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Original Research Article

A Prospective Analytical Research to Evaluate the Expenses and Benefits of Procalcitonin Testing in the Diagnosis of Early Onset Neonatal Sepsis

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Abstract

Aim: To compare between the expenses and benefits of procalcitonin testing in the diagnosis of early onset neonatal sepsis.

Methods: This prospective analytical study was carried out in the Department of Paediatrics, Visakha Steel General Hospital Visakhapatnam, Andhra Pradesh, India for 15 months. 100 Full-term neonates (>37 weeks gestational age) admitted to NICU with the clinical symptoms or signs of sepsis within 72 h of birth, and those with risk factors for EONS. For all patients included in the study, complete blood count with differential (CBC), CRP, blood culture, procalcitonin was done on admission to NICU, and was repeated after 8 hours from the initial one, while CRP was repeated after 24 hrs. Cultures from other sites (including CSF), chest x ray and imaging were done as appropriate.

Results: Their mean weight was 3.213 kg (range 2.2- 4.8 kg). Mean age of presentation was 14 hours (range 2-68) hours. About 90 (90 %) patients presented with more than one symptoms and signs of sepsis For CBC results, mean WBC count was 19.8± 9.1, the mean neutrophils count was 15.55± 10.57) and the mean platelet count was 171.21±69.21. This study includes neonates with early onset sepsis. In the present study the most frequent presenting sign was hypothermia (63%), hypo activity and mottling (54%), followed by feeding intolerance (43%). Blood cultures were positive in 37 patients, who compromised the group of proven sepsis. Klebsiella was present in 14 patient, E coli in 10 patients, while group B streptococcus in 8 patients. Pseudomonas infection present in 3 patients, while MRSA (methicillin resistant staphylococcus aureus) was detected in 2 patients. The initial CRP (CRP1) , was non-reactive in 42 patients (42%), The initial procalcitonin in the sepsis group (PCT1), was non-reactive 42 patients (42%) , <2.6 in 32 patients (32%), 2.6-10 in 20 patients (20%), and more than that in 6 patients (6%). Sensitivity= 93.5%, Specificity=54%, PPV= 66.3%, NPV= 87.5%. PCT2: Sensitivity=96.8%, Specificity=88.7%, PPV=91.1%, NPV=99%.

Conclusion: The expense of testing PCT twice is less than the expenses of one-day admission in NICU in developing countries. Application of such protocol could be of use in limiting period of stay in NICU.

Key words: NICU, Procalcitonin, Early Onset Neonatal

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Introduction

Death due to infections remains a major contributor to mortality in children younger than 5 years of age worldwide. The incidence and etiology of early- and late-onset sepsis in neonates is variable across countries, which necessitates that antibiotic therapy be tailored in an institution-specific manner. However, the difficulties in confirming the diagnosis of neonatal sepsis have led to the use of a variety of antibiotics for variable durations leading to the emergence of antibiotic-resistant microorganisms.[1,2] The symptoms and signs of NS mimicking that of other infections, co-existence of infections make the diagnosis difficult, particularly in countries battling with infectious diseases. With varying incidence in Asian countries, and ≈ 1.6 million neonates succumbing annually in developing countries indicates the need for an early diagnosis including clinical and laboratory evaluation.[3]There are many available markers for NS but each with limitations; Complete Blood Count (CBC), immature: total neutrophil count (I: T ratio) and absolute neutrophil count (ANC) do not have sensitivity especially if measured early in the course of sepsis.[4] Though culture is the gold standard for confirmation,[5] result is influenced by various factors such as prior use of antibiotics including in the prenatal period, bacterial load, laboratory standards and is time-consuming (up to 72 hours); it often fails to identify the causative organism in infected infants[6] given low culture yields. Several leukocyte indices and acute phase protein levels were evaluated for the diagnosis of sepsis. C-reactive protein though is a classical and sensitive marker of inflammation[7] is not useful to differentiate between bacterial and other infections, has a limited role in diagnosing early-onset sepsis compelling the use of combination tests for markers.[8] In the absence of a single ideal diagnostic & confirmatory laboratory test.[7] the quest and search for the same for early diagnosis of NS is the need of the hour. The paradigm in pediatric practice is a neonate is likely to suffer more if the infection is under-diagnosed and untreated than over-diagnosed and treated demanding a diagnostic test with high sensitivity than high specificity. Emerging shreds of evidence showing serum procalcitonin (PCT) as a measurable laboratory marker in the inflammatory response to the infection is promising due to its high sensitivity compared to CRP, but its specificity is still debated.[9] Considering the diversity in population, that is different from other global counterparts where the diagnostic utility of PCT is encouraging, there is a scarcity of studies on evaluation of the role of various biochemical markers in the diagnosis of NS especially PCT in Indian neonates. We attempted to document the effects of intrapartum risk factors, assess and compare the diagnostic role of Procalcitonin and CRP in early onset NS.

Material and methods

This prospective analytical study was carried out in the Department of Paediatrics, Visakha Steel General Hospital Visakhapatnam, Andhra Pradesh, India for 15 months. 100 Full-term neonates (>37 weeks gestational age) admitted to NICU with the clinical symptoms or signs of sepsis within 72 h of birth, and those with risk factors for EONS. The risk factors considered were: GBS infection during pregnancy, premature rupture of membrane, prolonged rupture >18 h before birth and Clinical syndrome of maternal intrauterine infection were included in this study.

Patients < 37 weeks gestational age, patients with congenital anomaly or metabolic inborn error of metabolism, patients received antibiotics before admission to NICU and refusal of the parents to sign the consent were excluded from this study. For all patients included in the study, complete blood count with differential (CBC), CRP, blood culture, procalcitonin was done on admission to NICU, and was repeated after 8 hours from the initial one, while CRP was

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repeated after 24 hrs. Cultures from other sites (including CSF), chest x ray and imaging were done as appropriate.

Under complete aseptic conditions 3 ml of venous blood was collected, 1 ml on ethylene diamine tetra acetic acid (EDTA) for complete blood count (CBC) and 2 ml was collected in plane tube and was left to clot then centrifuged to separate serum for estimation of serum CRP and detection of PCT. For serum PCT analysis Sera of the patients were analyzed for PCT using commercially available Enzyme-linked immunosorbent assay (ELISA) kit. PCT production was calculated from a standard curve of the corresponding recombinant human PCT. Manual broth-based blood culture systems was used, namely non-selective agar media. Growth of any organism in samples taken from symptomatic newborns was taken significant.

Criteria for the diagnosis of neonatal infection[10]

Temperature instability, Heart rate >180 beats/min or <100 beats/min, blood pressure 2 SD below normal for age, and capillary refill >3s. Respiratory problems: as apnea, dyspnea, retractions and cyanosis. Central nervous system affection: irritability, lethargy, abnormal Moro reflex, fontanel bulging, seizures, and hypotonia. Gastrointestinal system affection as feeding intolerance, abdominal dis- tension with repeated vomiting or frequent watery motions. leukocytosis (WBC>34000), leukopenia (WBC<5000), Thrombocytopenia<100000, CRP 2 SD above normal level, Procalcitonin 2 SD above normal value By the end of the first 48 h newborns were labeled proven infected if their blood culture showed growth of organism; and were continued on antibiotics. Another group were labeled infected but not proven and those include patients with positive CRP, leukopenia or leukocytosis, or if have clinical symptoms and signs of sepsis. The third group is the rest of the newborn suspected for EONS but without evidence, they were labeled suspected only, and their antibiotics were discontinued.

Statistical analysis

The EPI info programme was used for data entry and analysis. Chi square test was used for calculation of significance. P values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS 21.0.

Results

100 patients were included in the study, 68 females and 32 males. Their mean weight was 3.213 kg (range 2.2- 4.8 kg). Mean age of presentation was 14 hours (range 2-68) hours. 84 newborns (84%) presented within 28h of age, out of whom 54 newborns admitted at birth due to prolonged rupture of membrane (18 h and more). Prolonged rupture of membrane was the main risk factor, followed by maternal infections and fever (29 patients), fetal tachycardia (15 patients), and smelly liquor (7 patients).

Table 1 shows the clinical features of the newborns on admission to NICU About 90 (90 %) patients presented with more than one symptoms and signs of sepsis For CBC results, mean WBC count was 19.8± 9.1, the mean neutrophils count was 15.55± 10.57) and the mean platelet count was 171.21±69.21. This study includes neonates with early onset sepsis. In the present study the most frequent presenting sign was hypothermia (63%), hypo activity and mottling (54%), followed by feeding intolerance (43%).

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Table 1: clinical symptoms and signs of the patients

Clinical symptoms and signs	Number of patients	Percentage
Tachypnea	37	37
Tachypnea with intercostal retractions	20	20
Sudden desaturation	14	14
Tachycardia	8	8
Bradycardia	17	17
Feeding intolerance,	43	43
vomiting	12	12
Hypothermia	63	63
Hypo activity, mottling	54	54

Blood cultures were positive in 37 patients, who compromised the group of proven sepsis. Klebsiella was present in 14 patient, E coli in 10 patients, while group B streptococcus in 8 patients. Pseudomonas infection present in 3 patients, while MRSA (methicillin resistant staphylococcus aureus) was detected in 2 patients. The initial CRP (CRP1) , was non-reactive in 42 patients (42%), The initial procalcitonin in the sepsis group (PCT1), was non-reactive 42 patients (42%) , <2.6 in 32 patients (32%), 2.6-10 in 20 patients (20%), and more than that in 6 patients (6%).

Table (2) shows CRP1/PCT1 results in the group with proven sepsis and those infected but not proven in Table (3). CRP2/PCT2 for the two categories are shown in Table 4 and 5. Table 6 shows work out of the predictive values of CRP2 and PCT2.

Table 2: Results of CRP1 and PCT1 in newborns with proven sepsis

	Positive	Negative	Total
CRP1	26	11	37
PCT1	28	9	37
Total	54	20	74 Pvalue 0.31

Table 3: Results of CRP1 and PCT1 in newborns with not-proven sepsis

	Positive	Negative	Total
CRP1	8	34	42
PCT1	5	37	42
Total	13	71	84 Pvalue 0.063

Table 4: Results of CRP2 and PCT2 in newborns with not-proven sepsis

	Positive	Negative	Total
CRP2	32	5	37
PCT2	33	4	37
Total	65	9	74 Pvalue 0.061

Table 5: Results of CRP2 and PCT2 in newborns with not-proven sepsis

	Positive	Negative	Total
CRP2	21	21	42
PCT2	6	36	42
Total	27	57	84 Pvalue 0.004

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Table 6: The predictive value for CRP2 and PCT2 in diagnosis of EONI

	Positive	Treated group	Not treated group	Total
CRP2	Positive	56	24	80
	Negative	3	17	20
PCT2	Positive	52	5	57
	Negative	5	38	43

CRP2: Sensitivity= 93.5%, Specificity=54%, PPV= 66.3%, NPV= 87.5%. PCT2: Sensitivity=96.8%, Specificity=88.7%, PPV=91.1%, NPV=99%.

Managing infants in the three groups (proven, not-proven, and suspected infection) resulted in 412 inpatient days. The 37 patients in the group of proven infection (37%) were admitted for 10-14 days but 5 of them needed more than 2 weeks admission. 5 patients of this group died due to infection with GBS which caused bacteremia, followed by toxic myocarditis and heart failure, that didn't respond to the antibiotic course. The second group (not-proven infection) of 42 patients (42%) were discharged within 4 days of admission, and they did well on the follow up except for 10 patients who didn't show up. The third group 21 patients (21%) with suspected infection, treated with antibiotics for at least 10 days, and all of them did well on the follow up.

Discussion

This study includes neonates with early onset sepsis. In the present study the most frequent presenting sign was hypothermia (63%), hypo activity and mottling (54%), followed by feeding intolerance (43%). Mamta et al,[11] reported refusal to feed (77%), respiratory distress (44%), and hypothermia (47.5%), while Khatua et al.,[12] re- ported refusal to feed (92%), lethargy (74%), hypothermia (72%) and respiratory distress (24%) as common clinical presentation. In agreement with the study done by Muhammed et al, the most frequent recognized risk factor in the present study, was premature rupture of membrane (PROM).[13]

A study from Bangladesh showed that approximately one-third of all septicemia in neonates was attributable to premature rupture of membranes.[14] While a study from Thailand reported 27.9% of cases with EONI is due to PROM.[15] For such patients rapid diagnosis of early onset sepsis is needed, to avoid unnecessary stay in NICU, which in turn increase the economic and social burden in already poor settings. In agreement with the study done by Hornik[16] and Altunhan[17], CBC findings in this study was not informative, and didn't help in the diagnosis of sepsis. Also the British evidence update advisory group didn't recommend the use of CBC in the diagnosis of neonatal sepsis.[18] Camacho A,[19] stated that complete blood count is difficult to interpret in the aneonatal period because it varies significantly with day of life and gestational age, and they are poor indicator of sepsis.

In this study the predominate organism isolated from patients with proven sepsis was Klebsiella 14%, followed by E.coli 10%, this is in congruous with Zaidi et al [20] and Downie L,[21]who stated that Klebsiela is the most predominate organism in developing countries in both Hospital and community- acquired infection.

In contrast to other studies which stated that GBS is the most common pathogens of early onset sepsis in developed countries, but its burden in developing countries is less clear due to lack of studies using optimal diagnostic tools. [22] GBS present in 8% of the patients of this study, done in a developing country. This can be explained, as the most frequent risk factor detected in this

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study was premature rupture of membrane and maternal infection which in turn increase the possibility of neonatal infection with GBS.

MRSA was detected in 2% of the patients in this study, nasal swabs from the NICU staff and swabs from the incubators were taken to detect the source of infection. Two nurses from the staff were MRSA positive; they were isolated and received treatment for 10 days.
Zaidi et al [23]reported most pathogens isolated in the hospital setting before 72HR of life are similar to those isolated afterwards; it is likely that highly unclean delivery practices lead to infections with nosocomial agents very early in life.

Table 2 and 3 compare the results of CRP1 and PCT1 in the category of proven sepsis and not-proven sepsis. In both categories there was no significant difference between CRP1 and PCT1 in the ability to support the diagnosis of neonatal infection or refute it.
In the group with suspected sepsis there was no significant difference between PCT1 and CRP1 in distinguishing patients with neonatal sepsis.

Blommendahl J[24] stated that PCT was not a better marker than CRP levels because PCT is affected by perinatal factors within 48h of birth making its usefulness in diagnosing of early onset sepsis very limited. Other than infection, PCT levels increase in premature infants, hypoxia, RDS, and hemodynamic instability, decreasing its specificity in early onset sepsis. [25]In this study CRP was repeated over 24 hours, while PCT was repeated after 8 hours to increase their reliability, the same was done in the study of Blommendahl et al,[24]and the study done by Hengest 2003.[26] Analysis of table 4 revealed that both CRP2 and PCT2 tests were able to differentiate more between infected and not infected newborns but there was no-significant difference between the two tests (P value 0.061). For the group of newborns with not-proven sepsis as shown in table 5, there was significant difference between both tests (P value 0.004), PCT2 can distinguish more accurately between cases with possible sepsis and cases with no sepsis.

Compares predictive values for CRP2 and PCT2 Sensitivity= 93.5%, Specificity=54%, PPV= 66.3%, NPV= 87.5%. PCT2: Sensitivity=96.8%, Specificity=88.7%, PPV=91.1%, NPV=99%. Procalcitonin evaluation done by Chaurasiya et al,[27]demonstrated sensitivity of 96.25%, specificity of 85%, PPV of 96.25% and NPV of 85%. Claudio Chiesa et al [28]studied the reliability of PCT concentration in 28 infants with severe early onset sepsis. They observed that the sensitivity 92.6%, specificity 97.5%, PPV 94.3%, NPV 96.5% respectively.

It is clear from the previous data, that PCT didn't give additional information superior to that of CRP in diagnosing cases with suspected sepsis, in cases where blood culture results were not yet available. However, repeated PCT was more accurate than CRP in differentiating cases with sepsis. This means that the protocol of repeat test of PCT after 8 hours from the initial assessment could be of great benefit in diagnosing cases with early onset sepsis, thus reducing unnecessary stay in NICU, and over-use of antibiotics. WHO developed the ASSURED criteria for an ideal point of care test in resource-limited settings, taking its accuracy and reliability as the main features. PCT fulfills these criteria and the cost of assessment of PCT level twice for each patient is less than the cost of one day admission in NICU. Making use of accuracy of PCT2 in detecting cases with neonatal sepsis, could have saved about 32 % of total admission days. Comparing this to the cost of the test, the benefit of assessing PCT is clear.

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Conclusion

The expense of testing PCT twice is less than the expenses of one-day admission in NICU in developing countries. Application of such protocol could be of use in limiting period of stay in NICU.

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