Int.J.Curr.Microbiol.App.Sci (2018) *7*(4): 2747-2755

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 04 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.704.313

Evaluation of Procalcitonin, CRP and Blood Culture in the Diagnosis of Neonatal Sepsis

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Keywords

Blood culture, Neonatal sepsis, C-reactive protein, Procalcitonin

Article Info

Accepted:
23 March 2018
Available Online:
10 April 2018

A B S T R A C T

Sepsis is a major cause of morbidity and mortality among neonates. Neonatal sepsis may be categorized as early-onset or late-onset. Clinical manifestations of neonatal sepsis are non-specific, therefore, clinical diagnosis of sepsis is difficult and laboratory help is required. Present study was done to evaluate three different methods in diagnosis of neonatal sepsis. 3 ml of venous blood was collected employing strict aseptic precautions from all babies admitted to the NICU in our hospital during the study period. 2 ml of blood was aseptically added to BacT/ ALERT PF culture bottle. Bacteria grown from these bottles were identified by using the standard protocol. The antibiotic susceptibility for the isolates was tested by Kirby Bauer method following CLSI guidelines. Remaining blood sample was used for PCT estimation by immunochromatography assay and CRP estimation by chemiluminescence method. 50 blood samples were collected from neonates admitted to the NICU. The most common risk factor of suspected septicaemia in our study was birth asphyxia (32%), followed by preterm birth (28%). Blood culture was positive in 12 (24%) cases. The most common etiological agent was *Staphylococcus aureus*. PCT was positive in all blood culture positive (proven sepsis) cases. Out of 50 samples tested for CRP, 33(66%) were positive. A wide range of CRP values were obtained, ranging from 1.83 mg/dL to 120 mg/dL. Out of the 34 PCT positive cases, 33 (97.05%) were also CRP positive. Blood culture even though considered as gold standard it is time consuming and at times gives false negative results due to administration of antibiotics. PCT and CRP are reliable markers which aid in diagnosis of neonatal sepsis, which have the same diagnostic accuracy. CRP, when compared to PCT is affordable and can be conveniently used as a marker for the diagnosis of neonatal sepsis, especially in developing countries with poor resources

Introduction

Neonatal sepsis is invasive infection, usually bacterial, occurring during the neonatal period**.** Sepsis is a major cause of morbidity and mortality among neonates, especially in the developing countries (Shah *et al.*, 2006). An overall incidence of the culture proven sepsis of 1-8 cases per 1000 live births from the developed countries and 4.8-20.7 per 1000

live births from India has been reported (Ramesh Bhat and Lincy, 2011).

Neonatal sepsis may be categorized as early-onset or late-onset. Early onset occurs within first 5-7 days postnatally and is due to infection occurring through transplacental transmission within the uterus or an ascending infection from the cervix of the mother by organisms that colonize the genito-urinary tract. The neonate acquires the microorganisms as it passes through the colonized birth canal at delivery. Of newborns with early-onset sepsis, 85% present within 24 hours, 5% present at 24-48 hours, and a smaller percentage present within 48-72 hours. Onset is most rapid in premature neonates.

The microorganisms most commonly associated with early-onset infection include Group B *Streptococcus, Escherichia coli*, Coagulase-negative *Staphylococcus, Haemophilus influenza* and *Listeria monocytogenes* (Klinger *et al.,* 2009). The case fatality rate in early onset neonatal sepsis ranges from 16.7% to 19.4% (Baltimore *et al.,* 2001). Late onset sepsis develops 10–30 days after birth and the source of infection is the care-giving environment. Organisms that have been implicated in causing late-onset sepsis include Coagulase-negative staphylococcus, *Staphylococcus aureus, E. coli, Klebsiella, Pseudomonas, Enterobacter, Candida,* Group B *Streptococcus, Serratia, Acinetobacter* and anaerobic bacteria (Van den Hoogen *et al.,* 2010).

Most common predisposing and precipitating factors are premature rupture of membrane (PROM), prolonged second stage of labour or other maternal causes such as maternal fever within 2 weeks prior to delivery, meconium stained amniotic fluid (MSAF), foul smelling liquor and instrumental delivery. The foetal factors include low birth weight, prematurity and Apgar score (Shah *et al.,* 2006).

Clinical manifestations of neonatal sepsis are non-specific and include lethargy, poor feeding, temperature instabilities, respiratory distress, apnoea and shock (Lisa Ross De Camp *et al.,* 2009). However, these clinical signs also indicate the presence of many non-septic conditions like hypoglycaemia, hypothermia, electrolyte imbalance, inborn errors of metabolism, etc. Also, some neonates, especially premature ones may have very subtle features or even no features. Therefore, clinical diagnosis of sepsis is difficult and laboratory help is required.

In the laboratory, blood culture, owing to its specificity is considered the gold standard for detection of sepsis. However, it is very time consuming and there is always a possibility of false positivity, due to sample contamination or false negativity, when the neonate has already been exposed to antibiotic agents during labour. Also, studies on sepsis have underlined the concern about possible culture negative clinical sepsis, particularly in the setting of increasing maternal antibiotic use (Stoll *et al.,* 1996).

Recent studies have showed promising results with estimation of C-Reactive Protein and Procalcitonin. C-reactive protein (CRP) is an acute-phase reactant that is synthesized by the liver within six hours after the onset of inflammation and tissue necrosis. Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells and some types of bacteria in order to activate the complement system via the C1Q complex (Thompson *et al.,* 1999). Its rapid synthesis, short half-life and rapid decline with recovery, together with an association between increased blood values and serious bacterial infections, have made the CRP test popular. This test is often requested to help discriminate viral infections from bacterial infections or monitor the response to antibiotics (Joan M. Hengst, 2012).

It has been recently reported that procalcitonin (PCT), the prohormone of calcitonin, increases markedly in septic conditions and it appears to be a good predictor of infection severity. In this case, Procalcitonin is secreted mainly by the neuroendocrine cells of the lungs and intestine in response to bacterial endotoxin or inflammatory cytokines (TNF, IL-6) (Maruna *et al.,* 2000).

It does not rise significantly with viral or non-infectious inflammations, hence it is highly specific to bacterial sepsis. Furthermore, PCT is released into the circulation within 3 hours of onset of infection, plateaus at 6 hours, and remains elevated for 24 hours. This makes PCT a promising new agent for early and sensitive identification of infected neonates (José B López Sastre *et al.,* 2007).

In view of the above diagnostic merits of CRP and PCT, this study aims at investigating the feasibility of adapting these biomarkers into routine tests for neonatal sepsis along with the standard blood culture in the hospital settings. Lastly, an antibiotic sensitivity pattern needs to be drawn, to avoid re-emergence of infection and development of drug resistant strains.

Materials and Methods

3 ml of venous blood was collected employing strict aseptic precautions from all babies admitted to the NICU in our hospital during the study period. 2 ml of blood was aseptically added to BacT/ ALERT PF culture bottle. The bottle was loaded into the BacT/ ALERT Microbial Detection System after entering the relevant patient data. Blood culture bottle flagging indicates bacterial growth.

Sub cultures were made from such bottles on to MacConkey and Blood agar plates. Inoculated plates were incubated at 37 degrees for 24 hrs. Colony formed on these culture media were identified by using the standard protocol. The antibiotic susceptibility for the isolates was tested by Kirby Bauer method and CLSI guidelines were followed during testing and interpretation of susceptibility or resistance to the antibiotics.

Remaining blood sample was added to plain vacutainer (BD) and allowed to clot. The serum separated was used for PCT estimation by immunochromatography assay and CRP estimation by chemiluminescence method. Manufacturer's instructions were followed during testing and interpretation of the two serological acute phase markers.

Results and Discussion

In our study 50 blood samples were collected from neonates admitted to the NICU. Out of these, 15 (30%) were preterm and the remaining 35 (70%) were term babies. All the subjects in our study were evaluated for early onset sepsis that is, sepsis occurring before 7 days after delivery. Graph 1 depicts the number of cases evaluated on day 1 to day 7 after birth of the baby:

Various risk factors that were present in our study population are shown in table 1.

Most common risk factor of suspected septicaemia in our study was birth asphyxia (32%), followed by preterm birth (28%) and Meconium Stained Amniotic Fluid (28%). 3 deaths were reported during the study period in the study group.

Table 2 shows various concentrations of PCT in the 50 samples that were tested.

16 (32%) sample from clinically suspected sepsis cases, PCT was negative and from all these cases blood culture also yielded no growth. PCT was positive in all blood culture positive (proven sepsis) cases.

Table.1 Various risk factors in study population

Sl. No.	Risk Factors	Number of subjects	Percentage
1.	Meconium Stained Amniotic Fluid alone	10	20%
2.	Birth Asphyxia alone	11	22%
3.	Preterm birth alone	9	18%
4.	PROM alone	2	4%
5.	Obstructed labor alone	1	2%
6.	Hyperbilirubinaemia alone	3	6%
7.	Congenital anomalies alone	2	4%
8.	Fever	1	2%
9.	Preterm birth with obstructed labor	1	2%
10.	Neonatal depression with MSAF	1	2%
11.	Preterm birth with neonatal depression	2	4%
12.	Neonatal depression with birth asphyxia	2	4%
13.	Preterm birth with MSAF	2	4%
14.	Birth asphyxia with PROM	1	2%
15.	Birth asphyxia with MSAF	2	4%

Table.2 PCT concentration of serum samples

Sl. No.	PCT Concentration (µg/L)	Number of subjects	Percentage
1.	0	16	32%
2.	0-0.5	03	6%
3.	≥0.5 - <2	13	26%
4.	≥2 - <10	05	10%
5.	≥10	13	26%

Table.3 CRP concentration of serum samples

Sl. No.	1	2	3	4	5	6	7	8	9	10	11	12
CRP (mg/dL)	16.15	41.8	37.0	31.2	48	91.45	62.36	24.7	120	62.3	62.36	41.8

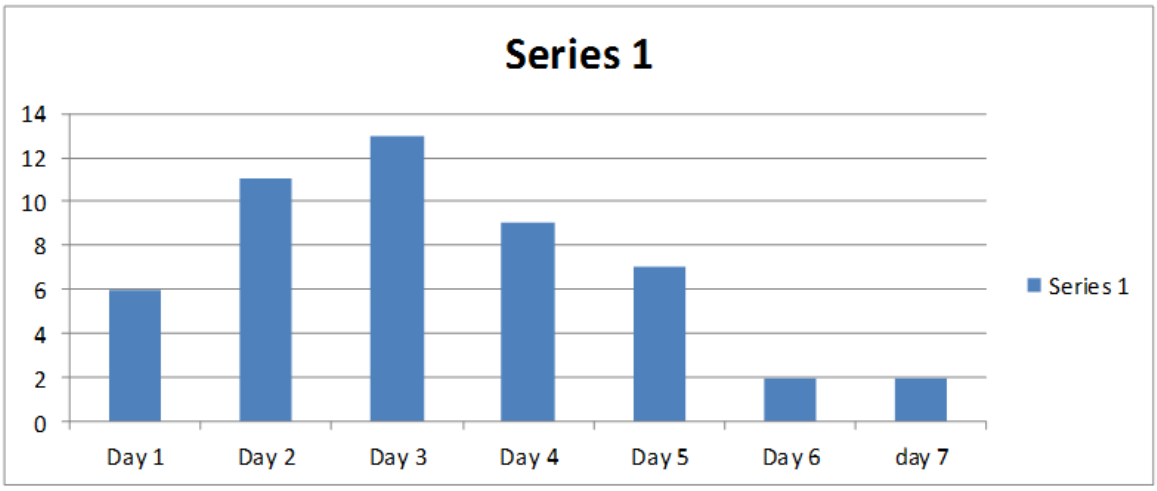
Table.4 For Gram positive organism's sensitivity pattern

Case No.	Causative agent	Penicillin	Gentamicin	Rifampicin	Ciprofloxacin	Linazolid	Cefoxitin
1	*S. aureus*	R	S	R	S	S	R
2	*S. aureus*	R	R	R	S	S	S
3	*S. aureus*	S	S	S	S	S	S
4	*S. aureus*	R	S	S	S	S	R

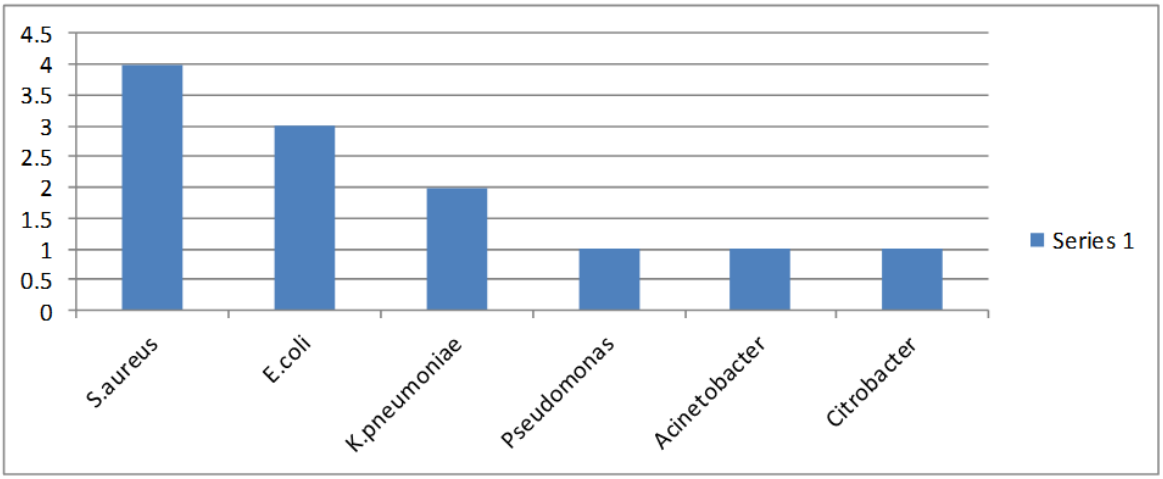
Table.5 For Gram negative organism's sensitivity pattern

Case No.	Causative agent	Antibiotic Sensitivity Pattern Ampicillin	Amikacin	Gentamicin	Imipenim	Ciprofloxicin	Cotrimoxazole	Cefotaxime
1	*E. coli*	R	S	S	S	S	R	R
2	*E. coli*	R	S	S	S	R	R	R
3	*E. coli*	R	S	S	S	S	R	R
4	*K.pneumoniae*	R	S	S	S	S	S	S
5	*K.pneumoniae*	R	R	R	S	S	S	S
6	*Pseudomonas*	S	S	R	S	R	R	R
7	*Acinetobacter*	R	R	S	S	S	S	S
8	*Citrobacter*	R	S	S	S	S	S	S

Graph.1 Showing day wise evaluated of cases



Graph.2 Showing different types of bacteria isolated from blood culture



Out of 50 samples tested for CRP, 33(66%) were positive. A wide range of CRP values were obtained, ranging from 1.83 mg/dL to 120 mg/dL. Out of the 34 PCT positive cases, 33 (97.05%) were also CRP positive.

CRP was positive with high values in all the proven sepsis cases. However, 21(63.63%) cases which were blood culture negative gave a positive CRP. Table 3 shows the different CRP concentrations in the blood culture positive cases.

Blood culture was positive in 12 (24%) cases. Graph 2 shows the various causative agents that were detected by culture in our study

The most common etiological agent was Staphylococcus aureus.

Table 4 and 5 shows the antibiotic sensitivity pattern of Gram positive and Gram negative bacteria isolated. Out of 4 *S. aureus* isolates 2 were MRSA. Among *E. coli* all the 3 isolates were resistant to Cefotaxime and sensitive to Imipenum.

Neonatal sepsis remains a diagnostic and treatment challenge for modern neonatal care providers, especially in developing countries where highest incidence and the highest mortality rates are reported. Infants presenting with clinical signs of infection are evaluated with a variety of diagnostic tests and treatment with broad-spectrum antibiotics initiated until a definitive diagnosis can be made. Isolation of microorganism(s) from one or more blood cultures is the gold standard to establish a definitive diagnosis of neonatal sepsis (Pourcyrous *et al.,* 1993).

The sole use of blood culture to diagnose neonatal infection has a number of limitations. It may take 24 to 72 hours to obtain culture results (Garcia-Prats *et al.,* 2000). The sensitivity of blood cultures may be impaired by exposure to intrapartum antibiotics, which are administered to 15% to 40% of mothers in labor (Bromberger *et al.,* 2000). Intrapartum antibiotic exposure can result in a partially treated infant, delaying the onset of clinical signs and symptoms of infection and further complicating the expedient definitive diagnosis of early-onset sepsis in the infant. With the development of multiple drug-resistant bacteria and the cost of therapy with multiple antibiotics, the ability to diagnose or rule out sepsis is an essential tool to limit inappropriate antibiotic exposure (Philip and Mills, 2001).

Variety of laboratory tests, other than blood culture has been developed to enhance the early and accurate identification and treatment of infants with suspected sepsis. In present study blood culture and two biomarkers CRP and PCT were evaluated.

Out of the 50 clinically suspected cases of neonatal sepsis, 12 were culture positive which accounts to 24% of cases in our study. This is comparable to a study conducted by (Kapoor *et al.,* 2005) and (Dechen C. Tsering *et al.,* 2011) who reported 20% and 22% respectively of culture proven cases of neonatal sepsis. A study conducted by (Kurien Ani Kuruvi *et al.,* 1998)' in Vellore reported a mortality rate of 16.7% and 13.6% in early and late onset sepsis respectively, unlike in our study where there were only 3(6%) deaths related to neonatal sepsis. Adequate anticipation, early diagnosis and treatment with appropriate antibiotics have contributed to this low level of mortality.

In gram negative bacteria, *E. coli* was the most common isolate (25%) followed by *Klebsiella* (16.66%), *Psuedomonas* (8.33%), *Acinetobacter* (8.33%) and *Citrobacter* (8.33%). *Staphylococcus aureus* (33.33%) was responsible for sepsis among gram positive organisms. This was comparable to

the study conducted by (Dechen C. Tsering *et al.,* 2011), however in their study Enterobactor species was leading cause of sepsis among gram negative bacteria. The antimicrobial sensitivity pattern differs in different studies as well as at different times in the same hospital in India and in overseas studies (Dechen C. Tsering *et al.,* 2011) which results from indiscriminate use of antibiotics. In present study all the *S. aureus* isolates were sensitive to Ciprofloxacin and Linazolid and only 25% were sensitive to Penicillin. This high rate of resistance to Penicillin was also seen in a study conducted in Lucknow (Roy *et al.,* 2002) All gram negative bacteria were sensitive to Imipenum, 75% to Amikacin and Ciproflaxacin.

Among the various risk factors that predispose the neonates to sepsis, the most important risk factor in our study was birth asphyxia (32%), followed by preterm birth (28%) and meconium stained amniotic fluid (28%) which is comparable to a study in Sikkim (Dechen C. Tsering *et al.,* 2011) while (Kurien Ani Kuruvi *et al.,* 1998) reported meconium stained liquor as the most common risk factor.

PCT was positive in 34 (68%) out of 50 cases. PCT showed a false positive rate of 64.7%, that is PCT was positive in spite of blood culture being negative, but the values of PCT in these cases ranged between 0.5-<10 µg/L. 6 cases showed significant levels in spite of being culture negative (significant levels being taken as >2-≥10µg/L. 66% (33) cases were positive for CRP in our study. 55.26% (21) cases which were blood culture negative were positive for CRP. Only one case which was blood culture negative with CRP negative gave a false positive PCT, however the PCT level was between 0.5-2µg/L.

False positive CRP results were seen in 55.26% of cases and false positive PCT test was seen in 64.7% of cases. This false positive raise in PCT levels may be attributed to the physiological peak among uninfected neonates (José B López Sastre *et al.,* 2007). In the present study we have not found any advantage of PCT over CRP which is comparable with the conclusions of many authors (Biommendahl *et al.,* 2002; Naglaa F. Boraey *et al.,* 2012). We would like to conclude that blood culture even though considered as gold standard it is time consuming and at times gives false negative results due to administration of antibiotics. PCT and CRP are reliable markers which aid in diagnosis of neonatal sepsis, which have the same diagnostic accuracy. CRP, when compared to PCT is affordable and can be conveniently used as a marker for the diagnosis of neonatal sepsis, especially in developing countries with poor resources.

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How to cite this article:

Rashmi Padmanabha Mahale, Morubagal Raghavendra Rao and Sowmya Govindanahalli Shivappa. 2018. Evaluation of Procalcitonin, CRP and Blood Culture in the Diagnosis of Neonatal Sepsis. *Int.J.Curr.Microbiol.App.Sci.* 7(04): 2747-2755.
doi: https://doi.org/10.20546/ijcmas.2018.704.313