JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH

How to cite this article:
R BHAT Y AND RAO A. THE PERFORMANCE OF HAEMATOLOGICAL SCREENING PARAMETERS AND CRP IN EARLY ONSET NEONATAL INFECTIONS. Journal of Clinical and Diagnostic Research [serial online] 2010 December [cited: 2010 December 10]; 4:3331-3336.

Available from
http://www.jcdr.in/article_fulltext.asp?issn=0973-709x&year=2010&volume=4&issue=6&page=3331-3336&issn=0973-709x&id=1054

ORIGINAL ARTICLE

The Performance Of Haematological Screening Parameters And CRP In Early Onset Neonatal Infections

RAMESH BHAT Y* AND AMITHA RAO**

ABSTRACT

Background: In neonates with early onset sepsis (EOS), the haematological screening parameters and C-reactive protein (CRP) have wide variations in performance.
Objective: To evaluate the performance of haematological screening parameters and CRP in blood culture positive neonatal EOS.
Methods: We retrospectively studied the neonates who were suspected to have bacterial infections within the first 48 hours of life, based on the risk factors and/or the clinical features in whom haematological screening parameters, CRP and blood cultures were obtained. The screening parameters included total leukocyte count (TLC), the ratio of immature to mature neutrophil count (B: N), micro-ESR, platelet count (PLT), toxic granules (TG) and cytoplasmic vaccuolations (CV) in peripheral smear, and CRP. The screening parameters were assessed for individual performance and in combination in culture positive neonates.
Results: Of the 1291 neonates who were screened for EOS, 212 (16.4%) had positive blood cultures. The male to female ratio was 1.08:1. Preterm, small for gestational age and symptomatic newborns constituted 33.9%, 17.9% and 39.2% of the total number of neonates respectively. Coagulase negative Staphylococcus, Klebsiella and Pseudomonas were the predominant culture isolates. Among the haematological parameters, the positivity was best with micro-ESR (44.8%) and the least with TG/CV (2.8%). Any 2 or more parameters were positive in one third of the subjects. TLC and micro-ESR had significantly more positivity among the symptomatic than the asymptomatic neonates ($P<0.01$). Odds of any 2 or more parameters which were positive for symptomatic relatives to the asymptomatic neonates was 3.89 (95% CI: 2.14 - 7.06; $P<0.001$)
Conclusion: The sensitivities of the traditional haematological screening parameters and CRP were not satisfactory in identifying the neonates with EOS. A relatively better performance is expected in the symptomatic than in the asymptomatic neonates.

Key words: neonates, early-onset sepsis, screening, haematological parameters, CRP

*MD (Paed), Associate Professor; **MD (Paed), Associate Professor, Department Of Pediatrics, Kasturba Medical College, Manipal. 576104, Udupi District, Karnataka, India

Corresponding Author
Ramesh Bhat. Y. MD (Paed)
Associate Professor
Department of Paediatrics
Kasturba Medical College,
Manipal-576104
Manipal University
Udupi District, Karnataka, INDIA
E-mail: docrameshbhat@yahoo.co.in
Tel: (91) 9448296564
Fax: (91) 820 2571934

Introduction

The successful treatment and good treatment outcome of bacterial infections in neonates depend on the early initiation of appropriate antibiotic therapy. Hence, clinicians are always compelled to make an early diagnosis. However, the early diagnosis of bacterial infections is a difficult task. [1] [2] [3] The positive blood culture report which is a gold standard for the diagnosis of neonatal sepsis, requires 48-72 hours of time. [2] [3] Further, the yield of the blood culture is also low [2] [3] [4] [5] [6] and in some hospitals, the facilities may not be available. Hence, for early identification, several screening tests and their usefulness, either individually or in combinations, have been reported. The published haematological screening parameters and C-reactive protein (CRP) have wide variations in performance. [2] [3] [6] [7] [8] [9] [10] Few authors have reported a poor prediction of sepsis by the individual parameters [11] [12], but a sensitivity of above 90% by a combination of parameters has been reported by others.[6] [9] Recently, few researchers suggested the need for the reassessment of the complete blood count. [13] They did not use CRP as a screening tool and reported a higher sensitivity of the physical examination. In this context, the present study was undertaken to evaluate the performance of various haematological screening parameters and CRP in neonates with blood culture proven early-onset sepsis (EOS) over a period of five years.

Methods

The study was conducted retrospectively in the neonatal unit of a teaching hospital between January 2000 and December 2004. Neonates who were clinically suspected to have bacterial infections within the first 48 hours of life, based on the risk factors and/or clinical features, were subjected to various haematological screening parameters and blood cultures. Neonates developing symptoms after 48 hours of life and blood cultures which grew fungi were excluded. The haematological screening parameters included total leukocyte count (TLC), platelet count (PLT), micro- erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), band to mature neutrophil ratio (B: N) and the presence of cytoplasmic vaccuolations (CV) and/or toxic granulations (TG) in peripheral smear examinations. The semi quantitative measurement of CRP by the slide agglutination technique was done between the first 24- 36 hours of life. The blood culture samples included a single sample from a peripheral vein/artery which was taken under aseptic conditions before commencing antibiotics. Chest X-ray and other investigations were performed whenever indicated.

For the study, a TLC of <5000 or >20000/mm^3 [7] [8] [9], a PLT of <150000/mm^3 [9], a CRP of >6mg/L, B: N ratios of ≥0.2 [8] [12] and the presence of CV or TG in the peripheral smears [2] [10] were considered to be abnormal. Micro-ESR [14] was considered to be positive if the readings were more than >97^{th} percentile/1^{st} hour. These haematological parameters were assessed for the performance individually and in combination in neonates having positive blood cultures. We also compared the positivity of the parameters in symptomatic and asymptomatic neonates. A neonate was considered to be symptomatic if he/she had lethargy, fever, hypothermia, poor feeding and/ or manifestations which were pertaining to the respiratory, gastrointestinal, cardiovascular, central nervous (CN) or the haematological systems. Other neonates with no abnormalities were considered to be asymptomatic.

Statistical analyses were performed by using the Statistical Package for Social Sciences for Windows version 11.5 software. Chi square (χ^2) tests were used to compare the categorical variables. A *p*-value of <0.05 was considered to be significant.

RESULTS

Of the 1291 neonates who were screened for early onset infections, 212 (16.4%) had positive blood cultures. Eighty three (39.2%) were symptomatic. Of the total, 140 (66.03%) were term and 72 (33.9%) were preterm. The mean birth weight was 2465.8 (±718.8) g. The male

to female ratio was 1.08:1. The small for gestational age (SGA) neonates were 38(17.9%). Coagulase negative Staphylococcus (42%), Klebsiella (20.1%), Pseudomonas (17.9%) and Acinetobacter (7.1%) were the predominant blood culture isolates. Among the haematological parameters, the positivity was best with micro- ESR (44.8%) and least (2.8%) with TG/CV [Table/Fig 1]. Any 2 or more parameters were positive in one third of the blood culture positive neonates.

[Table/Fig 1]: Performance of sepsis screen parameters (n=212)

Parameters	Positivity (n)	Percentage
Total leukocyte count	63	29.7
Platelet count	36	16.9
Micro-ESR	95	44.8
CRP	36	16.9
B: N ratio	33	15.6
Toxic granules/ cytoplasmic vaccuolations	6	2.8
Any two parameters	73	34.4

ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; B: N ratio, band to mature neutrophil ratio

TLC and micro-ESR had significantly more positivity among the symptomatic than the asymptomatic (P<0.01) neonates [Table/Fig 2].

[Table/Fig 2]: Performance of sepsis screen parameters in symptomatic vs. asymptomatic newborns

Parameters	Positivity (%)		OR (95%CI)	p-value
	Symptomatic (83) n (%)	Asymptomatic (129) n (%)		
Total leukocyte count	34 (40.9)	29 (22.5)	2.39 (1.31-4.37)	0.004
Platelet count	14 (16.9)	22 (17.1)	0.99 (0.47-2.06)	0.97
Micro ESR	47 (56.6)	48 (37.2)	2.20 (1.28-3.86)	0.005
CRP	15 (18.1)	21 (16.3)	1.13 (0.55-2.35)	0.73
B: N ratio	12 (14.5)	21 (16.3)	0.87 (0.40-1.88)	0.72
Toxic granules/ cytoplasmic vaccuolations	3 (3.6)	3 (2.3)	1.58 (0.31-7.99)	0.58
Any two parameters	44 (53.0)	29 (22.5)	3.89 (2.14-7.07)	0.000

ESR, erythrocyte sedimentation rate; CRP, C-reactive protein, B: N ratio, band to mature neutrophil ratio; OR, odds ratio; CI, confidence interval.

A, two or more parameters positivity was seen in a higher percentage of symptomatic (53%) than asymptomatic (22.5%) neonates, with blood culture positivity (Odds ratio: 3.89; 95% CI: 2.14 - 7.06; P <0.001).

DISCUSSION

Newborns are uniquely susceptible to overwhelming bacterial infections. The incidence of early-onset bacterial infections range from 1-10 per 1000 live births and the related mortality ranges between 15% and 30 %.[15] [18] The morbidity among the survivors is also high.[1] [4] [15] [16] [17] [18] Further, newborns who develop sepsis often deteriorate rapidly. Because the failure or delay in treatment is likely to result in significant mortality and morbidity, early and efficient diagnosis is challenging to the clinician. The blood culture not only takes time, but is also complicated, with a low yield. In the present study, the blood culture yield was 16.4%. This was low, as compared to the 20 % yield which was obtained by many authors.[2] [4] [5] [11] A much lower yield (14%) has been reported by Varsha et al. [6] In the present study, the treatment of the mothers of at -a -risk neonates and the single blood culture samples could have contributed to the low culture yield.

The traditional sepsis work up included various haematological parameters and CRP. The predictive ability of these parameters has been found to vary widely in the literature.[7] [8] [9] [10] [12] [13] [18] [19] [20] In the present study, the sensitivity of the haematological screening parameters and CRP varied from 2.8%-44.8%. Micro ESR had the best sensitivity, but it was only 45%. None of the screening parameters individually predicted the presence of bacteraemia satisfactorily. Similar observations were made earlier too. [11] [12]

The total leukocyte count and the B: N ratios have been correlated with an increased risk of bacterial infections in neonates.[7] [8] [9] However, they had a wide range of sensitivity (17-90%).[2] [6] [7] [8] [9] [19] In our study, the sensitivity was 29.7% for TLC and 15.6% for the B: N ratio. Neutropenia was observed in

1.9% of the culture proven sepsis cases. Degenerative changes in neutrophils like cytoplasmic vacuolization and toxic granulation were reported to be a valuable adjunct in the early detection of neonatal bacterial infection. A sensitivity of 81% to CV and 67% to TG has been found by Liu and his colleagues. [10] Sharma et al [2] found a sensitivity of TG in 60% and CV of 15% among the proven sepsis cases. In contrast, we observed a sensitivity of only 2.8% in TG/CV.

The micro-ESR is an inexpensive, easy bedside screening test. Its sensitivity ranges from 30% to 73% [2] [3] [8] in proven sepsis cases. An enhanced sensitivity of revised over traditional micro-ESR has been reported earlier. [14] In the present study, the revised micro-ESR had the best sensitivity (44.8%). Thrombocytopenia as an important parameter in supporting the diagnosis of sepsis has been described, although it appears to be a late finding and to be nonspecific. [3][18] The positivity of thrombocytopenia was 16.9% in our study. The reported sensitivity of CRP for the detection of bacterial infections varied widely from 47% to 100%.[6] [8] [12] [20] Further, Benitz [21] et al found that the sensitivity of CRP in culture proven EOS rose from 35% at the initial evaluation to 78.9% in next 24 hours. They also opined that the sensitivity of a normal CRP at the initial evaluation is not sufficient to justify withholding antibiotic therapy and they suggested serial CRP estimations. The CRP level done on day 2 of life in our study, showed a sensitivity of only 16.9%. The importance of the serial measurement of the septic screens was demonstrated by Gerdes [20] et al, who performed two separate screens (WBC, I/T, CRP, micoESR), 12 to 24 hours apart. Infants who had normal initial screens were positive on repeat testing and identified all septic neonates. Our study has been limited by a one- time evaluation of the screening parameters.

As the sensitivity and the specificity of the individual tests may not justify their individual use in newborn infants, a significant improvement of diagnostic capability when used in various combinations, has been studied. The greatest sensitivity when 2 or more of 5 tests (WBC, B:N, CRP, heptoglobin and micro-ESR) were done, was established by Philip et al.[8] To further improve the diagnostic efficacy, Rodwell[9] et al developed a seven-point haematological scoring system which had 96% sensitivity. An above 80% sensitivity by the combination of any 2 or more positive tests in culture positive EOS was also reported earlier from Indian studies.[2] [6] In contrast, we observed a sensitivity of 34.4% by the combination of any two or more parameters. This observation agrees with that of Fowlie and Schmidt [22]

An accurate and timely diagnosis of early onset neonatal sepsis remains challenging to the clinician and the laboratory. A test with a rapid turnaround time, with 100% sensitivity which allows accurate diagnosis and appropriate antimicrobial treatment, is desirable. A reasonable specificity is also required to allow the antibiotics to be safely withheld in non-infected infants. The sensitivity of individual tests or combinations varies widely. Escobar [7] et al did not use CRP in their evaluation because of a lack of the full assessment of its utility. They opined that the current recommendations were vague, that the B: N ratio was unreliable and that the risk of sepsis among asymptomatic infants was low. Further, they reported that the sensitivity of the physical examinations was much higher. They also suggested the reassessment of the complete blood count and the need for more research in the areas of clinician compliance, as well as the clinician perception of the risk of sepsis. Recently, measures of acute phase proteins, cytokines, cell surface antigens, and bacterial genomes have been used alone or in combination to improve the diagnosis of neonatal sepsis.[23]

Among the cytokines, interleukin (IL)-6 and IL-8 have been demonstrated to have good diagnostic utilities as early phase markers, while acute phase reactants such as C-reactive protein and procalcitonin have superior diagnostic properties during the later phases. Other markers, including inter-alpha-inhibitor proteins and IL-10 have been demonstrated to yield important prognostic information. The advent of flow cytometry and molecular techniques have

made crucial contributions to the field and have improved the diagnostic accuracy.[24, 25] Among the promising cytokines, interleukin (IL)-6 has been most intensively studied. Its sensitivity ranges from 70-80%. Khassawneh M et al . [26] reported that the IL-6 cut-off value of 18.2 pg/ml was associated with a sensitivity and specificity of 87% and 50% respectively. Procalcitonin (PCT) is another promising marker with a sensitivity in the range of 70–80% at birth.[25] In the immediate postnatal period however, although the sensitivity of IL-6 decreases over time, the sensitivity of PCT (as well as that of CRP) increases, making the serial measurements useful for those situations in which one needs to decide how long to treat the infant. Cutoff concentrations of >6.1 ng/ml of PCT for the optimum prediction of sepsis in neonates, were reported.[27]

Hassawneh M et al [26] also studied the role of immunoglobulin M (IgM). The sensitivity of IgM as a single test at a value of ≥10 mg/dl was 91.3% for patients with 'sepsis' or 'probable sepsis'. This sensitivity in identifying patients with sepsis was paralleled with a low specificity of 45%. They found that the performance of CRP was better than IgM and IL-6 in distinguishing between the neonates with sepsis and without sepsis.

Recently, attention has been directed to the leukocyte cell surface antigens as the diagnostic markers of sepsis. Neutrophil surface CD64 and CD11b expression have been studied. For culture-positive sepsis episodes, the CD64 index having the highest area under the curve (0.852) of all haematologic variables, with a sensitivity of 80% and a specificity of 79%, with a cutoff value of 4.02, was reported.[28] Dorothee et al [29] suggested that the expression levels of the Toll-like receptor (TLR)2 on monocytes by flow cytometry, is comparably more valuable than C-reactive protein, IL-8, and IL-6 as early sepsis markers. The routine availability of new tests at affordable costs is likely to enhance the rapid identification of neonatal sepsis.

CONCLUSION

The sensitivities of the traditional haematological screening parameters and CRP are not satisfactory in neonates with blood culture positive early onset infections. The sensitivity of a combination of any two or more parameters is also not satisfactory, although a relatively better sensitivity is likely in symptomatic than in asymptomatic neonates. Because an early onset neonatal infection is a serious but treatable condition whose treatment should not be missed or delayed, a test or a combination of tests with a high sensitivity is desirable. A better understanding of the neonatal inflammatory response to sepsis and the identification of sensitive and specific markers of inflammation or rapid microbe-specific diagnostic tests are needed to assist the clinician in the early detection of neonatal sepsis.

REFERENCES

[1] Stoll BJ, Gordon T, Korones SB et al. Early onset sepsis in very-low-birth-weight (VLBW) neonates. J Pediatr 1996; 129:72-80.
[2] Sharma A, Kutty CV, Sabharwal U, Rathee S, Mohan H. Evaluation of sepsis screen for diagnosis of neonatal septicemia. Indian J Pediatr 1993; 60:559-63.
[3] Misra PK, Kumar R, Malik GK, Mehra P, Awasthi S. Simple hematological tests for diagnosis of neonatal sepsis. Indian Pediatr 1989; 26:156-60.
[4] Baltimore RS, Huie SM, Meek JI, Schuchat A, O'Brein KL. Early-onset neonatal sepsis in the era of group B Streptococcal prevention. Pediatrics 2001; 108:1094-8.
[5] Gladstone IM, Ehrenkranz RA, Edberg SC, Baltimore RS. A ten-year review of the previous fifty-year experience. Pediatr Infect Dis J 1990; 9:819–25.
[6] Varsha, Rusia U, Sikka M, Faridi MM, Madan N. Validity of hematologic parameters in identification of early and late onset neonatal infection. Indian J Pathol Microbiol 2003; 46:565-8.
[7] Manroe BL, Weinberg AG, Rosenfeld CR, Browne R. The neonatal blood count in health and disease. 1. Reference values for neutrophilic cells. J Pediatr 1979; 75:89-98.
[8] Philip AGS, Hewitt JR. Early diagnosis of neonatal sepsis. Pediatrics 1980; 65; 1036-41.
[9] Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematologic scoring system. J Pediatr 1988; 112:761-7.
[10] Liu HC, Lehan C, Speer ME, Fernbach DJ, Arnold J. Rudolph AJ. Degenerative Changes in neutrophils: an indicator of bacterial infection. Pediatrics 1984; 74:823-827.
[11] Kumar V, Singhi S. Predictors of serious bacterial infection in infants up to 8 weeks of age. Indian Pediatr 1994; 31:171-80.

[12] Kite P, Millar MR, Gorham P, Congdon P. Comparison of five tests used in diagnosis of neonatal bacteraemia. Arch Dis Child 1988: 63:639-43.
[13] Escobar GJ, Li D, Armstrong MA et al. Neonatal sepsis workups in infants ≥ 2000 grams at birth: A population –based study. Pediatrics 2000; 106:256-63.
[14] Diwakar KK, Rosul G. Revised look at micro-erythrocyte sedimentation rate in neonates. Indian Pediatr 1999; 36:703-5.
[15] Kuruvilla KA, Pillai S, Jesudason M, Jana AK. Bacterial profile of sepsis in a neonatal unit in south India. Indian Pediatr 1998; 35:851-8.
[16] Horbar JD, Badger GJ, Carpenter JH et al. Trends in mortality and morbidity for VLBW infants 1991-99, Pediatrics 2002; 110:143-51.
[17] Stoll BJ, Hansen N, Fanaroff A et al. Changes in pathogens causing early onset sepsis in VLBW infants. N Engl J Med 2002; 347: 240-7.
[18] Ho LY Sepsis in young infants. Rational approach to early diagnosis and treatment. Singapore Med J 1992; 33:119-22
[19] Da Silva O, Ohlsson A, Kenyon C. Accuracy of leukocyte indices and CRP for diagnosis of neonatal sepsis: a critical review. Pediatric Infect Dis J 1995; 14:362-6.
[20] Gerdes JS. Clinicopathological approach to the diagnosis of neonatal sepsis. Clin Perinatol 1991; 18: 361-81.
[21] Benitz WE, Han MY, Madan A, Ramachandra P. Serial serum C-reactive protein levels in the diagnosis of neonatal infection. Pediatrics 1998; 102:E41.
[22] Fowlie PW, Schmidt B. Diagnostic tests for bacterial infection from birth to 90 days-a systematic review. Arch Dis Child Fetal Neonatal Ed 1998; 78:F92–8.
[23] Mishra UK, Jacobs SE, Doyle LW, Garland SM. Newer approaches to the diagnosis of early onset neonatal sepsis. Arch Dis Child Fetal Neonatal Ed 2006; 91:F208-12.
[24] Lam HS, Ng PC. Biochemical markers of neonatal sepsis. Pathology 2008; 40:141–8.
[25] Chiesa C, Panero A, Osborn JF, Simonetti AF, Pacifico L. Diagnosis of neonatal sepsis: a clinical and laboratory challenge. Clin Chem 2004;50: 279-87.
[26] Khassawneh M, Hayajneh WA, Kofahi H, Khader Y, Amarin Z and Daoud A. Diagnostic markers for neonatal sepsis: comparing C-reactive protein, interleukin-6 and immunoglobulin M. Scandinavian J Immunol 2007; 65: 171–5.
[27] Enguix A, Rey C, Concha A, Medina A, Coto D, Diéguez MA. Comparison of procalcitonin with C-reactive protein and serum amyloid for the early diagnosis of bacterial sepsis in critically ill neonates and children. Intensive Care Med 2001;27 (1): 211-5
[28] Bhandari V, Wang C, Rinder C, Rinder H. Hematologic profile of sepsis in neonates: Neutrophil CD64 as a diagnostic marker. Pediatrics 2008; 121: 129-34
[29] Dorothee V, Gabriele D, Susanne SE, Erik H, Clemens S, Johannes R. Expression of Toll-like receptors in neonatal sepsis. Pediatric Research 2005;58:654-9