional sepsis workup was done in all cases and the diagnosis of neonatal sepsis was proved based on the results of blood culture. The serum Procalcitonin was measured by quantitative Enzyme linked immunofluorescence assay and the results were compared to CRP levels between the neonates with or without proven sepsis.

Results: Of the total 220 babies admitted in NICU during that period 50 were eligible for study and analyzed. 24% babies had Definite Sepsis, 60% had Probable Sepsis and 16% babies had No Sepsis. Of the neonates with suspected sepsis 24% had positive culture and 76% were culture negative. Mean PCT level was 13.27 ± 33.2 ng/ml. The mean PCT levels were higher in Meningitis group (Mean PCT-26.45) than no meningitis group. (p value-0.216). The mean PCT levels were higher for Pneumonia group (Mean PCT-13.98) than that of NO Pneumonia group (Mean PCT-12.81). The mean PCT levels was highest in neonates whose TLC>5000 (Mean PCT-18.5) (p value-0.002). The mean PCT levels were higher in all 3 infection groups in neonates with CRP>0.5 mg/dl (positive) than that of neonates with CRP≤0.5 mg/dl (negative). Mean PCT levels were 0.433, 52.22 and 27.95 in no infection, probable infection and definite infection group respectively. (p value- 0.001) Evaluating CRP as a diagnostic marker for definite neonatal sepsis with cut off value as 0.5 mg/dl, had sensitivity of 41.67%, Specificity of 89.47%, Positive Predictive Value of 55.56% and Negative Predictive value of 82.93%. Evaluating PCT as a diagnostic marker for definite neonatal sepsis. The Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value were 83.3%, 26.32%, 26.32% and 83.3% respectively taking cut off level of procalcitonin to be >0.5 ng/ml.

Conclusion: The importance of procalcitonin in diagnosing neonatal septicaemia cannot be denied. But it becomes more useful when it is used along with other investigations for decision making. Especially in identifying the group of neonates who may not be infected and may not require antibiotics.

I. INTRODUCTION

Sepsis is the commonest cause of neonatal mortality and is probably responsible for 30-50% of the total neonatal deaths each year in developing countries. The reported incidence of neonatal sepsis varies from 7.1 to 38 per 1000 live births in Asia, 6.5 to 23 per 1000 live births in Africa, and 3.5 to 8.9 per 1000 live births in South America and the Caribbean. By comparison, rates reported in United States and Australia range from 1.5 to 3.5 per 1000 for EOS sepsis and up to 6 per 1000 live births for LOS sepsis, a total of 6-9 per 1000 for neonatal sepsis.

Neonatal sepsis is one of the commonest cause of morbidity and mortality in the neonates in India compared to the developed countries. Since the clinical sign and symptoms of sepsis in neonates are non-specific and associated with high morbidity and mortality, early suspicion and treatment before blood culture confirmation of it is crucial. However this approach, treating infants suspicious of neonatal sepsis, may lead to unnecessary increased antibiotic usage, higher incidence of side-effects due to their use, increased resistance to antibiotics, a long hospitalization and separation of infants from their mothers and increased health costs. To help in early diagnosis, batteries of investigations are used called sepsis screening, these help in making a decision whether to treat or not to treat an infant suspected of having sepsis. The routine sepsis screen includes a complete blood count (CBC) with total and differential white cell count, band cells count, band cell and neutrophil ratio, platelet count, CRP, micro ESR and blood culture.

However, to date no single laboratory test had provided rapid and identification of infected neonates. This inability had lead to a search for a new diagnostic marker like procalcitonin.

Procalcitonin: In recent times Procalcitonin (PCT) has emerged as a newest earlier marker of sepsis. Procalcitonin is the precursor protein of calcitonin (CT) and has no hormonal activity. Most CT precursor peptides, including PCT, are found in the serum of normal healthy persons. PCT is preferentially induced in patients with sepsis, especially in severe bacterial sepsis or septic shock. Patients with systemic inflammation of non-bacterial origin generally have low PCT levels. PCT is closely related to the severity of systemic inflammation and has reliable kinetics of induction and elimination; therefore levels have been shown to be highest at time of onset of sepsis and decline over a period of time with appropriate antibiotic therapy. Increases in PCT occur more rapidly than increase in CRP, and PCT can be detected in the plasma 2 hours after injection of endotoxins. Within 6-8 hours, PCT concentrations rise and a plateau is reached after approximately 12 hours, with levels then decreasing to
normal values after 2-3 days with appropriate management. This has enabled PCT to be used as a valuable marker for diagnosis, evaluating prognosis and response to therapy. C-reactive protein: CRP is another acute phase proteins, although it is a classical and sensitive markers of inflammation, it cannot be used to differentiate between bacterial and other infection. C-reactive protein is a rapidly responsive acute phase reactant synthesized in the liver within 6 to 8 hours of an inflammatory stimulus and is found in negligible concentrations in the sera of healthy neonates. Monitoring of CRP levels has been widely promoted as a way to reduce the duration of antibiotic therapy in infants with suspected and proven sepsis. Although sensitivity and negative predictive values are not high enough for CRP alone to be a definite diagnostic test.

It is absolutely necessary to diagnose early neonatal sepsis and its cause using clinical findings and rapid diagnostic method so that no time is wasted to start the appropriate treatment. If not recognized early, it can cause septicemia leading to, multiple organ dysfunction and invariably death. The neonates who develop sepsis often die rapidly. Although this approach is reasonable given the dire consequence of a missed diagnosis, improvement in our diagnostic accuracy should diminish the exposure to the risk of avoided antibiotic therapy, excess financial and emotional cost to the parents.

There is paucity of data regarding best diagnostic marker for neonatal sepsis, from this part of the world so our study was an attempt to clearly differentiate the infected from non infected neonates among the risk of infection by using Procalcitonin and CRP levels, also to identify those with infection at the earliest and compare between the two tests and response to treatment.

II. MATERIALS AND METHODS

This prospective, observational study was undertaken at a Level III neonatal intensive care unit in Chaitanya Hospital from Jan 2106 to Dec 2016. The study protocol and consent forms were Reviewed and approved by the site’s Institutional Review Board. To be eligible for the study, neonates (term and preterm) with suspicion of sepsis were enrolled in study with no antibiotic therapy prior to enrollment. Neonates with lethal congenital suspicion of sepsis were enrolled in study with no antibiotic therapy. 50 children were enrolled in our study and rests were excluded because parents did not give consent in 44 cases, and 6 babies had birth asphyxia and 6 had some congenital malformation.

During the study period of 5 months 220 children were admitted in NICU. 106 babies fulfilled our criteria for suspected sepsis. 50 children were enrolled in our study and rests were excluded because parents did not give consent in 44 cases, and 6 babies had birth asphyxia and 6 had some congenital malformation.

The neonates were classified into 3 groups as was done by (white et al, 2007) with at least one clinical sign of sepsis (as above), who had at least one of an abnormal white cell count, platelets count or CRP level, together with a negative blood culture.

Unclassified. Neonates who would not fall in any of the above groups will be placed here.

Procalcitonin level analysis were done using ENZYME LINKED IMMUNOFLOUORESCENCE ASSAY FOR PROCALCITONIN BY VIDAS BRAHMS PCT KIT manufactured by BIOMERIUX INDIA (P) LTD. This is a quantitative assay where levels less than 0.5ng/ml was low risk and greater than 2ng/ml as high risk.

CRP analysis was done by using IMMUNOTURBIDOMETRY METHOD. This is a quantitative analysis where levels greater than 5mg/litre was considered as positive.
babies, and 26% were normal weight. Around half (52%) of them were low birth weight. The mean birth weight was 2028.00 ± 718 grams (Range-0.750 -4.00 kg). Most of the babies were appropriate for gestational age(AGA) only 2 babies were large for gestational age(LGA) and 8(16%) were small for gestational age(SGA).

The table 1 shows the frequency of different signs and symptoms in 3 different categories of diagnosis. The most common symptom in definite infection group was respiratory distress (91.7%) which was also most common symptom in other categories. None of the baby had ear discharge and none of them had umbilical sepsis.

Table 1 Distribution of neonates according to clinical features

<table>
<thead>
<tr>
<th>Signs/symptom</th>
<th>Definite sepsis (n=12)</th>
<th>Probable sepsis (n=30)</th>
<th>No sepsis (n=8)</th>
<th>Total (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Convulsion</td>
<td>2 (16%)</td>
<td>3 (10%)</td>
<td>0 (0%)</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>Severe chest Indrawing</td>
<td>10 (83%)</td>
<td>18 (60%)</td>
<td>6 (75%)</td>
<td>34 (68%)</td>
</tr>
<tr>
<td>Nasal flaring</td>
<td>8 (66.7%)</td>
<td>16 (53.3%)</td>
<td>5 (62.5%)</td>
<td>29 (58%)</td>
</tr>
<tr>
<td>Grunting</td>
<td>4 (33.3%)</td>
<td>14 (46.7%)</td>
<td>3 (37.5%)</td>
<td>21 (42%)</td>
</tr>
<tr>
<td>Respiratory Distress</td>
<td>11 (91.7%)</td>
<td>23 (76.7%)</td>
<td>8 (100%)</td>
<td>42 (84%)</td>
</tr>
<tr>
<td>Bulging fontanelle</td>
<td>2 (16.7%)</td>
<td>3 (10%)</td>
<td>0</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>Ear discharge</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lethargy/ Unconsciousness</td>
<td>5 (41.7%)</td>
<td>11 (36.7%)</td>
<td>0</td>
<td>16 (32%)</td>
</tr>
<tr>
<td>Inability to feed</td>
<td>6 (50%)</td>
<td>14 (46.7%)</td>
<td>3 (37.5%)</td>
<td>23 (46%)</td>
</tr>
<tr>
<td>Redness of skin around umbilicus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reduced movements</td>
<td>6 (50%)</td>
<td>11 (36.7%)</td>
<td>2 (25%)</td>
<td>19 (38%)</td>
</tr>
<tr>
<td>Not Suckling at all</td>
<td>1 (8.3%)</td>
<td>6 (20%)</td>
<td>1 (12.5%)</td>
<td>8 (16%)</td>
</tr>
<tr>
<td>Temp &gt;37.7°C/&lt;35.5°C</td>
<td>1 (8.3%)</td>
<td>3 (10%)</td>
<td>1 (12.5%)</td>
<td>5 (10%)</td>
</tr>
</tbody>
</table>

2.) Details about procalcitonin as marker of sepsis

Procalcitonin was sent at admission in all suspect sepsis case. Procalcitonin level of >0.5 ng/ml was considered as positive. Mean PCT level observed in our study was 13.27±33.2 ng/ml. Minimum value observed was 0.05 and maximum 200 ng/ml with median value of 3.87 ng/ml (interquartile range 0.5- 8.7). The mean PCT levels were higher in meningitis group (Mean PCT-26.45±61.47) than that of No Meningitis group (Mean PCT-9.97±21.37). This difference, however, was not statistically significant (p value-0.216). The mean PCT levels were higher for Pneumonia group (13.98±36.91) when compared to that of NO Pneumonia group (12.81±28) of neonates. The difference, however, was not statistically significant (p value-0.350).

3.) Details of comparison of procalcitonin with various sepsis markers.

Comparison of procalcitonin with TLC counts

The mean PCT levels were in highest in neonates with definite sepsis and TLC 5000-20000 (M-18.5±37.8) followed by neonates in probable sepsis whose TLC was in same range (M-17.7±39.89). There was significant statistical difference in the mean levels (p value-0.002) for neonates with TLC between 5000-20000 in different sepsis categories. (Table 2)
Table 2 Comparison of Procalcitonin with Total leucocyte Counts

<table>
<thead>
<tr>
<th>Total count(TLC)</th>
<th>leucocyte</th>
<th>No Sepsis</th>
<th>Probable Sepsis</th>
<th>Definite Sepsis</th>
<th>p-value</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5000</td>
<td></td>
<td></td>
<td>0</td>
<td>3</td>
<td>1.69</td>
<td>0.076</td>
</tr>
<tr>
<td>5000-20000</td>
<td></td>
<td>0.3086</td>
<td>0.076</td>
<td>0.13</td>
<td>0.002</td>
<td>2</td>
</tr>
<tr>
<td>&gt;20000</td>
<td></td>
<td>0.050</td>
<td>0.076</td>
<td>0.13</td>
<td>0.368</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>df-1, p-value-0.127</td>
<td>df-2, p-value-0.730</td>
<td>df-2, p-value-0.420</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison of Procalcitonin with CRP

Among neonates with CRP>0.5mg/dl, the mean PCT levels were highest in Probable Infection group followed by Definite Infection group and the difference was not statistically significant (p value -0.461). Among neonates with CRP negative CRP<0.5 mg/dl the mean PCT levels were higher for neonates in probable sepsis group followed by definite sepsis group. The difference was statistically significant (p value-0.001).(Table 3)

Table 3 Comparison of Procalcitonin with CRP

<table>
<thead>
<tr>
<th>CRP</th>
<th>No Sepsis</th>
<th>Probable Infection</th>
<th>Definite Infection</th>
<th>p-value</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N Mean</td>
<td>SD</td>
<td>N Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>≤ 0.5 mg/dl (Negative)</td>
<td></td>
<td>0.276</td>
<td>0.15</td>
<td>10.20</td>
<td>0.001</td>
</tr>
<tr>
<td>&gt;0.5 mg/dl (Positive)</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>52.22</td>
<td>0.461</td>
</tr>
<tr>
<td></td>
<td>p-value-0.807</td>
<td></td>
<td>p-value-0.222</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.) Diagnostic value of CRP and Procalcitonin

Table 4 Diagnostic value of CRP and Procalcitonin

<table>
<thead>
<tr>
<th>Cut off value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procalcitonin</td>
<td>&gt;0.5ng/ml</td>
<td>83.3%</td>
<td>26.32%</td>
<td>26.32%</td>
</tr>
</tbody>
</table>
The diagnostic markers of neonatal sepsis include total WBC and differential counts; an immature-to-total neutrophil ratio, ≥0.2; neutropenia; thrombocytopenia; and levels of CRP, PCT, haptoglobin, fibrinogen, and cytokines (interleukin [IL] 6, IL-8, and tumor necrosis factor-α), etc., with the bacterial culture providing a definitive diagnosis). Among these markers, CRP is most commonly used in all hospitals during follow-up and diagnosis. Although CRP levels can be obtained easily and rapidly through an automatic method, and has a high sensitivity, its specificity is low, making it difficult to diagnose sepsis.

In the present study procalcitonin was sent at admission in all suspect sepsis case. Mean PCT level observed in our study was 13.27± 33.2 ng/ml. Minimum value observed was 0.05 and maximum 200 ng/ml with median value of 3.87 ng/ml. In study by Ali Am et al in Egypt they found that babies presented positive cultures had PCT levels greater than 0.5 mg/dl and in most of them were greater than 2mg/dl. The correlation of PCT with culture was highly significant (p=0.004) and the relative risk was much greater than just presence or absence of infection. It is generally acknowledged that some neonates with sepsis will have negative blood cultures; hence mere negative blood culture does not negate infection. These babies behave like those who have infection and therefore are categorized as probable infection neonates may have actual sepsis.

Our study shows mean PCT levels were higher in all 3 infection groups in neonates with CRP>0.5 mg/dl (positive) than that of neonates with CRP≤0.5 mg/dl (negative). Mean PCT levels were 0, 52.22 and 49.83 in no infection, probable infection and definite infection group respectively. However difference was not statistically significant.

In definite infection group, mean PCT levels were higher for neonates whose CRP>0.5 mg/dl (Mean PCT-27.95) than that of neonates with CRP≤0.5mg/dl (Mean PCT-6.78). Again the difference was not statistically significant (p value-0.222).

The diagnostic profile of procalcitonin is claimed to be superior to other acute phase reactants including CRP (Chiesa et al, 1998).

In our study, the sensitivity, specificity, positive predictive value and negative predictive value using cut off value of CRP as 0.5 mg/dl were 41.67%, 89.47%, 55.56% and 32.92% respectively. Sucilathangam et al (2012) reported the sensitivity, specificity, positive predictive value and negative predictive value using cut off value of CRP as 6 mg/L, 50.0%, 69.4%, 38.8% and 78.1% respectively. Boo et al (2008) reported the sensitivity, specificity, positive predictive value and negative predictive value of CRP as 55.6%, 89.9%, 58.8% and 88.6% respectively in confirmed sepsis cases while Sakha et al (2008) reported sensitivity, specificity, positive predictive value and negative predictive value of CRP (more than 3.5mg/L) as 70.4%, 72.2%, 43.2% and 89% respectively in the diagnosis of neonatal sepsis.

In our study, the Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value were 83.3%, 26.32%, 26.32% and 83.33% respectively taking cut-off level of procalcitonin to be >0.5 ng/ml. This is higher than the White D et al 2007 study of 194 neonates with suspected sepsis which reported the Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value as 48%, 74%, 39% and 80% respectively at cut-off level of procalcitonin >0.5 ng/ml.

A study by Ballot et al (2004) showed that the Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value in none vs. definite infection category as 76.9%, 50%, 14% and 95% respectively for PCT using a cut-off value of 0.5 ng/ml but also stated that although PCT was significantly related to the category of infection, it is not sufficiently sole marker of neonatal sepsis. PCT would be useful as part of full sepsis evaluation. They also concluded high negative predictive value on presentation may rule out sepsis.

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Vazzalwar et al (2005) found Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value of 97%, 80%, 92% and 92% respectively at PCT cut-off value of 0.5 ng/ml. Chiesa et al (1998) reported Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value of 92.6%, 97.5%, 94.3% and 96.8% respectively.


<table>
<thead>
<tr>
<th>CRP</th>
<th>&gt;5mg/dl</th>
<th>41.67%</th>
<th>89.47%</th>
<th>55.56%</th>
<th>82.93%</th>
</tr>
</thead>
</table>

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to be 66.7%, 50%, 28.6% and 83.3% respectively using cut-off of PCT>2ng/ml. In our study, 86% neonates were discharged, 8% neonates were taken away against medical advice (LAMA) and 3 (6 %) neonates had expired (table 9). Chacko and Sohi (2005) reported case fatality rate 19.4% in EOS and 13.3% in culture positive cases. Tallur et al (2000) reported higher rate (47.5%) of mortality in proven neonatal sepsis. In our study, the mortality was lower than expected because some very sick babies were taken away by the parents against medical advice.

However, a study by Sakha et al reported that CRP had a higher NPV than PCT In neonates, an elevated PCT level may help in predicting septicemia; furthermore, low PCT levels were useful in ruling out septicemia as a diagnosis. The good negative value found suggested that PCT can be tested rapidly and is highly discriminating means to rule out bacteraemia. Therefore, PCT assessment could help physicians limit the number of unnecessary prescriptions for antibiotics.

V. CONCLUSIONS

In the present study, the sensitivity of procalcitonin was very high, hence its use as a sole diagnostic marker could not be recommended. However it has a good negative predictive value, hence it has a utility in ruling out the possibility of infection in neonates. Hence we conclude that the importance of procalcitonin in diagnosing neonatal sepsicaemia cannot be denied. But it becomes more useful when it is used along with other investigations for decision making. Especially in identifying the group of neonates who may not be infected and may not require antibiotics.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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