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Haematological Parameters in Neonatal Sepsis

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Abstract
Background: *In neonatal sepsis, the haematological screening parameters and Creactive protein (CRP) have wide variations in performance.*
Objective: *To evaluate the performance of haematological screening parameters and CRP in blood culture positive neonatal sepsis.*
Methods: *This prospective study was done over a period of one year. Blood samples from 100 clinically suspected neonatal septicemia cases were subjected to aerobic culture and Sepsis screen tests like C-Reactive protein, micro-ESR, total WBC count, Absolute neutrophil count, Immature/Total neutrophil count (I/T) ratio, Immature/mature neutrophil count (I/T) ratio and platelet count. The culture results were correlated with the Sepsis screen tests.*
Results: *Of the 100 cases studied, 26%were blood culture positive. 72%were males. 56%were preterm and 40% were very low birth weight neonates. Early onset septicemia was more common, seen in 68% of cases than late onset septicemia (22.4%) cases. E. Coli was the commonest organism isolated in 38.46% of cases followed by klebsiella pneumoniae & staphylococcus aureus. Among the haematological parameters, the positivity was best with ANC & I/T Ratio (84.61%) and the least with platelet count (73.07%). Any 2 or more parameters were positive in 96.15% of the subjects.*
Conclusion: *The sepsis parameters in predicting neonatal septicemia clinically needs further evaluation. Blood culture remains the gold standard for the diagnosis of neonatal septicemia. Combination of two or more sepsis screen parameters has better results in diagnosing neonatal septicemia compared to a single test while awaiting the blood culture results.*
Keywords: *Blood culture, Neonatal septicemia, Sepsis screen.*

INTRODUCTION

Neonatal septicaemia encompasses various systemic infections of the newborn such as septicaemia, meningitis, pneumonia, arthritis, osteomyelitis & urinary tract infections.[1] The successful treatment and outcome of bacterial infections in neonates depend on the early initiation of appropriate antibiotic therapy. The

positive blood culture report, which is a gold standard for the diagnosis of neonatal sepsis, requires 48-72 hours. It is time consuming. Therefore, haematological parameters can be evaluated for early diagnosis of neonatal sepsis.[2] An ideal diagnostic test for neonatal sepsis should have maximum sensitivity and specificity. In recent years, various investigators have evaluated some highly sensitive and specific inflammatory markers (e.g. ELISA methods, haptoglobins, interleukins and counterimmuno electrophoresis, etc.) to diagnose neonatal sepsis.[3] Early diagnosis of neonatal septicaemia is still a great challenge. For early diagnosis of neonatal septicaemia, a hematologic scoring system (HSS) of Rodwell[4] [includes total & differential leukocyte count, total neutrophil count, immature & total neutrophil ratio (IT ratio), immature & mature neutrophil ratio (IM ratio), total immature polymorphonuclear cell (PMNs) count & platelet count] is preferable because it includes all parameters. Haematological parameters accurately predict the presence or absence of infection and are reliable.[5]

MATERIAL & METHODS

Present study was conducted on new born babies aged 0-28 days admitted in Bal Chikitsalay with signs & symptoms of septicaemia to evaluate the usefulness of the total & differential white blood cell count, morphology of neutrophils, IT ratio, IM ratio, band cell count, platelet count, ESR and the C-reactive protein as an early indicator of neonatal septicaemia in 100 cases. This study was undertaken because these are simple bed-side and cost effective tests which can be done even if the baby is on antibiotic therapy.

After the first clinical suspicion of infection, blood was taken for blood culture, blood cell count with differential count & quantitative CRP & micro ESR.

Blood cultures were done in all cases & correlative evaluation between haematological parameters, C reactive protein & blood culture reports was done. 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.

Sepsis screen was considered positive if more than two criteria were positive out of the following parameters:

a) Elevated C –reactive protein [> 16 mgm /lit. on day 1 and 2 of life or more >10 mgm /lit. on subsequent days of life]
b) Elevated micro ESR i.e. > [age + 3] mm in first hour in less than 3 days old neonate or > 15 mm in first hour at any age.
c) Leukopenia i.e. TLC < 5000/cu mm.
d) Neutropenia i.e. Absolute neutrophil count < 1500/cu mm.[6]
e) Elevated ratio of immature granulocyte [band cells] to the sum of all neutrophils, granulocytes [i.e. I/T ratio > 0.20.]
f) Presence of toxic granules [eosinophilic granules in cytolplasm of neutrophil], cytoplasmic vacuolation & Dohles bodies [aggregates of rough endoplasmic reticulum which stains light blue on Giemsa stain] on the smear are suggestive of sepsis.
g) Decreased platelet counts < 1.5 lacs /cu mm.
h) Positive blood culture

OBSERVATIONS

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. Out of 100 cases number of early onset sepsis cases were more than late onset sepsis cases & they were 68 percent. Out of 100 cases studied, 72 were male and 28 were female with a ratio of 2.57:1.

In the present study out of 100 cases only 26 cases were culture positive, amongst which 22 cases of early onset sepsis & only 4 cases of late onset sepsis showed culture positivity.

Leucopenia was found in more cases than leucocytosis.

Neutropenia, I/T ratio and CRP were positive in higher proportion of culture positive cases in percent of 84.61, 84.61 & 92.30 respectively.

Neutropenia, Leucopenia/Leucocytosis, increased immature cells and Thrombocytopenia were positive in higher proportion in culture proven cases compared to culture negative cases.

Raised micro ESR & CRP showed higher number of false positive cases in respect to culture positivity.

All screening parameters were significant with respect to culture proven sepsis. The findings of various sepsis screen parameters in relation of blood culture positive & negative cases have been tabulated in Table 1

Table 1: Distribution Of Cases According To Sepsis Screen Perameters In The Present Study

S. No.	Parameters	Culture positive (n=26)		Culture negative (n=74)		Total cases	
		No.	Percentage	No.	Percentage	No.	Percentage
1	Raised micro ESR (> 15 mm in 1st hr or Age + 3 in 1st hr in < 3 days old)	20	76.92	50	67.56	70	70
2	TLC Less than 5000/cmm or 25000, 30000 & 21000 at birth, 12-24hrs & day 2 onwards.	21	80.76	20	27.02	41	41
3	ANC (< 1500/cmm)	22	84.61	15	20.27	37	37
4	I/T Ratio(>0.2)	22	84.61	10	13.51	32	32
5	I/M Ratio(>.3)	21	80.76	15	20.27	37	37
6	Platelet count (<1.5 lakh)	19	73.07	25	33.78	44	44
7	PBF (toxic granules, cytoplasmic vacuolations & Dohle bodies)	20	76.92	18	24.32	38	38
8	CRP (> 16mg/dl on 1st & 2nd day, > 10 mg on subsequent days)	24	92.30	66	89.18	90	90
9	>2 parameters positive	25	96.15	9	12.16	34	34

In present study CRP (92.30 percent) followed by Neutropenia & I/T ratio (84.61 percent) showed good sensitivity.

Highest specificity shown by I/T ratio (86.49 percent) followed by I/M Ratio & Neutropenia (79.72 percent).

If two or more of the above tests were positive, sensitivity of the screening tool increased above 90 percent & specificity above 80 percent. The sensitivity & specificity pattern of various sepsis screen parameters & their combination has been shown in Table 2.

Table 2: The Sensitivity & Specificity Of Sepsis Screen Parameters

S. No.	Screening parameters	Sensitivity (%)	Specificity (%)
1.	Raised micro ESR (> 15 mm in 1st hr or Age + 3 in 1st hr in < 3 days old)	76.92	32.43
2.	TLC (Less than 5000/cmm or 25000, 30000 & 21000 at birth, 12-24hrs & day 2 onwards.)	80.76	72.97
3.	ANC (< 1500/cmm)	**84.61**	**79.72**
4.	I/T Ratio(>0.2)	**84.61**	**86.49**
5.	I/M Ratio(>.3)	80.76	**79.72**
6.	Platelet count (<1.5 lakh)	73.07	66.21
7.	PBF (toxic granules, cytoplasmic vacuolations & Dohle bodies)	76.92	75.67
8.	CRP (> 16mg/lt on 1st & 2nd day, > 10 mg on subsequent days)	**92.30**	10.81
9.	>2 parameters positive	96.15	87.83

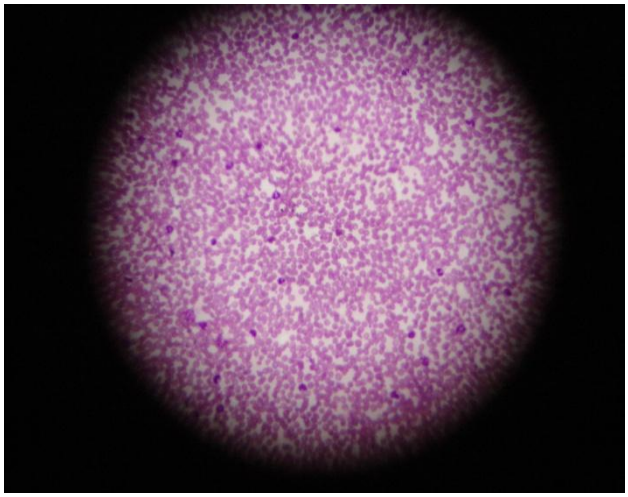

Figure 1: Showing leucocytosis in giemsa stain; X x 400

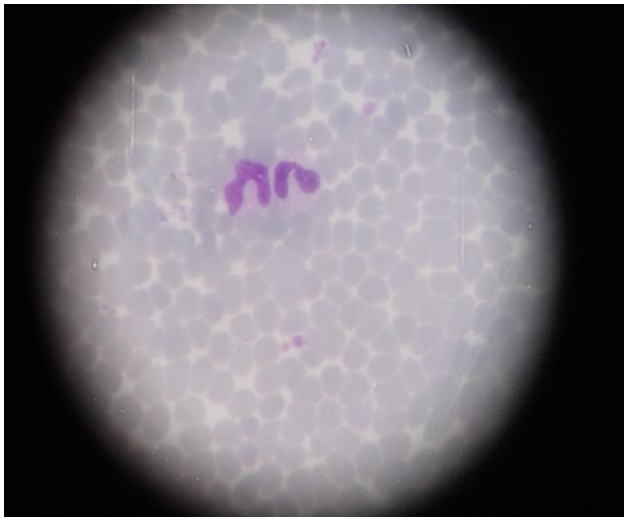

Figure 2: Showing band cells in giemsa stain; X x 1000

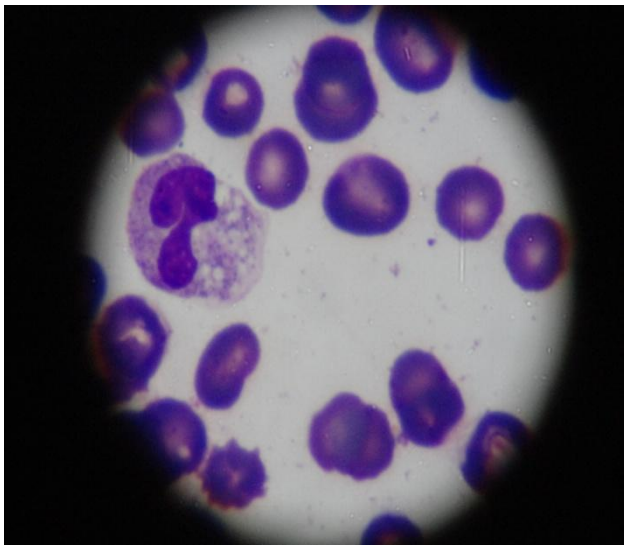

Figure 3: Showing immature polymorph with cytoplasmic vacuolations in giemsa stain; X x 1000

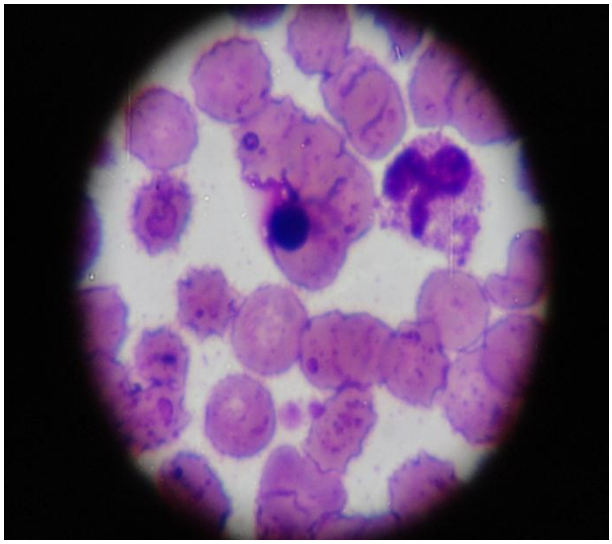

Figure 4: Showing immature polymorph with toxic granulations in giemsa stain; X x 1000

DISCUSSION

Neonatal septicemia is still a leading cause of mortality and morbidity in developing countries like India. The value of the sepsis screen is of prime significance in the diagnosis of neonatal septicaemia.

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. Blood culture is still the "Gold standard" for the diagnosis of septicemia in neonates and should be done in all cases of suspected septicemia.

In the present study Maximum neonatal sepsis cases (68%) were found of less than 3 days old age neonates (early onset septicemia) as compared to neonates aged more than 3 days (late onset septicemia) (32%), Similar observations were seen in the study done by National Neonatal and Perinatal Database[7] & Sriram[8].

Out of 100 cases, 72 patients were male & 38 were female with a high prevalence in the age group of 24-48 hours. Present study showed male preponderance with a ratio of 2.57:1. Khair et al[5] & Bhat & Rao[9] observed male, female ratio as 1.39:1 & 1.08:1 respectively.

In the present study, the blood culture yield was 26%. This was similar to the 28.6 - 42.2 % yield which was obtained by many authors (7,10). A much lower yield (14%) has been reported by Varsha et al[2]. 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.

Gram negative organisms formed the majority of the isolates as compared to Gram positive organisms (65.38% Vs 34.62%, respectively) in the present study. This is comparable with studies conducted by NNPD[7], Jaswal et al[10] & Sriram[8]. The most common organism isolated in present study was E.coli while in other studies it was Klebsiella Pneumoniae.

The traditional sepsis work up included various haematological parameters and CRP. In the present study, the sensitivity of the haematological screening parameters and CRP varied from 73.03-92.30%.

The total leukocyte count and the B: N ratios have been correlated with an increased risk of bacterial infections in neonates. However, they had a wide range of sensitivity (17-90%).[9] In our study, the sensitivity was 80.76% for TLC and B: N ratio. Neutropenia was observed in 84.61%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. Sharma et al[11] found a sensitivity of TG in 60% and CV of 15% among the proven sepsis cases. In contrast, we observed a sensitivity of 76.92% in TG/CV.

The micro-ESR is an inexpensive, easy bedside screening test. Its sensitivity ranges from 30% to 73% in proven sepsis cases.[2,9] In the present study, sensitivity of micro-ESR was 76.42%.

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.[9] The positivity of thrombocytopenia was 73.07% in our study.

Benitz et al[12] 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 et al[13], 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.[9] 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.[9] We observed a sensitivity of 96.15% by the combination of any two or more parameters.

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.

CONCLUSION

We concluded by present study that the value of the sepsis screen is more for excluding the diagnosis of neonatal septicaemia which can be done reasonably if two screens 12-24 hours apart are negative. The Hematologic profile that we studied is a simple, quick and cost effective tool in the early diagnosis of neonatal sepsis. The sensitivities of the traditional haematological screening parameters and CRP are satisfactory in neonates with blood culture positive early onset infections. The sensitivity of a combination of any two or more parameters is more satisfactory, 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. Blood culture is still the "Gold standard" for the diagnosis of septicemia in neonates and should be done in all cases of suspected septicemia. Since symptoms suggestive of sepsis may be caused by a variety of other illnesses, confirmation of sepsis by the sepsis screen tests may help in avoiding unnecessary antibiotic therapy.

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