International Journal of Contemporary Pediatrics
Ahmad I et al. Int J Contemp Pediatr. 2016 Feb;3(1):107-112
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20160140

Research Article

Changing role of neonatal sepsis markers in the era of BacT/Alert: is there a need to devise a new septic screen

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Received: 02 October 2015
Accepted: 17 December 2015

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ABSTRACT

Background: Emergence of newer culture methods and other diagnostic aids for neonatal sepsis makes evaluation of known sepsis markers imperative to define their changing role. This study was designed to assess role of CRP, leukopenia, neutropenia and thrombocytopenia in neonatal sepsis in this changing era.
Methods: A prospective observational study was conducted in a level-3 NICU in north India in 2013. Study duration was 1 year. All patients visiting our hospital with non-specific symptoms and signs suggestive of sepsis were admitted and evaluated. Neonates with confirmed sepsis were allotted to cases group and others to control group. CBC and CRP were taken from both groups; sensitivity, specificity, negative and positive predictive value of leucopenia, neutropenia and thrombocytopenia were assessed. Relationship of these markers with neonatal mortality was also assessed. Statistical analysis used was Graphpad Instat statistical software was used for analysis. Comparisons were made by chi-square test and Fischer's exact test. A p-value <0.05 was taken as significant.
Results: 120 neonates had confirmed sepsis and 76 were in the control group. CRP had sensitivity, specificity, negative and positive predictive values of 67%, 19%, 27% and 57%. Sensitivity, specificity, negative and positive predictive values for leukopenia were 22%, 68%, 29% and 59%, respectively. For neutropenia and thrombocytopenia these values were 16%, 81%, 34% and 61% and 41%, 71%, 42% and 70%, respectively. These markers also bore a significant association with mortality in both cases and controls (p-Value <0.05).
Conclusions: Haematological parameters are poor screening tools for neonatal sepsis; however they have a definite role as prognostic indicators. CRP appears to be a good screening tool for sepsis; however its low sensitivity is a drawback. There is a need to combine CRP with other markers like procalcitonin in newer septic screens to aid early diagnosis of neonatal sepsis.

Keywords: Septic screen, Bactec/BactAlert, Neonatal sepsis

INTRODUCTION

Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection in the first month of life with or without accompanying bacteremia.[1] Sepsis is the commonest cause of neonatal mortality; it is responsible for about 30-50% of the total neonatal deaths in developing countries.[2,3] It is estimated that up to 20% of neonates develop sepsis and approximately 1% die of sepsis related causes.[3] In India, the incidence of neonatal sepsis according to the data from National Neonatal Perinatal Database (NNPD, 2002-03) is 30 per 1000 live births. The NNPD network comprising of 18 tertiary care neonatal units across India found sepsis to be one of the

commonest causes of neonatal mortality contributing to 19% of all neonatal deaths.[4] Because of its high incidence and bad prognosis, sepsis continues to be a huge challenge for paediatricians. The outcome can be improved by early diagnosis, rational antimicrobial therapy and aggressive supportive care.[1,5,6] However, early recognition of sepsis in the neonate is very difficult. Such infants often present with non-specific symptoms and signs so that failure or delay in treatment may result in significant morbidity and mortality.[7,8] Diagnosis of neonatal sepsis based on clinical symptoms alone is not possible.[9,10] Blood culture, considered as the gold standard, is the most specific method to diagnose neonatal sepsis.[5] But it is time-consuming and requires the expertise of a trained microbiologist, the facilities for which are unavailable in many rural and community settings.[5] Newer culture methods like Bactec and BacT/Alert PF have emerged as an advancement over conventional culture methods. They have improved yield especially for fastidious organisms and in patients already on antibiotic therapy.[11] However, even though they give faster results than conventional culture methods, the time cultures take make decisions regarding initiation of therapy difficult on the basis of cultures alone. Besides they are not available everywhere.[12]

Septic screen, a group of readily available and rapid tests based on impact of infection on inflammatory pathways and haematological systems, was devised to circumvent the drawbacks of culture and aid decision making in suspected sepsis when culture results are not readily available. Various tests included are estimations of total leucocyte count (TLC), absolute neutrophil count (ANC), platelet count, immature to total (I/T) neutrophil ratio, micro ESR and CRP.[13] Different researchers have reported different sensitivities and specificities of different screening parameters for neonatal sepsis.[10,14] We designed this study to assess the sensitivity, specificity, positive and negative predictive value of these screening parameters in the era of newer culture methods like Bactec, Bact/Alert PF. Besides, emergence of useful newer screening tests like procalcitonin, various cytokines, cell surface markers and molecular methods makes it all the more important to assess the validity of recommended older methods.[15]

METHODS

This study was a prospective study conducted in a level-3 neonatal intensive care unit of a tertiary care referral hospital in north India in the year 2013. All neonates admitted with a suspicion of sepsis or in whom sepsis was suspected anytime during hospital stay, were evaluated for sepsis. Neonates with positive blood culture or diagnosed with pneumonia, meningitis or urinary tract infection (UTI) were considered to have confirmed sepsis and included in the study. Any organism, including *Coagulase-negative Staphylococcus* (CONS) was considered the causative agent of sepsis and not a contaminant if the criteria of the Vermont Oxford Network Database were present: clinical signs of sepsis, positive blood culture for CONS, and intravenous antibacterial therapy for at least five days after obtaining blood culture or until death, in case it occurs within five days after obtaining blood culture.[16] Neonates who didn't have confirmed sepsis and for whom an alternative diagnosis that could explain their symptoms was established, were assigned to the control group. Neonates with life-threatening congenital malformations, chromosomal disorders or who were extremely premature (<28 weeks gestation) were excluded from both groups. Neonates who could not be allotted to either cases or controls were also excluded. Total leucocyte count (TLC), absolute neutrophil count (ANC), platelet count and C-reactive protein levels (CRP) were collected from all patients, cases as well as controls. Meningitis was defined when CSF culture was positive or when CSF analysis revealed pleocytosis, low glucose or high protein. Pneumonia was diagnosed by suggestive signs and symptoms in addition to radiological evidence. UTI was confirmed by urine culture. Among investigations, leucopenia was defined as WBC count <5000/µl and thrombocytopenia as platelet count <150000/µl. The ANC varies considerably in the immediate neonatal period and the normal reference ranges are available from Manroe's charts.[17] For very low birth weight infants, the reference ranges are available from Mouzinho's charts.[18] Neutropenia was defined in comparison to references values in these charts. CRP levels > 6 mg/dl were taken as positive. These parameters were compared between cases and controls. Graphpad Instat statistical software was used for analysis. Comparisons were made by chi-square test and Fischers exact test. A p-value <0.05 was taken as significant.

RESULTS

Over the study period, 212 patients were evaluated for sepsis on the basis of clinical features. 120 patients satisfied our case definition and were alloted to the sepsis group. 76 patients who didn't satisfy our case definition and in whom an alternative diagnosis that would explain their symptoms could be established were allotted to the control group. Rest 16 patients, whose diagnosis remained obscure and in whom a diagnosis of sepsis or other disease couldn't be established with certainty, were excluded from both groups. 28 babies with sepsis died giving a mortality of 23.3%. It was comparable to the mortality of 23.6% in control group (p-value 1.0).

The baseline characteristics of the study (cases) group are given in Table 1.

Lethargy and refusal of feeds were the commonest presenting symptoms in cases. Other symptoms are listed in Table 2.

76 (63.3%) patients had culture-positive sepsis and Coagulase negative Staph was the commonest organism

isolated followed by Candida species. The microbiology profile of the patients is given in Table 3.

The complications observed in the cases are listed below in Table 4.

Table 1: Characteristics of the study group.

Mean gestational age (weeks)	36.3 (Range 29-39)
Mean birth weight (Kg)	2.6 (Range 0.9-4.5)
Mean age at admission (days)	10 (Range 1-27)
Males	72 (60%)
PROM in mother	5 (4%)
Delivered by LSCS	48 (40%)
Medical illness in mother	16 (13%)
Obstetric problems in mother	14 (12%)
Leaking>18 hours	6 (5%)
UTI in mother	4 (3%)

Table 2: Presenting complaints of patients.

Presenting complaint	No. of patients (Percentage)
Refusal of feeds	68 (57)
Lethargy	60 (50)
Respiratory difficulty	30 (25)
Fever	22 (18)
Oliguria	12 (10)
Jaundice	10 (8)
Irritability	6 (5)
Skin abscess	6 (5)
Loose stools	4 (3)

Table 3: Organism isolated in blood culture.

Organism isolated in blood culture	Total no of patients *(n=120)* (%) Total cultures positive *(n=76)*
Coagulase negative Staph	18 (15)
Candida	12 (10)
Enterococcus	11 (9.2)
E.coli	7 (5.8)
Staph aureus	8 (6.6)
Enterobacter	6 (5)
Morganella	4 (3.3)
Klebsiella pneumonae	4 (3.3)
Aerococcus	2 (1.7)
Acinetobacter	2 (1.7)
Leuconostoc	1 (0.8)
Kukoria krusei	1 (0.8)

CRP was positive in 80 cases and 61 controls. Among the screening parameters studied, CRP had the highest sensitivity (67%); however it was least specific (19%). Leucopenia, neutropenia and thrombocytopenia were present in 26, 19 and 49 patients in the cases group, respectively. They were also present in 24, 14 and 22 patients in the control group, respectively. Haematological indices used in septic screen had uniformly poor sensitivity, but good specificity and positive predictive value. Sensitivity, specificity and negative and positive predictive values of different indices used are given in Table 5.

Table 4: Complications developing in septic neonates.

Complication	Patients who survived	Patients who died
Meningitis	14	4
Hydrocephalus	2	
Seizures	10	3
Renal failure	4	3
Hyponatremia	2	8
Hypernatremia	6	3
Neonatal jaundice (unconjugated)	6	
Cholestasis	1	
Hypoglycemia	10	2
Hypocalcemia	1	
Coagulopathy	2	6

Table 5: Diagnostic value of screening tests for sepsis.

Parameter	Sensitivity	Specificity	PPV	NPV
CRP	0.67	0.19	0.57	0.27
WBC	0.22	0.68	0.59	0.29
ANC	0.16	0.81	0.61	0.34
PLT	0.41	0.71	0.70	0.42

We also studied relationship of these screening tests with outcome, in terms of mortality, in cases as well as controls. CRP, an acute phase reactant didn't show any significant relationship with mortality (p-Value >0.05). However, all hematological indices like leucopenia, thrombocytopenia and neutropenia, showed a significant association with mortality in both cases and controls (p-Value <0.05). The association was strong with a p-value <0.001 in all cases and a high odds ratio Table 6.

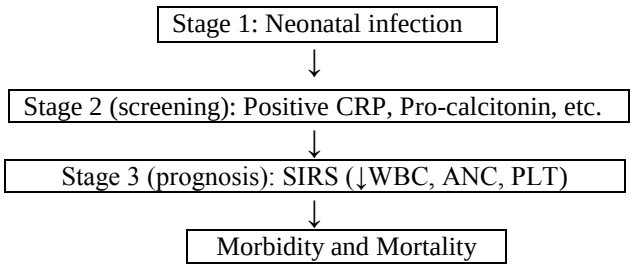
Table 6: Relationship of various haematological and biochemical markers with mortality.

Parameter	Patients	Odds Ratio	P-Value
Leucopenia	Cases	13.9	<0.0001
	Control	26	<0.0001
Neutropenia	Cases	13.4	<0.0001
	Control	14.7	0.0006
Thrombocytopenia	Cases	19	<0.0001
	Control	8	0.003
CRP +ive	Cases	2.28	0.40
	Control	1.7	0.32

DISCUSSION

Outcome of neonatal sepsis is closely related to early diagnosis. The automated culture systems Bactec and BacT/Alert are reliable blood culture systems for pediatric blood culture specimens and may offer improved and faster recovery of microbes, particularly from patients on antimicrobial therapy.[11] Newer diagnostic tests including acute phase reactants like procalcitonin, fibronectin and neopterin, cell surface markers like neutrophil CD11b and CD64, granulocyte colony stimulating factor and cytokines like interleukins, tumour necrosis factor α and chemokine's are emerging.[15] Molecular methods like PCR have shown promise.[15,19] It becomes imperative to test the validity of our existing diagnostic tools in this changing scenario. WBC count, ANC, platelet count and CRP are included in many septic screens and their diagnostic value was checked in our study.

In our study CRP had sensitivity, specificity, NPV and PPV of 67%, 19%, 57% and 27%. In a systematic review of the literature of studies evaluating the usefulness of CRP and leukocyte indices the authors have found a wide range of sensitivity (17% to 90%) and specificity (31% to 100%).[8-10] This variation could be because of the different methodologies used to measure CRP, the cut off used and the difference in microbiologic techniques like culture methods used. Overall sensitivity of CRP is highest among all markers we checked and its specificity the least (19%), making it a better screening tool; sensitivity of only 67% though leaves a lot to be desired. Hematological indices used in septic screen on the other hand, had uniformly poor sensitivity, but good specificity and positive predictive value. Thus presence of leucopenia, neutropenia and thrombocytopenia are poor screening tools for neonatal sepsis and their absence is not strong evidence against neonatal sepsis. However when present, they are strong pointers to presence of sepsis. This should come as no surprise as presence of these hematological abnormalities are defining criteria for systemic inflammatory response syndrome (SIRS) and severe sepsis in early infancy.[20] Infection is the commonest cause for SIRS resulting in high specificity and positive predictive value of these markers for sepsis. However, every infection doesn't progress to SIRS, so many cases may be missed in early stages and thus resulting in lower sensitivity of hematological parameters when used for sepsis screening. So, putatively occurrence of leucopenia, neutropenia and thrombocytopenia represent a relatively advanced stage of infection. Association of these parameters with increased mortality further supports the argument. The hematological parameters studied have not shown very high sensitivity or specificity in many other studies as well and the results have been varied.[21-23] The variations may be partly explained by differences in case definition, in the blood sampling time, the severity of infection, the age of the neonates, culture methods used and the diagnostic criteria followed. Pertinently, many researchers previously have reported very high negative predictive values for both leucopenia and neutropenia, meaning absence of these parameters would make sepsis unlikely.[21,23] However, we found uniformly poor negative predictive value of all hematological parameters studied. This could be explained by the use of Bact/Alert culture system in our study which is more sensitive than conventional culture systems, especially for fastidious organisms and in patients already on antibiotics.[11] Besides, in a country like India where most of the sick neonates are initially treated at places where culture facilities aren't available and patients referred to neonatal centres have already received one or more antibiotics. As demonstrated in the flow-chart below, it appears that using hematological parameters as screening tools, delays the diagnosis and contributes to worse outcome. For better prognosis and rational management, we need to diagnose sepsis at an earlier stage. That is why CRP appears to be a better screening tool; its drawbacks can be overcome by combining it with other newer inflammatory markers, to give a septic screen which picks more patients at an earlier stage and avoids the danger of relying on parameters which may be negative in early sepsis. In recent years measurement of procalcitonin and other inflammatory mediators have been reported as sensitive parameters for the early diagnosis of neonatal sepsis.[24]



The different indices used were also assessed for their relationship with outcome. It was seen that all hematological parameters were poor prognostic factors and predicted mortality in both groups of patients. It should not be surprising because leucopenia is a known component of SIRS and neutropenia is suggestive of bone marrow dysfunction or consumption at infection site in severe sepsis.[25] Platelet counts drop in sepsis, possibly because of disseminated intravascular coagulation and the damaging effects of endotoxin.[25,26] Association of leucopenia, neutropenia & thrombocytopenia with increased mortality has been found by others as well.[27,28] Though SIRS is usually caused by infection, it can also be associated with other conditions like metabolic disorders, necrotizing enterocolitis, shock and asphyxia. So, hematological indices like leucopenia, etc. when present define more advanced stages of infection and are thus poor tools for screening purposes as already discussed. Because of their easy availability and association with poor prognosis, these hematological abnormalities still have a huge role to play in neonatal sepsis management. Their presence warrants early aggressive management, frequent monitoring and early referral to a centre with better neonatal intensive care

facilities. Besides, there always remains a subset of neonates with 'suspected' sepsis where cultures are sterile and infection cannot be confirmed. Among these patients of suspected sepsis, it appears safer to treat patients with hematological abnormalities with antibiotics because of the prognostic significance of these parameters and their high specificity.

CONCLUSION

Hematological parameters are poor screening tools for neonatal sepsis; however they have a definite role as prognostic indicators. CRP appears to be a good screening tool for sepsis; however its low sensitivity is a drawback. There is a need to combine CRP with other markers like procalcitonin in newer septic screens to aid early diagnosis of neonatal sepsis.

Limitations

Micro-ESR and immature to total neutrophil ratio were not studied.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Ahmad I, Ahmed A, Abrar SS, Lone MR, Khanday RM, Bhat JI, et al. Changing role of neonatal sepsis markers in the era of BacT/Alert: is there a need to devise a new septic screen. Int J Contemp Pediatr 2016;3:107-12.