ORIGINAL RESEARCH

To study CD64 expression on neutrophils as a novel biomarker in early diagnosis of sepsis in pediatric age group 0 - 18 year

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ABSTRACT

Introduction: 'Sepsis is defined as the systemic inflammatory response syndrome (SIRS) in the context of a suspected or confirmed infection, according to the international paediatric sepsis consensus conference.' CD64 is a leukocyte surface antigen that is expressed at low levels on nonactivated neutrophils' surfaces. The Fc receptor, which is increased during infection and sepsis, has a high affinity for CD64. In the event of bacterial infection, CD64 expression in preterm and term new born is comparable to that in older children and adults. Furthermore, CD64 expression is constant at room temperature for more than 30 hours, in contrast to CD11b and other PMN antigens, which are labile. Paediatric sepsis is rarely discussed as a primary cause of death in under developing nations. Early onset sepsis can be challenging because of overlapping clinical manifestations of a range of non-infectious illness such as aspiration syndrome, maladaptation & respiratory distress syndrome. **Materials and Methods:** This cross sectional study was conducted in Paediatric Emergency Ward, Neonatal and Paediatric Intensive Care Unit, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum, a teaching hospital affiliated with Jawaharlal Nehru Medical College, Belagavi from January 2020 to August 2021. Patients clinically suspected of having sepsis admitted to Paediatric Emergency Ward, Neonatal and Paediatrics Intensive Care Unit, Belagavi. All cases of clinically suspected sepsis admitted in Emergency paediatrics ward, NICU & PICU were included in the study. **Result:** Results shows that majority of the cases were in the age group of less than 1 month (64%), followed by 18% in 1 to 5 years.56% of patients were females and 44% were males. The female to male ratio was 1.27.out of total 50 cases, 46 cases show deranged white blood cell count in which 38 cases were blood culture positive and 8 cases were negative.27 cases show deranged absolute neutrophil count in which 21 cases were blood culture positive and 6 cases were negative. The general sensitivity of all the septic screenings is good, as can be observed. Sensitivity has been claimed to be as high.

INTRODUCTION

'Sepsis is defined as the systemic inflammatory response syndrome (SIRS) in the context of a suspected or confirmed infection, according to the international paediatric sepsis consensus conference.' The incidence of neonatal sepsis is 30 per 1000 live births in India, according to the national neonatal-perinatal database (2002–2003)[1,2].

Despite great advancements in care, sepsis remains the most prevalent critical condition in new-borns and is a significant source of morbidity and death.[3] When the immune system is stimulated, such as by bacterial infection, the immune response is closely regulated by balanced pro- and anti-inflammatory phases, which is assumed to be the mechanism or cause of sepsis. Given the high mortality rate of sepsis, effective therapy requires a diagnostic marker with high sensitivity and a near-100 percent negative predictive value. To identify neonatal sepsis various physiological markers, haematological indices and acute phase reactants have been studied[4,5]. Acute phase reactants test including C-reactive protein (CRP), Procalcitonin and cytokines and microbiologic cultures are still used in the laboratory evaluation of suspected sepsis infection[6]. Various leukocyte cell surface antigens have recently been investigated as possible diagnostic indicators for new born sepsis. CD11b, CD64, CD59, CD45RO, and CD25 are among them. CD64 has the best sensitivity and specificity of all these cell surface markers for identifying late-onset bacterial infection at the beginning and up to 24 hours after the first clinical manifestation[7]. CD64 is a leukocyte surface antigen that is expressed at low levels on nonactivated neutrophils' surfaces. The Fc receptor, which is increased during infection and sepsis, has a high

affinity for CD64. In the event of bacterial infection, CD64 expression in preterm and term new born is comparable to that in older children and adults[8]. Furthermore, CD64 expression is constant at room temperature for more than 30 hours, in contrast to CD11b and other PMN antigens, which are labile[9,10,11]. Paediatric sepsis is rarely discussed as a primary cause of death in under developing nations[12]. Early onset sepsis can be challenging because of overlapping clinical manifestations of a range of non-infectious illness such as aspiration syndrome, maladaptation & respiratory distress syndrome.

OBJECTIVE

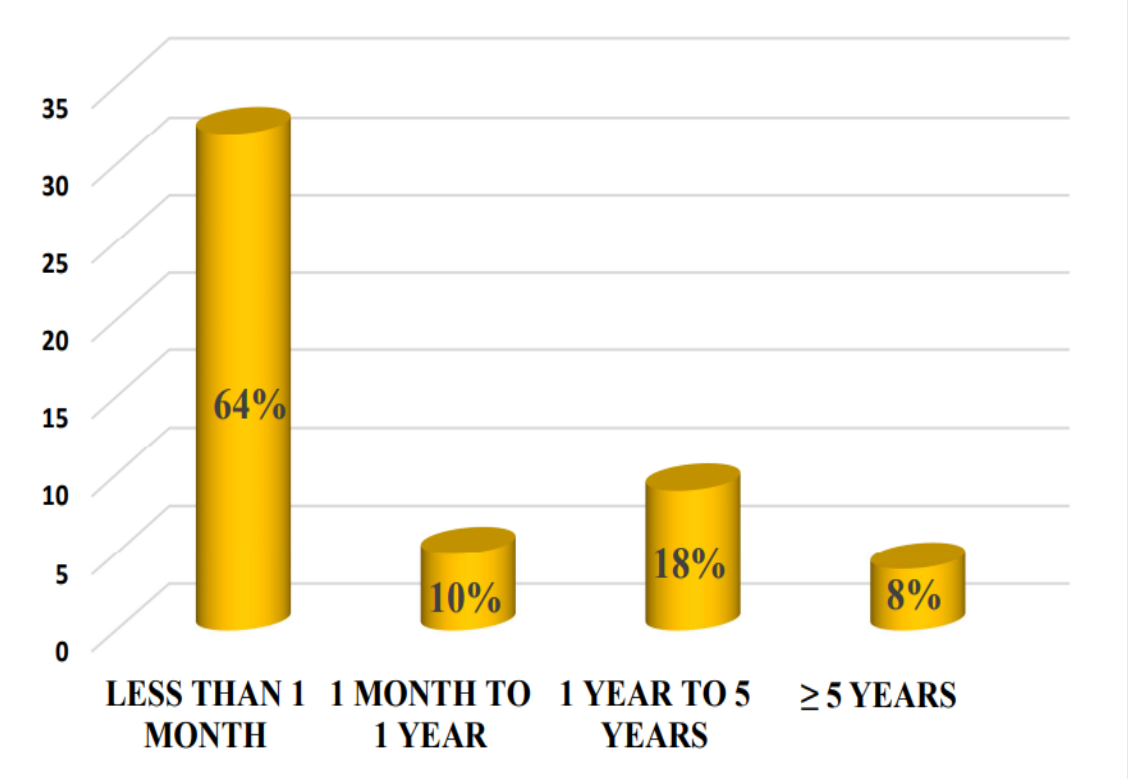
To study CD64 expression on neutrophil granulocyte as a novel biomarker in early diagnosis of sepsis.

MATERIALS AND METHODS

This cross sectional study was conducted in Paediatric Emergency Ward, Neonatal and Paediatric Intensive Care Unit, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum, a teaching hospital affiliated with Jawaharlal Nehru Medical College, Belagavi from January 2020 to August 2021. Patients clinically suspected of having sepsis admitted to Paediatric Emergency Ward, Neonatal and Paediatrics Intensive Care Unit, Belagavi. All cases of clinically suspected sepsis admitted in Emergency paediatrics ward, NICU & PICU were included in the study. Patients who have already received or receiving antibiotics and patient who developed sepsis post-surgery were excluded from the study. Study was carried out after obtaining permission from Institutional Ethics Committee. Sample size of the study was 50. Demographic data and clinical details relevant to the study were collected using a specially designed data collection instrument. An extensive clinical examination was performed, including anthropometry and vital signs, as well as a systemic examination. These findings were documented on a proforma that had been created and evaluated in advance. The blood samples were collected by the Neonatal and paediatric Emergency and ICU staff nurses/ residents under aseptic precautions and sent to the central laboratory immediately. The data was entered in the form of a data matrix in Microsoft Excel® and statistically analysed using IBM Statistical Package for the Social Sciences (SPSS) software version 23.0. Paired t-test were used for comparing mean values.

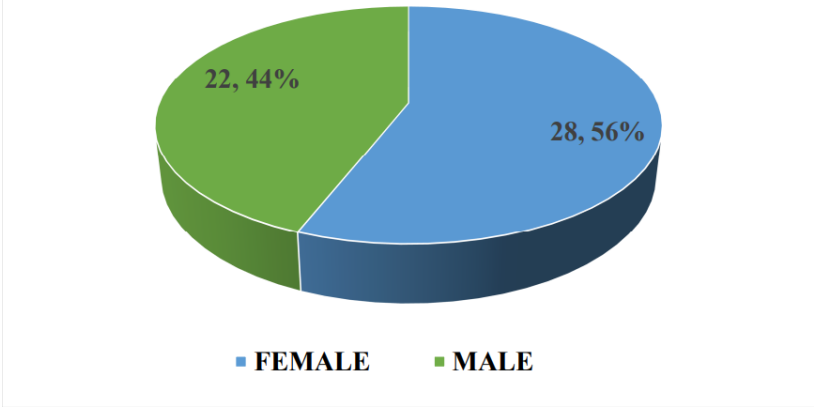
RESULT

Figure 1. Age Distribution



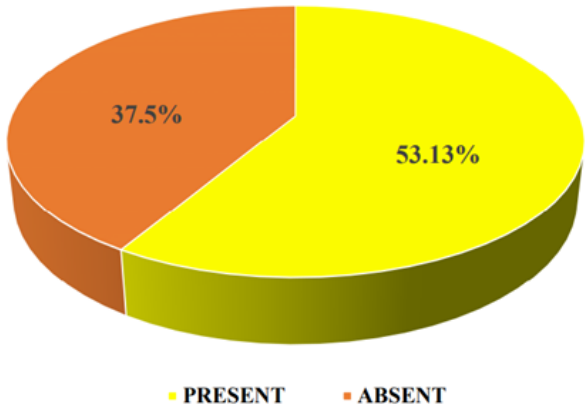
Results shows that majority of the cases were in the age group of less than 1 month (64%), followed by 18% in 1 to 5 years.

Figure 2: Gender Distribution Of The Sample



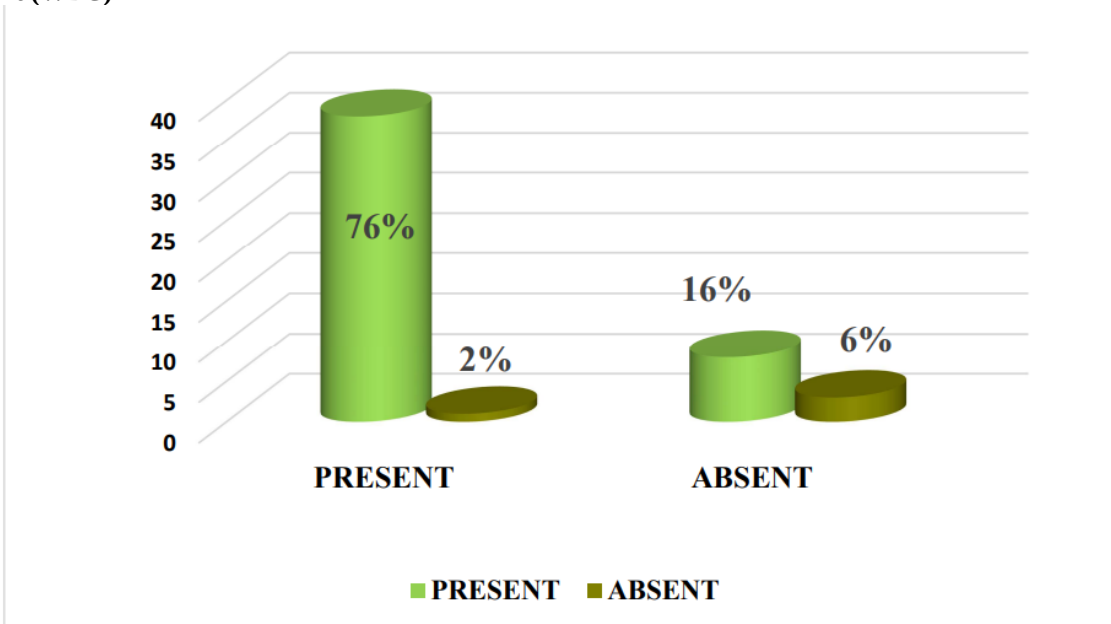
In present study, 56% of patients were females and 44% were males. The female to male ratio was 1.27.

Figure 3: Distribution Of Sepsis Cases For Age < 1 Month According To Hematological Scoring System (HSS)



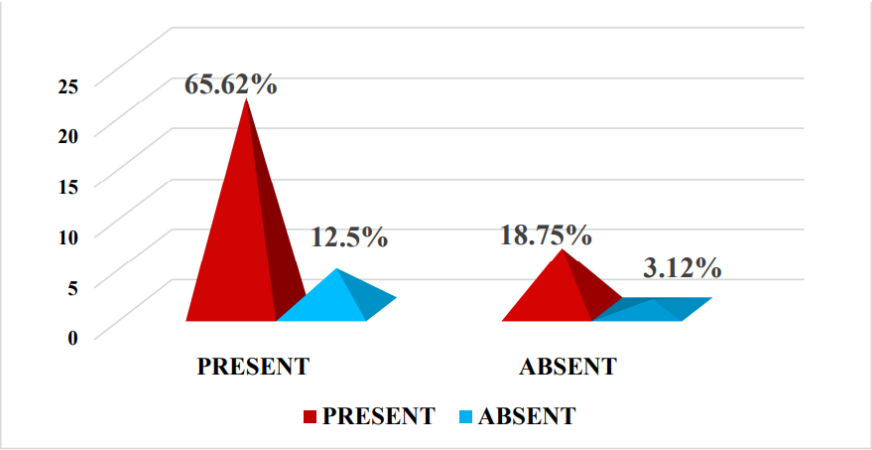
Result showed that 37.5% had sepsis cases for age < 1 month according to hematological scoring system (HSS)

Figure 4. Distribution Of Sepsis Cases For Age 0 – 18 Years With Respect To Total White Blood Cell Count (WBC)



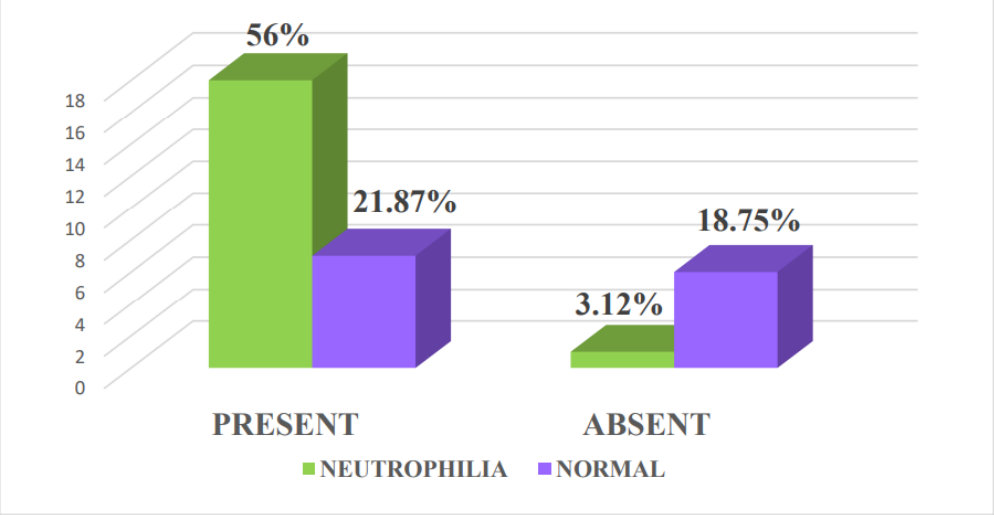
In present study, out of total 50 cases, 46 cases show deranged white blood cell count in which 38 cases were blood culture positive and 8 cases were negative.

Figure 5. Distribution Of Sepsis Cases For Age < 1 Month With Respect To Absolute Neutrophil Count (ANC)



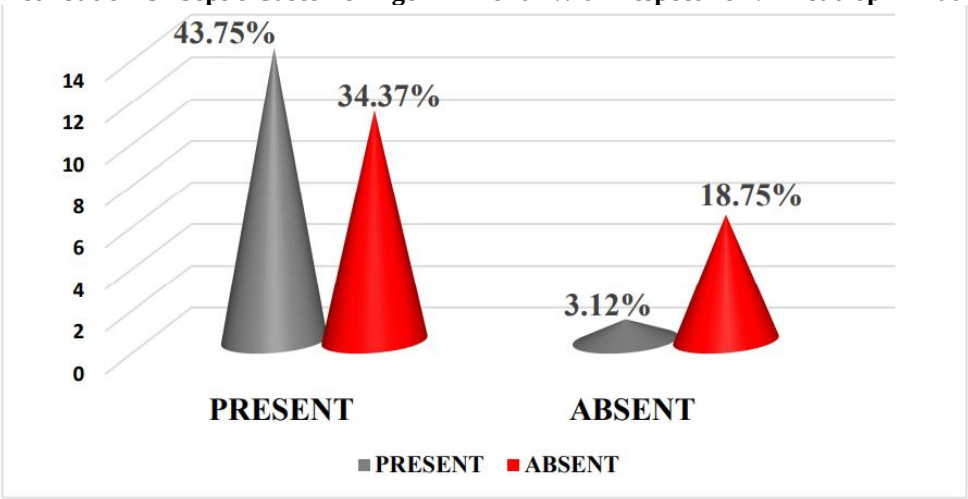
In present study, out of total 32 cases, 27 cases show deranged absolute neutrophil count in which 21 cases were blood culture positive and 6 cases were negative.

Figure 6. Distribution Of Sepsis Cases For Age < 1 Month With Respect To Immature Neutrophil Count (INC)



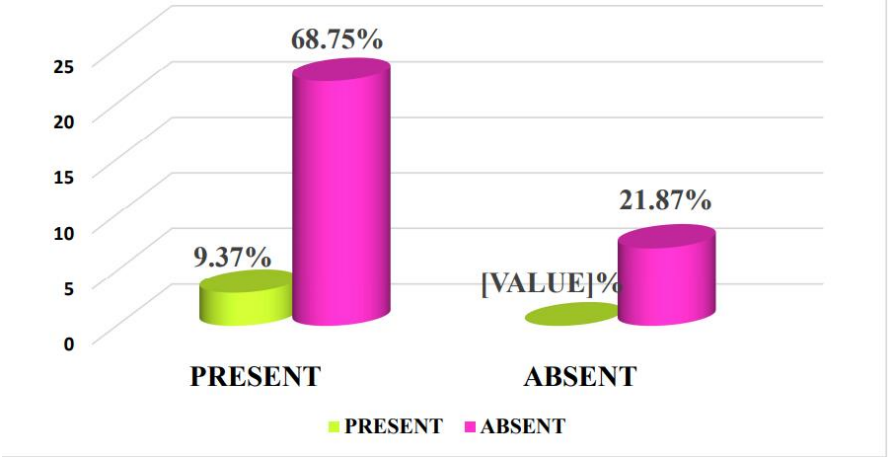
In present study, out of total 32 cases, a positive test result, i.e. an increased immature neutrophil cell count was seen in total 19 cases, out of which 18 cases were blood culture positive and 1 case was negative.

Figure 7. Distribution Of Sepsis Cases For Age < 1 Month With Respect To I:T Neutrophil Ratio



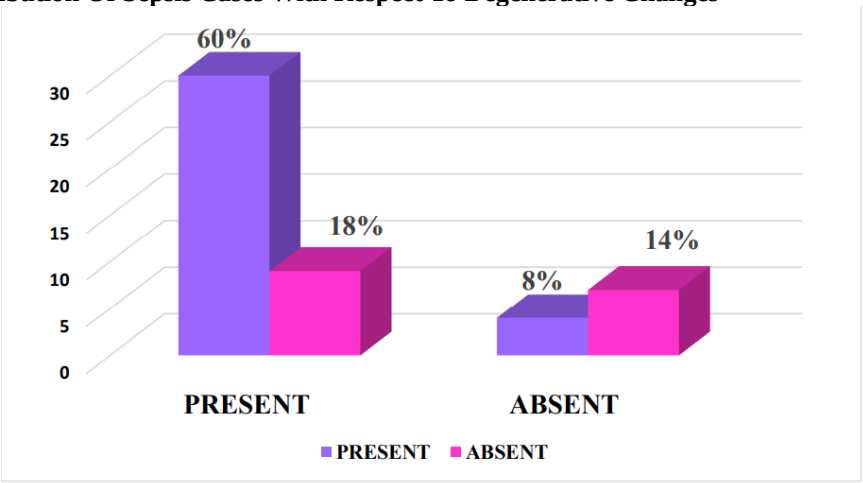
In present study, out of total 32 cases, an abnormal increased I:T ratio was observed in 15 cases, out of which 14 cases were blood culture positive and rest 1 case negative.

Figure 8. Distribution Of Sepsis Cases For Age < 1 Month With Respect To I:M Neutrophil Ratio



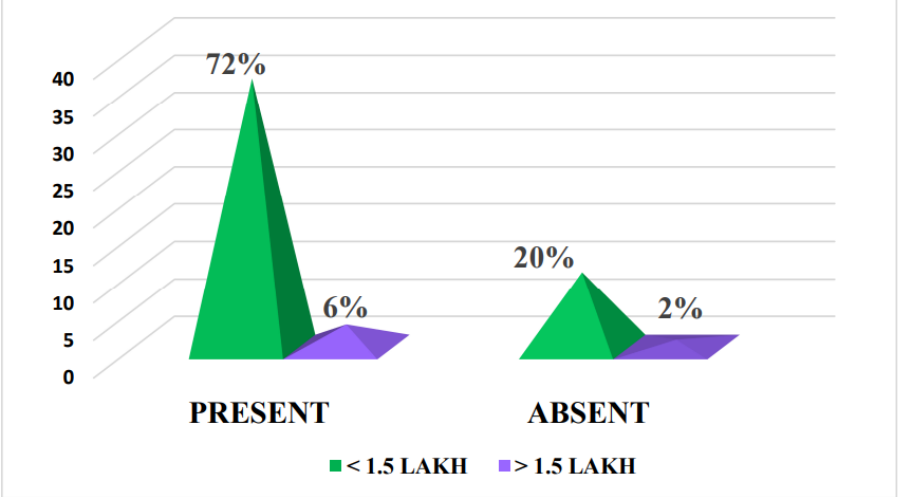
In present study, out of total 32 cases, an abnormal increased I:M ratio was observed in 3 cases, out of which all 3 cases were blood culture positive.

Figure 9. Distribution Of Sepsis Cases With Respect To Degenerative Changes



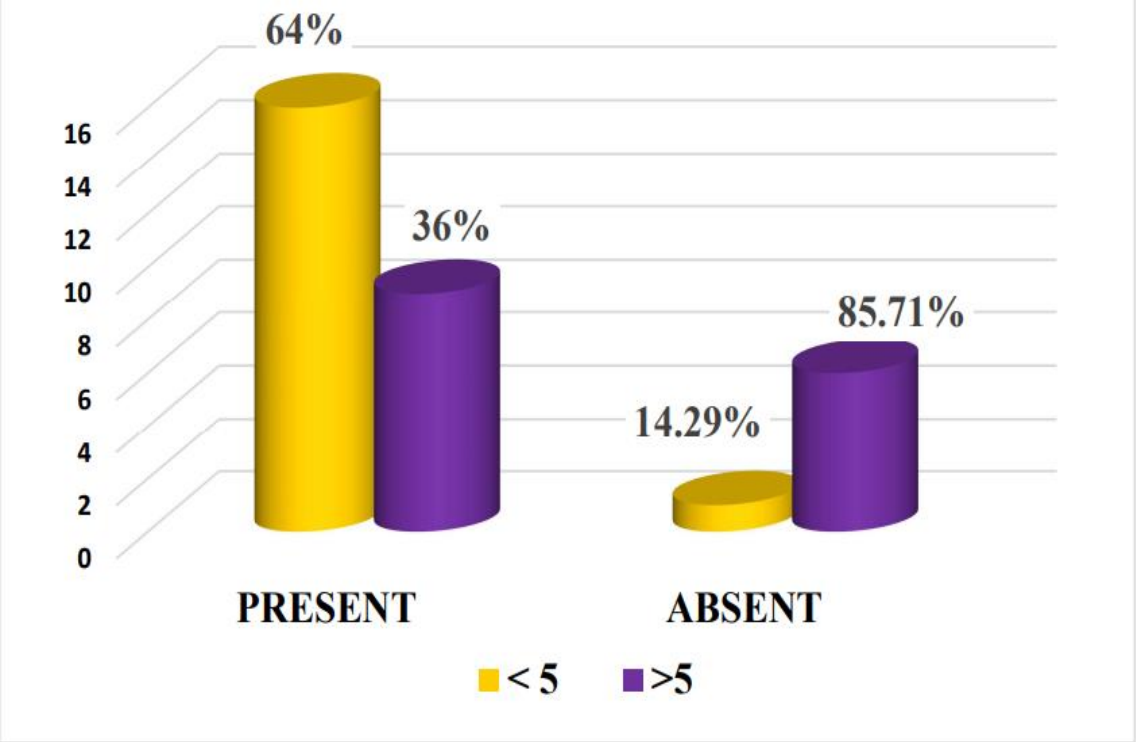
Out of total 50 cases, degenerative changes were observed in 34 cases, out of which 30 cases were blood culture positive and rest 4 cases were culture negative.

Figure10. Distribution Of Sepsis Cases With Respect To Platelet Count



Out of total 50 cases, reduced platelet count was seen in 46 cases, out of which 36 cases were blood culture positive and 10 cases were culture negative.

Figure 11. Distribution Of Sepsis Cases For Age < 1 Month With Respect To Hss Scoring System



In present study, out of total 32 cases, 15 cases show HSS Score ≥ 5 in which 9 cases were blood culture positive and 6 cases were negative.

Figure 12. Roc Curve Plotted For Hss Score, I: M Neutrophil Ratio And I:T Neutrophil Ratio

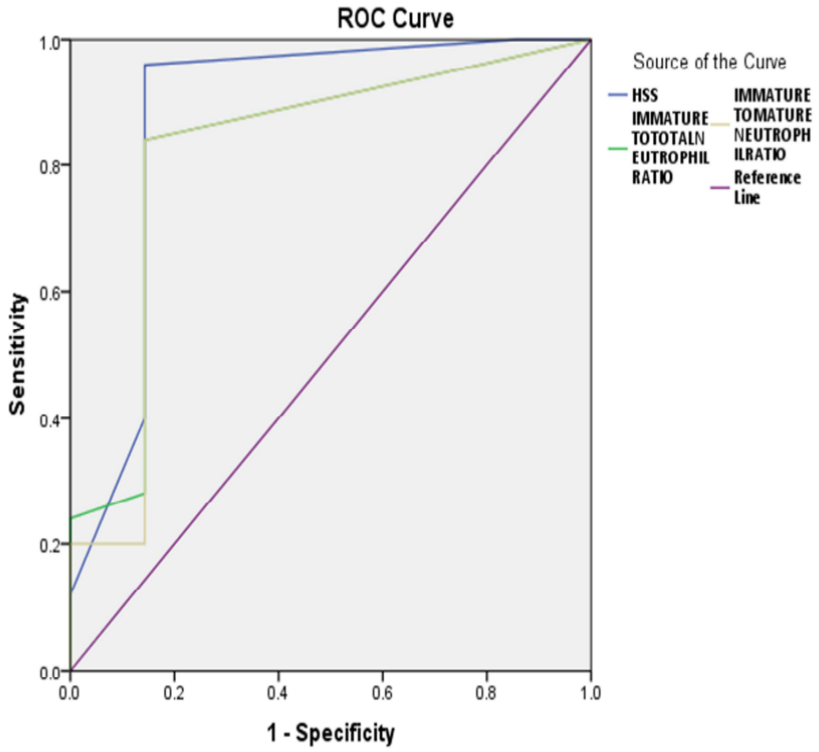


Table 1: Area Under The Curve

Test Result Variable(s)	Area
HSS SCORE	0.8800
IMMATURE TO TOTAL NEUTROPHIL RATIO	0.8257
IMMATURE TO MATURE NEUTROPHIL RATIO	0.8171

Figure 13. ROC Curve Plotted For WBC, ANC, Platelet Count, C Reactive Protein And Cd64

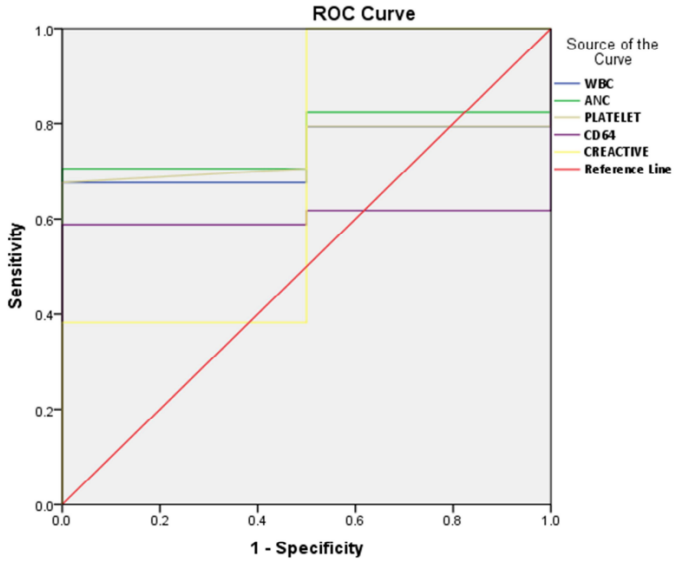


Table 2: Area Under The Curve

Test Result Variable(s)	Area
WBC	0.7353
ANC	0.7647
PLATELET	0.7426
CD64	0.6029
C REACTIVE	0.6912

Table 3: Comparison Of Diagnostic Values Of Various Tests

	SENSITIVITY	SPECIFICITY	PPV	NPV	P VALUE
TYPE OF ABNORMALITY	%	%	%	%	<0.05
ABNORMAL WBC COUNT	97.44	27.27	82.61	75	0.0079 (significant)
ANC	84	14.29	77.78	20	0.9122 (not significant)
INC	72	85.71	94.74	46.15	0.0058 (significant)
I : T RATIO	56	85.71	93.33	35.29	0.0509 (not significant)
I : M RATIO	12	100	100	24.14	0.3362 (not significant)
DEGENERATIVE CHANGES	76.92	63.64	88.24	43.75	0.0113 (significant)
PLATELET COUNT	92.31	9.09	78.26	25	0.8796 (not significant)
HSS SCORE > 5	64	85.71	94.12	40	0.0204 (significant)
C REACTIVE PROTEIN	100	87.5	97.14	100	0.0001 (significant)
NEUTROPHIL CD64	100	63.64	90.7	100	0.0001 (significant)

Figure 14: Toxic Vacuolations & Toxic Granules In Neutrophils

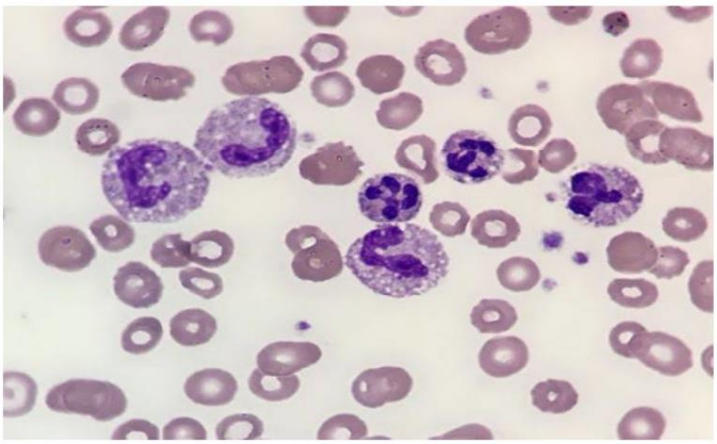
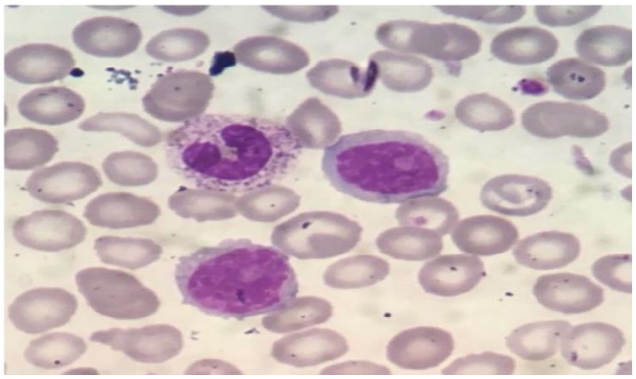


Figure 15. Band Form Of Neutrophil With Toxic Granulations



DISCUSSION

Parameters used in hematological scoring profile total leucocyte count: Individual assays for neonatal sepsis screening did not produce test findings that indicated a high sensitivity or specificity, thus they could not be relied on to establish a correct and trustworthy diagnosis. The Haematological Scoring System, often known as the Septic Screen, was established as a result of this. Many research on testing for new born sepsis have been conducted throughout the years. The majority of these scoring systems employ changes in WBC quality and quantity, as well as changes in platelets, as criteria. Micro-ESR and CRP have also been used in other research as part of the grading system[13,14]. The seven parameters listed above were included in the current investigation. As shown below, the diagnostic value of platelet count was compared to that of previous studies: The general sensitivity of all the septic screenings is good, as can be observed. Sensitivity has been claimed to be as high as 100% by certain studies. The scores also influence sensitivity and specificity. It's clear that when the cut-off score rises, the sensitivity drops slightly, followed by a dramatic rise in specificity. A score of more than 5 on the HSS was shown to be a better choice between sensitivity and specificity. Because no single haematological marker is preferable than another in terms of accurately predicting neonatal sepsis, a combination of all of these indicators is utilised for HSS. The results of our study didn't match with other previous studies except Rodwell et al. This can be because of previous studies conducted tool HSS score >3 – 4 as their sepsis criteria which differ from our study.[15,16,17]

Table 42: Comparative Study Of Diagnostic Value: HSS/Septic Screen

Study	Year	Parameter	Sen %	Spe %	PPV %	NPV %
Namdeo et al[115]	1985	Score≥2	88	50	61	67
Rodwell et al[54]	1988	Score≥3	96	78	31	99
Rodwell et al[54]	1988	Score≥4	89	89	45	99
Rodwell et al[54]	1988	Score≥5	41	96	52	94
Buch et al[116]	2011	Score≥3	80	87	88.14	78.69
Saleem et al[111]	2014	Score≥3	90	74.5	65.9	93.2
Present Study	2021	Score≥5	64	85.71	94.12	40

C REACTIVE PROTEIN (CRP): This test has a significant high true positive (deranged c reactive protein values and blood culture positive) and had a high true negative (no deranged C reactive protein values and blood culture negative). Thus this test has a high sensitivity but moderate sensitivity as seen in

all previous studies.The results of our current study i.e specificity and negative predictive value matched with Khassawneh M et al but not sensitivity because CRP is a non-specific marker of inflammation and elevated during minor infections and may not reflect infection severity.[18]

NEUTROPHIL CD64: This test has a significant high true positive (deranged neutrophil CD64 expression values and blood culture positive) and had a high true negatives (few or no deranged neutrophil CD64 expression values and blood culture negative). Thus this test has a high sensitivity but low specificity as seen in all previous studies.The results of our current study was not comparable with previous studies because there is a wide range of criteria for choosing patients, as well as discrepancies in the diagnosis of the disease and many of the previously done studies were retrospective meta analysis of confirmed blood culture patients only[19,20].

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