Research Article

Evaluation of Septic Screen as a Diagnostic Tool for Neonatal Sepsis in a Tertiary Hospital at Mysore

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Abstract: This is a hospital based cross sectional study to evaluate the validity of septic screen in detecting neonatal sepsis conducted over Nov 2012 to Nov 2013. 60 newborns admitted with suspected sepsis on the basis of clinical presentation were enrolled for the study. All the babies underwent septic screen (TLC, ANC, CRP, I/T Ratio, Platelet count and micro ESR) and Blood culture. Septic screen was considered positive if any 2 parameters were abnormal. Using blood culture as gold standard in diagnosing neonatal sepsis, the sensitivity, specificity, PPV and NPV for septic screen were calculated. The study population consisted of more male (40), preterm (41) and LBW babies (42). Nearly 70% of the cases were outborn and only 30% were inborn babies. 54 (90%) were early-onset, only six (10%) were late-onset sepsis. Out of 60 cases, 44 (73.3%) were septic screen positive i.e., two or more parameters were abnormal and 16 (26.7%) were negative. A total of 48 (80%) cases were blood culture proven sepsis and 12 (20%) were negative. 37 (61.2%) cases had positive blood culture and septic screen, only five (8.2%) cases were negative for both. Out of 48 culture positive cases, 11(18.2%) were negative on septic screen and seven (10.2%) septic screen positive cases didn't grow anything in blood culture. A positive septic screen had sensitivity of 77%, specificity of 41%, PPV of 84% and NPV of 31% when blood culture is considered as gold standard to detect neonatal sepsis.

Keywords: Neonatal sepsis, Septic screen, micro ESR, CRP, Blood culture.

INTRODUCTION

Sepsis is the commonest cause of neonatal mortality; responsible for about 30-50% of the total neonatal deaths in developing countries [1, 2]. It has been reported that approximately 1% neonates die of sepsis related causes and it has been estimated that up to 20% of neonates develop sepsis [2]. The mortality due to sepsis can be prevented with early diagnosis, rational antimicrobial therapy and aggressive supportive care [3].

Neonatal sepsis (NNS) is a clinical syndrome characterized by signs and symptoms of infection in the first month of life with or without accompanying bacteremia [3]. National Neonatal Perinatal Database (NNPD, 2002-03) reported neonatal sepsis in 30 per 1000 live births and reported as the commonest causes of neonatal mortality that contributes to 19% of all neonatal deaths [4]. In spite of adequate treatment with modern antibiotics has been a challenge because of its high incidence and its bad prognosis [5]. Optimal diagnosis and treatment strategies are difficult to define. The signs and symptoms are protean with high mortality and thus there is a urgent need to know whether the baby has sepsis in order to initiate treatment as quickly as possible, but confirmation of diagnosis by definitive blood culture is not possible rapidly.

Early diagnosis of NNS has remained a frustrating experience even in the developed countries. Due to the subtle and non-specific signs and symptoms, prompt and correct diagnosis of NNS is difficult. The blood culture is of gold standard for diagnosis but, it is costly and delay of at least 48 hours before preliminary results are received. The yield of blood culture is between 30%-70%. Therefore, some neonates with sepsis may go undetected. Additionally, inability to adequately exclude the diagnosis of neonatal sepsis early results in prolonged and unnecessary exposure to antibiotics [6].

Newer inflammatory markers such as interleukin-6, interleukin-8, and plasma elastase are highly sensitive and specific to diagnose neonatal sepsis and septic shock, but they require sophisticated and

expensive kits [7]. Therefore, impractical for routine clinical work-up in community health delivery systems, particularly in developing countries like India. A simple, quick, inexpensive laboratory test which may assist the diagnosis of sepsis (or its exclusion) would ensure early treatment and prevent unnecessary antibiotic therapy and hence this study was planned.

METHODOLOGY
Study Design: Cross sectional study.

Source of Data: Neonates admitted to our NICU with clinical suspicion of sepsis during Nov 2012 to Nov 2013.

Sample Size: All the babies satisfying the inclusion criteria and admitted during the study period were included in the study. This came up to 60 newborns.

Inclusion Criteria: Neonates (<30 days) admitted to our NICU with clinical suspicion of sepsis.

Exclusion Criteria:
- Neonates who received antibiotics before admission.
- Neonates who died before work up were complete.
- Neonates who underwent surgery.

Data Collection:
Institutional Ethical committee clearance was taken prior to the study and Parental written consent was taken before enrolling newborn to the study.

All the babies underwent sepsis screen and blood culture. Blood samples were obtained under strict aseptic precautions from peripheral venepuncture in all neonates within 24 h of admission, before initiation of antibiotic therapy.

Sepsis screen included following tests: Total Leucocyte Count (TLC), absolute Neutrophil Count (ANC), Platelet Count (PC), Immature: Total Neutrophil ratio (I: T ratio), Micro Erythrocyte Sedimentation Rate (mESR), C-reactive protein (CRP) tests.

Cell counts were obtained from an EDTA anticoagulated sample using Coulter cell counter. CRP was estimated using Latex agglutination slide test. Micro -ESR was estimated with capillary blood obtained by heel prick, collected in a standard 75 mm heparinised micro-hematocrit tube with internal diameter of 1.1 mm. Air was not allowed to interrupt the column of blood to avoid false normal result and one end of the tube was sealed with 2-3 mm of soap. The capillary tubes were placed on vertical lines drawn on a wall of each ward using 45 degree set square (rule). The distance from the highest point of the plasma column to the meniscus of the packed red cell column

(height of the plasma column) of each tube was measured with a rule. Mini-ESR was considered to be elevated if the height of plasma column is more than 15mm.

Sepsis screen was considered positive if any 2 of the following were present [8-12]:
- Total Leucocyte Count (TLC) of <5000/cu mm or >20000/cumm
- Absolute Neutrophil Count of < 1800/cumm
- I/T ratio of > 0.2,
- Micro ESR >15mm in 1st hour
- Platelet Count of < 150000/cumm
- CRP value of >1 mg/L.

The cut-off value taken for CRP was 1 mg/dl, as recommended by the manufacturers was considered as marker of infection.

Blood culture was performed under strict sterile precautions. A single blood sample (2 ml) was inoculated into the culture bottle. The BacT alert microbial detection system was used for blood culture.

Apart from the above tests, babies underwent other relevant tests like chest X ray, Urine analysis, urine culture, lumbar puncture and CSF analysis depending on the clinical presentation. All the babies with positive blood culture underwent CSF analysis.

After initiating antibiotics, babies were monitored for the response. Depending on the blood culture report and clinical response, therapy was modified. In case of positive blood culture, antibiotics were narrowed down to target specific organism depending on the sensitivity pattern. If blood culture was negative in a septic screen positive baby, decision on antibiotics was made depending on clinical condition. If there is a strong clinical suspicion, antibiotics were continued irrespective of septic screen or blood culture report.

Statistical analysis
All the study parameters were entered in the excel sheet and were analysed using epi-info software. Descriptive parameters were used for the univariate analysis. Sensitivity, specificity, NPV and PPV of septic screen was compared with culture outcome (gold standard) using a contingency table.

RESULTS
A total of 60 neonates admitted to our NICU on first come first serve basis formed the study group. All the babies underwent work up; none of the babies were excluded from the study. The study group consisted of more no of males (66.6%), preterms (68.4%), low birth weight (70%) and outborn babies (68.4%). As high as 90% of cases were early onset sepsis (table 1).

Table 1: Demographic and maternal data of the study group

Parameters	Values (%)
Sex	
Males	40 (66.6)
Females	20 (33.4)
Gestational age	
Term	19 (31.6)
Preterm	41 (68.4)
Birth Weight	
Low birth weight	42 (70)
Normal birth weight	18 (30)
Age at onset	
Earl onset sepsis	54 (90)
Late onset sepsis	6 (10)
Place of delivery	
Inborn	19 (31.6)
Outborn	41 (68.4)
Maternal data	
PROM > 24 h	15 (25)
Meconium stained liquor	5 (8.3)
Foul smelling liquor	5 (8.3)
Maternal fever	5 (8.3)

Out of 60 suspected sepsis cases, 44 (73.3%) were septic screen positive i.e., two or more parameters were abnormal and 16 (26.7%) were negative. A total of 48 (80%) cases were blood culture proven sepsis and 12 (20%) were negative. 37 (61.2%) cases had positive blood culture and septic screen, whereas only 5(8.2%) cases were negative for both. Out of 48 culture positive cases, 11(18.2%) were negative on septic screen and 7 (10.2%) septic screen positive cases didn't grow anything in blood culture. A positive septic screen had sensitivity of 77%, specificity of 41%, PPV of 84% and NPV of 31% when blood culture is considered as gold standard to detect neonatal sepsis.

Table 2: Relation between septic screen and blood culture

	Blood culture Positive	Blood culture Negative	Total
Septic screen Positive	37	7	44
Septic screen Negative	11	5	16
Total	48	12	60

p- 0.137, CI 95%

When considered individually, the sensitivity, specificity, PPV and NPV value of each of septic parameters varies widely. CRP individually had better sensitivity (81.2%), specificity (50%), PPV (86.6%) and NPV (40%) than a positive septic screen.

12 neonates (20%) were treated as clinical sepsis, in which 5(8%) neonates had both blood culture and septic screen negative.

Among 48 culture proven sepsis, 9 (18%) had meningitis. None of the CSF culture showed any growth. Blood culture positive rate in Early Onset Sepsis was 77.8% (42) and 100% in Late Onset Sepsis.

There was one death recorded among study population, which grew Staphylococcus.

DISCUSSION

Neonatal sepsis is a clinical syndrome. It is characterized by signs and symptoms of infection with or without bacteremia in the first month of life [13]. The diagnosis of neonatal infection is difficult to establish based on the clinical picture alone, yet it is imperative that treatment is instituted early because of the high mortality associated with neonatal infection.

Of the infected babies, 66.6% were boys and 70% were low birth weight. This was possibly due to impaired defense mechanisms and low immunoglobulin G levels in boys and low birth weight neonates [14-16]. More number of out born babies emphasizes the fact that a good hygienic care in peri partum period prevents neonatal sepsis.

Non-infectious disorders may produce haematological changes similar to those seen with infection, thereby compromising the specificity and PPV of the hematological screening tests. However, a combination of hemotological changes and/or a rise is CRP as septic screen, can be used to improve the diagnosis. Previously many authors have tried to find

out the credibility of septic screen with blood culture as gold standard to detect neonatal sepsis.

As no single individual haematological parameter is superior in comparison to another in predicting neonatal sepsis, a combination of these parameters in the form of septic screen has been recommended [17-19].

Total Leucocyte Count in response to sepsis, varies widely. Cut off values for normal range is not defined and offers little help in diagnosis. A TLC < 10 × 10^9/L or ≥20 × 109/L had a sensitivity of 86%, and a TLC < 5 × 109/L had a sensitivity of 32% as per Spector *et al.* [20]. Whereas, Chandna *et al.* [15] and Liu *et al.* [21] reported sensitivities of 17% and 29% respectively for a TLC < 5 × 10^9/L. In the present study TLC < 10 × 10^9/L had a sensitivity of 58%, an NPV of 28%, with a PPV of 87%.

In neonatal sepsis, probably because of utilization at the infection site and adhesion to endothelial cells, neutropenia is a more common finding than neutrophilia [22]. Berger *et al.* recommended a value <4 × 109/L (sensitivity 78%, PPV 25%) to detect early onset sepsis [23]. In the present study, ANC < 1750/mm^3 had a sensitivity of 77% and a PPV of 80.4% in detection of sepsis. Neutropenia in newborns can occur in cases of asphyxia, certain inborn errors of metabolism and also in Pregnancy induced hypertension in mother [22]. Therefore, its use as a sole predictor of sepsis is misleading. The variations between the results in different studies may be due to different criteria used, timing of sample, severity of infection, and the age of presentation and the reduced sensitivity of these tests in first week of life.

A 'left shift' of neutrophils happens during sepsis because of immature netrophils released from marrow which increases the ratio of Immature to Total neutrophils. Manroe *et al.* [24] observed that in healthy neonates, I/T ratio was 0.16 in the first 24 h, which fell to 0.13 by 60 h and remained so until 28 days of age. Christensen *et al.* [26] suggested that neutrophil ratios were often abnormal during neonatal sepsis. In the present study, I/T ratio > 0.2 had a sensitivity of 52%, while Rodwell *et al.* [26] had a sensitivity of 47% with same cut off. The reported cut-off value of I/T ratio is variable in different studies, possibly due to the inter observer variation in interpretation of peripheral smear [27, 28]. Rodwell *et al.* [26] used I/M > 0.30 as a predictor of infection. Unlike ANC, I/T ratio will not

increase in cases of neonatal asphyxia. Thus, neutrophil ratios overcome the limitations of neutropenia and give fewer false negative results compared with band count.

Platelet counts drop in sepsis, possibly because of disseminated intravascular coagulation and the damaging effects of endotoxin [29]. In the present study, the platelet count was not found to have good sensitivity (41.6%) and Specificity (41.6%).

Gerdes [30] has recommended normal value of micro-ESR as "day of life +3" corresponding to the 95th percentile value reported by Adler and Denton [36]. It would imply that 95th percentile values for micro-ESR on postnatal days 1, 3, 5 and 7 would be 4mm, 6mm, 8mm and 10 mm, respectively. 10mm has been considered as highest normal range for newborns more than 7 days. In our study, we considered micro ESR more than 15mm in 1st hour as positive. With this cut off, we had sensitivity of 43%, Specificity of 75%, Positive predictive value of 87% and Negative predictive value of 25%. Other studies Walliullah *et al.* [31] and Mondal *et al.* [32] found Sensitivity of micro ESR to be 63% and 63.2% respectively.

The result of the similar studies varies widely probably because of the differences in the sample collection method and inter-observer variations. In the present study, only peripheral blood samples were used while Rodwell *et al.* [26] obtained blood from umbilical cord, heel stick, peripheral venipuncture and umbilical artery catheter.

In the present study, CRP was the single best diagnostic test of the various indicators of sepsis. When considered with any of the hematological parameter, the sensitivity, specificity, PPV and NPV reduced. Da Silva *et al.* [33] too found the same. Sharma *et al.* [34] observed that CRP had 80% sensitivity and 93% specificity. Chandana *et al.* [15] observed 83% sensitivity but only 42% specificity for CRP. This variation could be because of the different methodologies used to measure CRP and the cut off used.

We had a high blood culture positive rate of 80%, probably because of low antenatal antibiotics and BacTec culture methods used. Probably because of high blood culture positivity rate, septic screen sensitivity, specificity and NPV were low compared to other studies (Table 3).

Table 3: Comparison of sensitivity, sensitivity, PPV and NPV of septic screen in comparison with blood culture

Sl. No.	Author	Year	Sensitivity	Specificity	PPV	NPV
1	Philip *et al.* [35]	1980	93%	88%	39%	-
2	Chandna *et al.* [15]	1988	88%	23%	51%	-
3	Gerdes *et al.* [30]	2004	100%	83%	27%	100%
4	Present study	2013	77%	41%	84%	31%

Strengths of the present study:
- High culture positive rate.
- We used Micro ESR as one of the parameter of septic screen, which is a simple, easy to perform, cost effective, and bed side test.

Limitations:
- No documentation of intrapartum antibiotic administered. However, culture positive rate was very high in our study.
- Sample size is low compared to other studies.

CONCLUSION
A positive septic screen has sensitivity of 77%, specificity of 41%, positive predictive value of 84% and negative predictive value of 31% when blood culture is considered as gold standard test to diagnose neonatal sepsis.

REFERENCES
1. Bang AT, Bang RA, Bactule SB, Reddy HM, Deshmukh MD; Effect of home-based neonatal care and management of sepsis on neonatal mortality: field trial in rural India. Lancet, 1999; 354(9194): 1955-1961.
2. Stoll BJ; The global impact of neonatal infection. Clin Perinatol., 1997; 24(1): 1-21.
3. Sepsis in the Newborn. Available from http://www.newbornwhocc.org/2014_pdf/Neonatal%20sepsis%202014.pdf
4. National Neonatal Perinatal Database; Report 2002-03. Published by NNP Nodal centre, Departmnet of Pediatrics, Al Indian Institute of Medical Science, New Delhi.
5. Vinod Kumar CS, Neelagaud YF; Incubation period for culture positivity to detect septicaemia in neonates. Indian Journal of Medical Microbiology, 2005; 23(4): 270-275.
6. West BA, Tabansi PN, Ugwu RO, Eneh AU; The predictive value of micro-erythrocyte sedimentation rate in neonatal sepsis in a low resource country. Pediatr Therapeut., 2012; S2:002.
7. Mayuga WAB, Isleta PFD; Clinical correlation of neonatal and maternal hematological parameters as predictors of neonatal sepsis. PIDSP Journal, 2005; 9(2): 36-43.
8. Datta S, Oberoi JK, Chgh TD; Laboratory diagnosis of neonatal sepsis. J Neonatology India, 2006; 20(1): 16-23.
9. Basu R, Bandyopadhyay S; Study on correlation between sepsis screening and blood culture in neonatal sepsis. IOSR Journal of Dental and Medical Sciences, 2014; 13(5): 52-56.
10. Pereira S, Pereira BJG, Bhakoo ON, Narang A, Sakhuja VS, Chugh KS; Peritoneal dialysis in neonates with acute renal failure. Ind J Pediatr., 1988, 58(6): 973-978.
11. Malik A, Hui CP, Pennie RA, Kirpalani H; Beyond the complete blood cell count and C-reactive protein: A systemic review of modern diagnostic tests for neonatsl sepsis. Arch Pediatr Adolesc Med., 2003; 157(6): 511-516.
12. Da Silva O, Ohlsson A, Kenyon C; Accuracy of leukocyte indices and C- reactive protein for diagnosis of neonatal sepsis: A critical review. Pediatr Infect Dis J., 1995; 14(5): 362-366.
13. Sankar MJ, Agarwal R, Deorari AK, Paul VK; Sepsis in the newborn. Indian J Pediatr., 2008; 75(3): 261–266.
14. Khatua SP, Das AK, Chatterjee BD, Khatua S, Ghose B, Saha A; Neonatal septicemia. Indian J Pediatr., 1986; 53(4): 509–14.
15. Chandna A, Rao MN, Srinivas M, Shyamala S; Rapid diagnostic tests in neonatal septicemia. Indian J. Pediatr. 1988; 55(6): 947–53.
16. Sharma A, Kutty CV, Sabharwal U, Rathee S, Mohan M; Evaluation of sepsis screen for diagnosis of neonatal septicemia. Indian J Pediatr., 1993; 60(4): 559–563.
17. Deorari AK; Neonatal sepsis; Manageable daunting issue for India. Journal of Neonatalogy, 2009; 23 (1): 7-11.
18. Ahmed Z, Ghafoor T, Ali S, Aziz S, Mahud S; Diagnostic value of C-reactive protein and haematological parameters in neonatal sepsis. JCPSP, 2005; 15(3): 152-156.
19. Zwar MP, Tambekar RG, Deshpande NM, Gadgil PA, Kalekar SM; Early diagnosis of neonatal septicemia by septic screen. Indian J Pathol Microbiol., 2003; 46(4): 610-612.
20. Spector SA, Ticknor W, Grossman M; Study of the usefulness of clinical and hematologic findings in the diagnosis of neonatal bacterial infections. Clin Pediatr., 1981; 20(6): 385–392.
21. Liu CH, Lehan C, Speer ME, Fernbach DJ, Rudolph AJ; Degenerative changes in neutrophils: an indicator of bacterial infection. Pediatrics, 1984; 74(5): 823–827.
22. Engle WD, Rosenfeld CR; Neutropenia in high risk neonates. J Pediatr., 1984; 105(6): 982–986.
23. Berger C, Uehlinger J, Ghelfi D, Blau N, Fancani S; Comparison of CRP and WBC count with differential in neonates at risk for septicemia. Eur J Pediatr., 1995; 154(2): 138–144.
24. Manroe BL, Weinberg AG, Rosenfeld CR, Browne R; The neonatal blood count in health and disease. I. Reference values for neutrophilic cells. J Pediatr., 1979; 95(1): 89–98.
25. Christensen RD, Bradley PP, Rothstein G; The leukocyte left shift in clinical and experimental neonatal sepsis. J Pediatr., 1981; 98(1): 101–105.
26. Rodwell RL, Leslie AL, Tudehope DI; Early diagnosis of neonatal sepsis using a hematological scoring system. J Pediatr., 1988; 112(5): 761–767.
27. Namedo UK, Singh HP, Rajput VJ, Kushwaha JS; Hematological indices for early diagnosis of neonatal septicemia. Indian J Pediatr., 1985; 22(4): 287–292.
28. Cohen P, Gardner FH; Thrombocytopenia as a laboratory sign and complication of gram-negative

bacteremic infection. Arch Intern Med., 1966; 117: 113–124.

29. Corrigan JJ; Thrombocytopenia: a laboratory sign of septicemia in infants and children. J Pediatr., 1974; 85(2): 219–221.

30. Gerdes JS, Polin RA; Sepsis screen in neonates with evaluation of plasma fibronectin. Pediatr Infect Dis J., 1987; 6(5): 443–446.

31. Walliullah SM, Islam MN, Siddika M, Hossain MA, Chowdhury AK; Role of micro-ESR and I/T ratio in the early diagnosis of neonatal sepsis. Mymensingh Med J., 2009, 18(1): 56-61.

32. Mondal SK, Nag DR, Bandyopadhyay R, Chakraborty D, Sinha SK; Neonatal sepsis: Role of a battery of immunohematological tests in early diagnosis. Int J Appl Basic Med Res., 2012; 2(1): 43-47.

33. Da Silva O, Ahisson A, Kenyon C; Accuracy of leukocyte indices and C-reactive protein for the diagnosis of neonatal sepsis: a critical review. Pediatr Infect Dis J., 1995; 14(5): 362–366.

34. Sharma A, Kutty CV, Sabharwal U, Rathee S, Mohan M; Evaluation of sepsis screen for diagnosis of neonatal septicemia. Indian J Pediatr., 1993; 60(4): 559–563.

35. Philip AJ, Mills PC; Use of C-reactive protein in minimizing antibiotic exposure. Experience with infants initially admitted to a well baby nursery. Paediatrics, 2000; 106(1): E4.

36. Adler SM, Denton RL; The erythrocyte sedimentation rate in newborn period. J Pediatr., 1975; 86(6): 942-948.