International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Implications of Procalcitonin as a Prognostic Marker in Neonatal Sepsis

Arghyadip Sahoo[1], Priyanjalee Banerjee[2]

[1]Consultant, DESUN Hospital And Heart Institute, Kolkata, India

[2]Senior Research Fellow, Institute of Post Graduate Medical Education and Research (IPGME&R), Kolkata, India

Abstract: ***Background:*** *Though there are several methods present for diagnosis of neonatal sepsis, however, till date, search for definite prognostic marker for monitoring treatment outcome is still on.* ***Objective:*** *Aim of the present study is to detect a prognostic marker for neonatal sepsis.* ***Material and methods:*** *The present study was conducted on neonates admitted to pediatric ward of Desun Hospital and IPGME&R, Kolkata for a period of six months from January to July, 2014. Before and after administration of antibiotic course blood samples were collected and hematological parameters (MicroESR, total WBC count) were investigated. Serum procalcitonin (PCT) and C reactive protein (CRP) were also estimated by immunoassay kits as per manufactures protocol. Antibiotic sensitivity and blood culture for isolation of microorganisms were also performed. Results were expressed as mean ± SEM and analysis was done by SPSS version 12.* ***Results:*** *Out of 54 neonates, 40.7% have shown criteria of proven sepsis, 27.7% suspected sepsis and 31.4% clinical sepsis. 15 out of 22 neonates with proven sepsis have been reported with strongly positive serum PCT values. However, after 5 days antibiotic treatment, more than 60% neonates have shown reduced PCT levels while no change in serum CRP and micro ESR were detected.* ***Conclusions:*** *Though procalcitonin has been well reported as a diagnostic marker for neonatal sepsis, it is however not well established as a prognostic marker. In the present study, we have reported serum PCT as a good prognostic marker for newborn sepsis. However, these results should be further validated with a large sample size.*

Keywords: Procalcitonin, neonates, sepsis, prognosis, C reactive protein

1. Introduction

Neonates are specially susceptible to sepsis with non specific clinical manifestations [1]. Worldwide, neonatal sepsis is an alarming condition resulting in high morbidity and mortality [2, 3]. To reduce mortality rate early and prolonged antibiotic treatment often leads to several other complications including antibiotic resistance development. This leads to the requirement of a highly specific clinical diagnostics and prognostic criteria.

For diagnosis of neonatal sepsis, the most traditional method is to isolate the causal microorganisms from blood cells culture [4]. Estimations of the white-blood cell count (WBC), the absolute neutrophil count (ANC), micro ESR and the I/T ratio are usually used for the diagnosis of neonatal sepsis, other than culture of blood cells [5, 6]. However, these tests lack high specificity in diagnosing neonatal sepsis [7]. In this regard, currently procalcitonin has been known to play a major role for early diagnosis of neonatal sepsis [8, 9]. Procalcitonin, synthesized primarily in thyroid gland besides some other neuroendocrine tissues, gets converted to calcitonin which is inhibited by the action of various cytokines and endotoxins. Hence, when endotoxins secreted by cell walls of gram negative bacteria increases, the level of serum procalcitonin eventually rises and thus serves as a marker for septic shock [10].

In several studies, beneficial diagnostic role of procalcitonin has been thoroughly investigated and positively reported [11, 12]. However, the role of procalcitonin as a prognostic marker remains elusive till date. Thus, the main objective of this study is to highlight prognostic value of procalcitonin in treatment of neonatal sepsis.

2. Materials and Methods

Study Design: Neonates admitted to newborn ward at Desun Hospital and Institute of Post Graduate Medical Education and Research (IPGME&R) Kolkata, West Bengal, for a period of 6 months (from Jan, 2014 to July, 2014) were included in this present study. Parent's consents were taken and the institutional human ethical committee approved this study. Particular exclusion criteria were infants already undergoing antibiotic treatment, or has been reported to have congenital anomalies or inborn error of metabolism or birth asphyxia.

Sepsis was concluded if the following clinical criteria were found in the neonates:

1) Maternal risk factor such as fever, prolonged rupture of amniotic membrane >24 hr
2) Neonatal history: low birth weight (< 2000 grams), premature birth (<37 weeks).
3) Symptoms of sepsis: temperature instability, apnea, respiratory distress, seizures, tachypnea, bradycardia, abdominal distension and vomits.

Only neonates with any features from I and II in addition to more than two clinical symptoms of sepsis were taken for screening of sepsis.

Blood samples for blood culture (1-2 ml) and PCT measurements were obtained by venous puncture before and after administration of antibiotics. Serum was then isolated by centrifugation and stored at -20°C for measurement of procalcitonin.

Microorganisms Screening: Blood culture media (Biphasic) was taken in bottles and blood samples collected for prospective neonates were added and then incubated at 37 °C for 5-7 days [13, 14]. Sub cultures were done on blood agar (Himedia, India) and EMB media taking samples from

bottles with positive results. The isolated microbes (Klebsiella, Acinetoobacter, CONS, Pseudomonas, Staphylococcus, Citrobacter) were identified by standard bacteriological methods. Sepsis work up included haematological parameters like the erythrocyte sedimentation rate (microESR), total laeukocyte count and C-Reactive Protein (CRP) estimations [15, 16].

Neonates were then classified in to following three groups according to microbiologic examination reports and clinical symptoms of sepsis:

1) Proven sepsis (N= 22): positive blood culture along with clinical symptoms of sepsis.
2) Suspected sepsis (N= 15): with clinical symptoms but negative blood culture. Also at least 2 positive hematological screening parameters (elevated microESR or CRP or abnormal WBC count) must be present.
3) Clinical sepsis (N= 17): Symptoms of sepsis must be present with both bacterial culture and screening test parameters being negative.

3. PCT level measurement in serum

The serum PCT level was measured by using a quantitative immuno-luminometry method using the Lumitest kit (BRAHMS Diagnostic, Berlin, Germany). PCT level of ≥0.5 ng/ml was considered as pathological and below that negative in the present study. PCT levels of 0.5-10 ng/ml and >10 ng/ml were considered as positive, and strongly positive, respectively.

3.1 Serum CRP

Serum CRP level was measured by using the A-15 CRP Kit (Bio-system, Costa Brava, Barcelona, Spain). CRP from the serum was measured by an immunoturbidimetric method in the laboratory according to the manufacturer's instructions. The linearity was maintained up to 150 mg/L and 6 mg/L was taken as the reference value.

4. Statistical Analysis

To compare means of the variables, one-way ANOVA test was done by SPSS (version 12). Diagnostic efficiency was defined as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). A *P*-value< 0.05 was considered as significant. The correlation of serum PCT and the CRP level with the haematological parameters (Total WBC count, Micro ESR and the CRP value) and the blood culture for an early diagnosis of neonatal sepsis was compared statistically and the results were analyzed by using SPSS, version 12. By using the blood culture results as the gold standard, the sensitivity, specificity, positive predictive values and the negative predictive values of the PCT-Q and CRP for diagnosing sepsis were calculated. The sensitivity of a test was defined as the proportion of infants with sepsis and this was correctly identified by the test.

5. Results

A cross sectional study with 54 neonates who fulfilled the study criteria were selected and further classified into 3 subgroups according to the outcomes of microbiological and hematological parameters examinations: proven sepsis (n=22); suspected sepsis (n=15) and clinical sepsis (n=17).
Among the microorganisms isolated from blood samples of the prospective neonates, the most frequently detected was Staphylococcus (7/22), followed by Klebsiella (5/22), Acinobacter (4/22), Pseudomanas (3/22), CONS (2/22) and citrobacter (1/22) (Figure 1).
In Table 2, parameters like gender and birthweights were compared among 3 different study groups. Out of 54 neonates, 26 were males and rest 29 neonates were females. Also 22 neonates were born with low birth weight ie. below 2000g.

In Table 3, hematological parameters like micro ESR, CRP levels and WBC count has been compared among 3 study groups. Elevated micro ESR was observed mainly in suspected and proven sepsis group while in suspected sepsis total WBC count is maximally increased. CRP level was elevated in proven and suspected sepsis group also.

Table 4 depicts that when 22 neonates presenting criteria of proven sepsis were treated with 5 days antibiotic course, significant improvement in clinical symptoms was observed. While before commencement of 5 days antibiotic course 15/22 have shown strongly positive procalcitonin levels, this number decreases to 6/22 which is statiscally significant.

6. Discussion

Proper diagnosis and prognosis of neonatal sepsis still pose a great problem to clinicians. Through early and proper diagnosis unnecessary antibiotic administration that may lead to various harmful side effects may be avoided. However the present diagnostic markers are highly insufficient. Microorganisms count in blood culture takes much longer time. Not only diagnosis but also prognosis can be predicted by procalcitonin. So that antibiotic resistance can be predicted and change of antibiotic can be considered. Newborn resuscitation in case of sepsis can be promptly undertaken.

Generally, C reactive protein and procalcitonin has been the most common factor for bacterial infection identification [17]. CRP mediates its action by activation of immune system through opsonization of bacteria and thus elicits further inflammatory response [18, 19, 20]. However PCT exhibits greater sensitivity than other proinflammatory biomarkers since the level of PCT comes back to its normal level more quickly after bacterial infections.CRP generally reaches a platue but this problem is not encountered in case of procalcitonin [21, 22].

Also total WBC count was normal in 9 out of 15 neonates blood samples in cases of proven sepsis also with micro ESR and CRP. Hence these cannot be considered as a definitive prognostic marker [23].

In the present study, serum level of procalcitonin has also been estimated to confirm PCT as diagnostic marker of neonatal septicemia as reported by others. The findings in our study are in good agreement with other experimental studies. [24] It was found that PCT levels were significantly

high in neonates with proven sepsis and also in suspected sepsis group. Serum PCT level can be well correlated with p value of < 0.001. This is in well accordance with report by Koksal et al. [25].

Although, from several other studies the role of PCT as a marker for neonatal sepsis is well established, Ballot et al stated that the level of PCT as insufficiently reliable and thus it can be a contributor to the outcome of sepsis examination but not primarily the major parameter [26]. Also Koksal et al. inferred that serum PCT is also superior in comparison to CRP as a diagnostic marker of neonatal sepsis and also can reflect the degree of illness [27].

Taking all these views under consideration, we must admit that search for a valuable and definite prognostic marker for neonatal sepsis is still on. Based only a reliable prognostic marker, clinicians can outline an effective treatment protocol. Since antibiotic resistance poses a huge obstacle, so if a definitive prognostic marker could be identified, chances of futile antibiotic treatments will be reduced manifold. In our present study it was found that when 22 neonates with proven sepsis were administered 5-days antibiotic course, improvement of clinical symptoms were observed in around 60% patients where procalcitonin level became negative which is highly significant. Whereas full course of antibiotic treatment cannot convert ESR and CRP level to normal (data not shown). Thus serum PCT level can be considered to be responsive to 5 days antibiotic treatment and thus can further be valued as a good prognostic marker [28, 29].

However, a much detailed intervention and a larger scale cross sectional study with greater sample size is required to further confirm these findings.

7. Conclusion

Routine use of serum procalcitonin as a diagnostic marker is widespread and well established. However, PCT may be also looked upon as a valuable prognostic marker in neonatal sepsis, in comparison with other less specific sepsis biomarkers like CRP, IL-6, etc. In near future, procalcitonin may occupy the center stage in sepsis prognosis in other infectious diseases also that includes symptoms of septic shock. Thus these findings must be further investigated to obtain a definite diagnostic as well as prognostic marker for treatment of neonatal sepsis with high effectiveness.

8. Acknowledgement

The authors would like to thank director of Desun Hospital and heart institute and Institute of Post Graduate Medical Education and Research (IPGME&R) for providing the lab facilities for conducting this study.

References

[1] Barbara S. Infections of the neonatal infant. In: Behrman Re, Kliegman R, Jensen HB, editors. Behrman: Nelson Textbook of Pediatrics. Philadelphia: WB Saunders CO; 2008.p.794-811.
[2] Afroza S. Neonatal sepsis -a global problem: an overview. *Mymensingh Med J.* 2006;15(1):108-14.
[3] Friedman G, Silva E, Vincent JL: Has the mortality of septic shock changed with time? *Crit Care Med* 1998; 26:2078–2086.
[4] Nwadioha SI, Nwokedi EOP, Kashibu E, Odimayo MS, Okwori EE. A review of bacterial isolates in blood cultures of children with suspected septicemia in a Nigerian Tertiary Hospital. Afric J Microbiol Res 2010; 4:222-225.
[5] Rivers E, Nguyen B, Havstad S, et al: Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med* 2001; 345:1368–1377.
[6] Wheeler AP, Bernard GR: Treating patients with severe sepsis. *N Engl J Med* 1999; 340: 207–214.
[7] Oberhoffer M, Vogelsang H, Russwurm S, et al: Outcome prediction by traditional and new markers of inflammation in patients with sepsis. *Clin Chem Lab Med* 1999; 37:363–368.
[8] Müller B, Becker KL, Schächinger H, et al: Calcitonin precursors are reliable markers of sepsis in a medical intensive care unit. *Crit Care Med* 2000; 28:977–983.
[9] Nijsten MW, Olinga P, The TH, et al: Procalcitonin behaves as a fast responding acute phase protein in vivo and in vitro. *Crit Care Med* 2000; 28:458–461.
[10] Müller B, Becker KL: Procalcitonin: How a hormone became a marker and mediator of sepsis. *Swiss Med Wkly* 2001; 131:595–602.
[11] Zahedpasha Y, AhmadpourKacho M, Hajiahmadi M, Haghshenas M. Procalcitonin as a marker of neonatal sepsis. *Iran J Paediatr* 2009;19:117-22.
[12] Carol ED, Thomason AP, Hart CA. Procalcitonin as a marker of sepsis. *Int J Antimicrob Agents* 2002;20:1-9.
[13] Zipursky A, Palko J, Milner R, Akenzua GI. The hematology of the bacterial infections in premature infants. *Paediatrics* 1976,57: 839- 53.
[14] Basu S, Guruprasad, Narang A, Garewal G. The diagnosis of sepsis in high risk neonates by using a hematologic scoring system. *Indian J Hematolo Blood Transfusion* 1999;17:32-34.
[15] Rodwell RL, Leslie AL, Tudehope DL. Early diagnosis of neonatal sepsis by using a hematological scoring system. *J Paediatr* 1988;112:161- 66.
[16] Kafetzis DA, Tigani GS, Costalos C. Immunologic markers in the neonatal period: their diagnostic value and accuracy in infection. *Expert Rev Mol Dign* 2005;5:231-39.
[17] Black S, Kushner I, Samols D. C-reactive protein. Minireview. J Biol Chem 2004; 279:48487-48490.
[18] Jaswal RS, Kaushal RK,Goel A, Pathania K. Role of the C-reactive protein in deciding the duration of the antibiotic therapy in neonatal septicaemia. *Indian Paediatrics* 2003;40:800-83.
[19] Hatherill M, Tibby SM, Sykes K, et al: Diagnostic markers of infection: Comparison of procalcitonin with C-reactive protein and leukocyte count. *Arch Dis Child* 1999; 81: 417–421.
[20] Casado-Flores J, Blanco-Quiros A, Asensio J, et al: Serum procalcitonin in children with suspected sepsis: A comparison of procalcitonin with C-reactive protein and leukocyte count. *Pediatr Crit Care Med* 2003; 4:190–195.

[21] Manneret G, Labaune JM, Isaac C, Bienvenu F, Putet G, Bienvenu J. Procalcitonin and C-reactive protein levels in neonatal infections. *Acta Paediatr* 1997;86:209-12.
[22] Chin YL, Tseng CP, Tsay PK, Chang SS, Chiu TF, Chen JC. Procalcitonin as a marker of bacterial infection in the emergency department. Critic Care 2004; 8:R12-R20.
[23] Blommendahl J, Janas M, Laine S, Miettinen A, Ashorn P. Comparison of procalcitonin with CRP and the differential white blood cell count for the diagnosis of culture-proven neonatal sepsis. *Scand J Infect Dis* 2002;34: 620-22.
[24] Gendrel D, Assicot M, Raymond J. Procalcitonin as a marker for the early diagnosis of neonatal infections. *J Paediatr* 1996;128:570-73.
[25] Koksal N, Harmanci R, Getinkaya M. The roles of procalcitonin and CRP in the diagnosis and the follow up of neonatal sepsis cases. *Turk J Paediatr* 2007;49:21-9.
[26] Ballot DE, Perovic O, Galpin J, Cooper PA. Serum procalcitonin as an early marker of neonatal sepsis. *S. Afr Med J* 2004;94:851-54.
[27] Zeni F, Vialon A, Assicot M, et al: Procalcitonin serum concentrations and severity of sepsis. *Clin Intensive Care* 1994; 5(Suppl 2):89–98.
[28] Chiesa C, Panero A, Rossi N. Reliability of the procalcitonin concentrations in the diagnosis of sepsis in critically ill neonates. *Clin Infect Dis* 1998;26:664-72.
[29] Vincent JL. Procalcitonin: The marker of sepsis? Crit Care Med 2000; 28:1226-1228.

Figure 1:

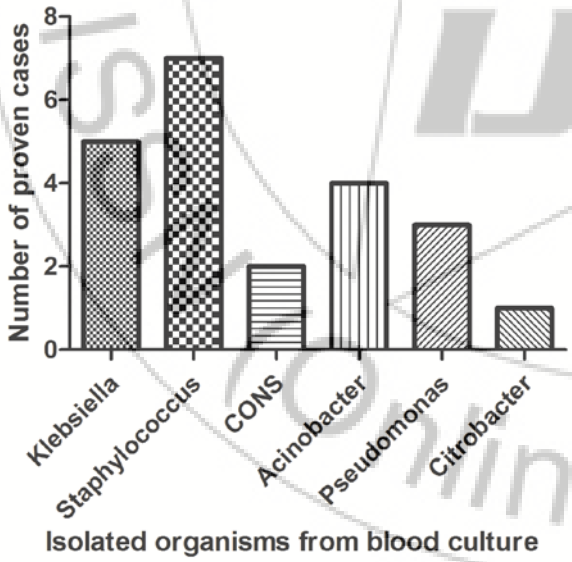

Table 1

Types of sepsis	*No. of cases*	*Male*	*Female*	*Birth weight(Low)*	*Birth weight(Normal)*
Clinical sepsis	17	10	7	6	11
Proven sepsis	22	9	13	9	13
Suspected	15	7	9	7	8
Total cases	54	26	29	22	32

Table 2

Group	*PCT levels (ng/ml)*	*No. of cases (n=54)*	*Clinical sepsis (n=17)*	*Suspected sepsis (n=15)*	*Proven sepsis (n=22)*
Negative	<0.5	19	14	11	1
Positive	0.5-10	6	3	3	6
Strongly positive	>10	29	0	1	15

Table 3

Sepsis group investigations	*Clinical Sepsis (N=17)*	*Suspected sepsis (N=15)*	*Proven sepsis (N=22)*
Total WBC count	4	9	7
Elevated level of micro ESR	2	12	8
Elevated serum CRP level	3	9	8

Table 4

Group	*Proven sepsis*	*Before initiation of 5 days antibiotic course*	*After completion of 5 days antibiotic course*	
			Improvement of clinical symptoms	*Detoriation of clinical symptoms*
Negative	1	1	10	0
Positive	6	6	6	0
Strongly	15	15	2	4