Evaluation of blood culture and serum procalcitonin for diagnosis of septicaemia in paediatric patients

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Abstract

Background: Septicaemia is a major cause of mortality and morbidity in paediatric age group in our country. The present study was aimed to evaluate the usefulness of procalcitonin assay in critically ill children with suspected sepsis taking blood culture as gold standard of diagnosis. The initial presentation of septicaemia may be subtle and therefore, it is important not only to recognise the children with septicaemia but also to manage the patients early with proper antibiotics.

Materials and Methods: 192 patients from NICU (Neonatal Intensive Care Unit) and PICU (Paediatric Intensive Care Unit) with suspected sepsis were enrolled in the study. Blood culture and estimation of serum procalcitonin were done in all the patients. Different risk factors associated with septicaemia in paediatric and neonatal age group were also analysed using three different cut-off values for PCT (≥0.5 ng/ml, ≥ 2 ng/ml and ≥10 ng/ml). Sensitivity, specificity, PPV (Positive predictive value), NPV (Negative Predictive value) were determined for PCT ≥0.5 ng/ml, ≥ 2 ng/ml and ≥10 ng/ml considering blood culture as gold standard diagnosis of septicaemia. Bacteriological profile and antibiogram of septicaemia were also determined in our study.

Results: Out of 192 suspected cases, 90(47%) patient were blood culture positive. Out of 90 proven cases of septicaemia, 51(57%) cases were neonates. In our study sensitivity, specificity, PPV (Positive predictive value), NPV (Negative Predictive value) for ≥ 2ng/ml were 70%, 81%, 77%, 75% respectively. For cut off ≥10 ng/ml specificity increased to 91% and sensitivity decreased to 40%. Gram negative septicaemia was encountered in 69% of culture positive cases and the most common isolate was found to be *Escherichia coli.*

Conclusion: It is challenging to distinguish sepsis from non-infectious condition in critically ill children. Serum procalcitonin assay is one of the important biomarkers of sepsis. In our study we observed moderate sensitivity and specificity for procalcitonin as a marker for sepsis. Procalcitonin can help in avoiding unwarranted antibiotic usage. Blood culture with antimicrobiobial susceptibility testing still remains the gold standard diagnosis of septicaemia.

Keywords: Procalcitonin, Septicaemia, Blood culture

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Introduction

Sepsis and its complications due to infectious diseases remain the leading cause mortality worldwide.[1] Complications of sepsis are significant contributors to child death in India.[2,3]

Early detection of sepsis is crucial as delay in treatment may lead to increase in mortality and morbidity. Identification of sepsis in critically ill patients is still a challenge, particularly in children. Physical signs and symptoms, though useful identifying possible cases have limited specificity. Definitive diagnosis of sepsis is done by blood culture.[4] Increase in mortality of 7.6% was observed for every hour delay in administration of antimicrobials in case of septic shock.[5] The Surviving Sepsis Campaign's 2008 "International guidelines for the management of severe sepsis and septic shock" also recommend administration of antimicrobial therapy within 1 hour of recognition of severe sepsis or septic shock.[6]

Though the gold standard for a systemic bacterial infection (bacteraemia) is a positive blood culture, result is ready only 24-72 hrs after the sampling. Early detection of sepsis is important during this period. If we cannot rule out sepsis, unwarranted use of antimicrobial therapy may lead to increased resistance to antibiotic, increased duration of hospital stay and cost of treatment. Furthermore, there are concerns about possible blood culture–negative clinical sepsis, particularly in the setting of increased prophylactic and empirical antibiotic use.[7] Therefore, using fast diagnostic methods including specific laboratory markers could be beneficial for early the diagnosis of neonatal sepsis.[8]

There are several markers of sepsis, like C-reactive protein, serum procalcitonin (PCT), IL- 6, IL-8, lactate, etc., of which PCT has been found to be the most effective.[7- 12]

Due to its ability to help differentiate between viral and bacterial infections, PCT has been evaluated for its ability to guide decisions for appropriate antibiotic therapy. India has one of the highest rates of infectious

diseases and has alarmingly high rates of antibiotic resistant bacteria. Marker procalcitonin would help for the correct indication of antibiotic therapy in such cases and indirectly prevent development of drug resistance by reducing unnecessary use of antibiotics.[13,14]

The present study was aimed to assess the usefulness of serum PCT as a marker of sepsis in paediatric age group of 0 to 18 year using the semi-quantitative, rapid immunochromatographic kit in a tertiary care centre. This cross sectional study was carried out to assess the risk factors associated and etiological agents for paediatric septicaemia and their antibiotic susceptibility pattern.

Materials and Methods

The study included 192 patients from the NICU (Neonatal Intensive Care Unit) and PICU (paediatric Intensive Care Unit) with suspected sepsis over a period of 6 months in Gauhati Medical College and Hospital. Sepsis was confirmed clinically and by positive blood culture (BacT/Alert system from Biomerieux). Laboratory and clinical findings helped to identify patients as having "sepsis syndrome" (sepsis, severe sepsis, septic shock) or no sepsis based on the ACCP (American College of Chest Physicians) recommendations.[15]

An episode of bacteraemia or sepsis was defined as the recovery of any significant, pathogenic bacterial species in 1 or 2 sets of blood cultures (aerobic and anaerobic bottles) obtained in the Emergency Department. Organisms commonly considered as blood culture contaminants (e.g., Coagulase-negative staphylococci, aerobic and anaerobic diphtheroids, *Micrococcus* spp., *Bacillus* spp.) and were excluded from this definition.[16]

This study was conducted in Department of Microbiology in collaboration with the Department of paediatrics. 90 proven septicaemia cases out of 192 were evaluated for risk factors, signs, symptoms. Blood culture and serum procalcitonin level were done for all 192 cases. Neonatal sepsis is a systemic infection occurring in infants at 28 days of life and is an important cause of morbidity and mortality of newborns. Early-onset neonatal sepsis (EOS) has been variably defined based on the age at onset, with bacteraemia or bacterial meningitis occurring at 72 hours in infants hospitalized in the neonatal intensive care unit (NICU), versus 7 days in term infants. In preterm infants, EOS is most consistently defined as occurring in the first 3 days of life and is caused by bacterial pathogens transmitted vertically from mother to infant before or during delivery. Late-onset sepsis (LOS) is sepsis occurring after 72 h in NICU infants and 7 days of life in term infants, has been variably defined as occurring up to the age of 90 or 120 days, and may be caused by vertically or horizontally acquired pathogens.[17]

Antimicrobial susceptibility testing (AST) was done according to CLSI guideline (2011). *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 were used as standard strains. Screening for MRSA (Methicillin resistance Staphylococcus aureus) was done using cefoxitin (30µg) disc. Resistance to cefotaxime (30µg) disc was used as screening method for detection of ESBL (Extended spectrum beta lactamase producer) confirmed by double disc synergy test.[18]

Serum PCT was detected semi-quantitatively by rapid immunochromatographic technique using a commercially available test kit (PCT-Q, BRAHMS Diagnostica GmbH, Berlin, Germany). The result was read and interpreted as per the manufacturer's recommendations:

i. PCT > 10 ng/ml: Severe bacterial sepsis or septic shock
ii. PCT 2 to 10 ng/ml: Severe systemic inflammatory response, most likely due to sepsis unless other causes are known
iii. PCT 0.5 to 2 ng/ml: A systemic infection cannot be excluded
iv. PCT < 0.5 ng/ml: Local bacterial infection possible; sepsis unlikely

The clinical condition, signs and symptoms of sepsis, antibiotics used, blood culture and final outcome of patients were recorded for all patients.

Statistical analysis

Sensitivity, specificity, PPV(Positive predictive value), NPV(Negative predictive value) of the PCT assay were analyzed using three different cut-off values for PCT (≥0.5 ng/ml, ≥ 2 ng/ml, ≥10 ng/ml) taking blood culture as gold standard. Statistical analysis was carried out by SPSS version 16.0(IBM Corp, Armonk NY). A *P* value less than 0.05 was considered statistically significant.

Results

Of a total of 192 children including neonates were investigated for serum procalcitonin level and blood culture. Ninety (47%) patients were found to be positive for proven septicaemia.

Table 1: Age distribution of 90 proven cases septicaemia

Age group	Number of cases	Percentage
0-28 days	51	57%
>28 days - 1year	9	3%
> 1 year -18 year	30	10%

Out of 90 proven cases of septicaemia, 51(57%) cases were neonates [Table 1]. Again among these cases, 54(60%) were males and 36(40%) were females.

Table 2: Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of PCT level ≥0.5 ng/ml, ≥ 2 ng/ml, ≥ 10ng/ml

PCT value	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
≥0.5 ng/ml	87%	52%	61%	81%
≥ 2 ng/ml	70%	81%	77%	75%
≥10ng/ml	40%	91%	80%	53%

Table 3: Neonatal and maternal risk factors associated with neonates with proven septicaemia

	Risk factors	No of cases	Percentage
Neonatal	LBW (Low birth weight)	51	100%
	Prematurity	24	47%
	Perinatal asphyxia	24	47%
	Invasive procedure	42	82%
Maternal	Febrile illness of mother within 2 weeks prior to delivery	18	35%
	Foul smelling /meconium stained liquor	18	35%
	Prolonged rupture membrane >24 hours	6	11%

LBW was found to be the most important predisposing factor for neonatal septicaemia. 100% babies with septicaemia in our study had LBW. Febrile illness of mother (35%) and meconium stained liquor (35%) were also two important risk factors involved in case of neonatal septicaemia [Table 3]. Twenty (39%) neonates had early onset septicaemia (EOS) and 31(61%) had late onset septicaemia (LOS).

Table 4: Species distribution of blood cultures isolates

Gram negative(n)=62	No of isolates
Escherichia coli	23
Klebsiella spp.	22
Pseudomonas aeruginosa	10
Acinetobacter spp.	7

Gram positive (n)=28	No of isolates
Staphylococcus aureus	18
Enterococcus spp.	6
Group B Streptococci	4

In our study most common organism isolated was *Escherichia coli* followed by *Klebsiella spp.* [Table 4]. *Escherichia coli* and *Klebsiella spp.* showed maximum resistance to ampicillin (91%) and out of 45 Enterobacteriaceae 15 strains were ESBL (Extended spectrum beta lactamase producer). *Escherichia coli* and *Klebsiella spp.* showed 70% and 64% sensitivity to ciprofloxacin respectively. Again *Pseudomonas spp*. showed 60% sensitivity to ciprofloxacin. Out of 8 *Pseudomonas* strains 2(25%) strains were resistant to imipenem and meropenem. *Escherichia coli, Klebsiella spp.* and *Pseudomonas spp*. showed 87%, 68% and 60% sensitivity to amikacin respectively. Most of the gram negative isolates were sensitive to imipenem and meropenem. All 18 *Staphylococcus* strains were resistant to ampicillin. Six out of 18 *Staphylococcus* strains were MRSA (Methicillin Resistant Staphylococcus aureus). Gram positive strains showed 100% sensitivity to vancomycin and linezolid. In our study mortality rate in neonatal group was found to be more (7.7%) than non-neonatal group (3.3%).

Discussion

Septicaemia remains a significant cause of mortality and morbidity in children including neonates. Clinical diagnosis of septicaemia from other non-infectious causes of systemic inflammation is often difficult as it presents with non-specific signs and symptoms. In the present study procalcitonin has been used as early marker for sepsis using blood culture as gold standard for diagnosis of sepsis. This study also evaluated the bacterial isolates in case of sepsis in paediatric age group including neonates. There are very few studies on serum PCT and sepsis from India. To the best of our knowledge this is the first study from North East India on serum procalcitonin as a marker of septicaemia in paediatric population. In some studies >2ng/ml has been used as diagnostic threshold for infection and sepsis.[19,20] Semi-quantitative PCT assay is a rapid immunochromatographic assay and it is easy to perform. The method shows good sensitivity and specificity in diagnosing bacterial sepsis at PCT levels of ≥2 ng/ml in several studies.[21,22]

A study by Sinha *et al.* revealed sensitivity (86%) and high specificity (95%) at a cut-off ≥ 2 ng/ ml and using a cut-off of above 0.5 ng/ ml revealed higher sensitivity but with a reduction in the specificity to 84%.[23] Hence serum PCT of 2ng/ml or more using the rapid immunochromatographic method is an effective marker of sepsis and may help in aggressive management of such patients along the lines of sepsis. A retrospective study among children demonstrated PCT value of ≥1 ng/mL predicted having serious bacterial infection with PPV 28%, NPV 93%, sensitivity 70%, and specificity 68%[24]. Bossink *et al.* reported 90% NPV using a 0.5ng/ml cut off value for procalcitonin among the hospitalised febrile patients.[25]

Considering PCT levels ≥2 ng/ml the sensitivity, specificity, PPV and NPV of current study were found to be 70%, 81%, 77% and 75% respectively. In contrast to other studies our study showed lower sensitivity and specificity. For cut off ≥10 ng/ml specificity increased to 91% and sensitivity decreased to 40%.

In a prospective cohort study, PCT level of > 1.63 ng/mL had 85% sensitivity and 83% specificity for determining the presence of sepsis in paediatric ICU patients.[26]

In our study out of 90 proven cases of septicaemia, 63(70%) had procalcitonin level ≥2ng/ml and 36(40%) had ≥10ng /ml. Among 98 suspected patients of neonatal septicaemia, 51 had proven septicaemia (52%). Similarly Ahmed *et al.* also reported 30 culture positive cases out of 86 neonates of suspected sepsis (34.8%).[27]

Febrile illness and foul smelling or meconium stained liquor of mother were two important risk factors in development of neonatal septicaemia. In the current study 18% neonates had mother with febrile illness within 2 weeks prior to delivery. In another study, 18.1% neonates had their mothers near term with pyrexia, of which 21.9% had EOS (Early onset sepsis) and 12.9% had LOS (Late onset sepsis).[28]

In our study, 69% of septicaemia was caused due to gram negative bacteria followed by 31% by gram positive bacteria. Fifteen out of 45 Enterobacteriaceae strains were ESBL (Extended spectrum beta lactamase producers). Most of the gram negative isolates were sensitive to imipenem and meropenem. In another study 40% *Pseudomonas spp*. were resistant to imipenem and meropenem.[29] Among the 6 *Staphylococcus aureus*, 2(33.33%) were detected as Methicillin resistant *Staphylococcus aureus* (MRSA).[28]

All gram positive bacteria were sensitive to vancomycin and linezolid. Studies by Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group reported prevalence of MRSA is 41%.[30]

The mortality rate among neonatal group in the current study was 7.7% which is much less than other study (46.6%).[31]

In our study we observed 70% sensitivity and 81% specificity for PCT value ≥ 2 ng/ml as a marker of sepsis. Blood culture is still the gold standard for diagnosis of septicaemia in paediatric patients. Procalcitonin can guide the clinician regarding use of empirical antibiotic in a suspected case of septicaemia as delay in start of treatment may contribute to increased morbidity and mortality.

Conclusion

There is modest variation in reported diagnostic accuracy of PCT in various studies. In our study also we observed moderate sensitivity and specificity for procalcitonin as a marker of sepsis. Blood culture remains the gold standard for diagnosis of septicaemia. A positive blood culture with antibiotic susceptibility tests are the best guide to the clinician in choosing appropriate antimicrobial therapy. For taking decision on start of empirical antibiotic treatment we can use procalcitonin as marker of septicaemia. Procalcitonin as a marker of septicaemia clinically needs further evaluation.

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