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# Role of modified haematological sepsis score and red cell distribution width in early diagnosis of neonatal sepsis

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**Abstract:** *Background:* Neonatal sepsis is one of the leading causes of morbidity and mortality in neonates. The subtle clinical signs may sometimes go unnoticed. Blood culture is considered to be the gold standard, however it is time consuming. This may result in unnecessary and prolonged exposure to antibiotics. Haematological sepsis score and red cell distribution width have been known since long, however not being used routinely. These are easily available, rapid and economical. *Objectives:* To assess the predictive role of Modified Haematological Sepsis Score and Red Cell Distribution Width in early diagnosis of neonatal sepsis. *Methods:* We conducted a cross sectional, descriptive study, involving 200 neonates with sepsis, admitted in postnatal wards or neonatal intensive care unit (NICU) of a tertiary care hospital in Southern India, over a period of 5 years. Modified haematological sepsis score (HSS-M) and red cell distribution width (RDW) reports of these neonates were evaluated against blood culture and C-reactive protein (CRP), using Receiver Operating Characteristic (ROC) curve, sensitivity and specificity. A p value of <0.05 was considered statistically significant. *Results:* Out of the 200 neonates with sepsis, 143 (71.5 %) had early onset neonatal sepsis. Of all the sepsis cases, only 27 (13.5%) were culture proven. Pre labor rupture of membranes (PROM) was the most common maternal risk factor (16.5%) and prematurity (33%) along with low birth weight (30%) were the most common neonatal risk factors for sepsis. Most common manifestation of sepsis in neonates was respiratory distress (93.5%). HSS-M showed excellent diagnostic predictability when compared with blood culture [Area under curve (AUC) – 0.946] and CRP (AUC – 0.882), and it was observed to be a highly sensitive screening test (100%). RDW showed a fair sensitivity and a high negative predictive value when compared against blood culture and CRP. *Conclusion:* HSS-M and RDW have a good diagnostic predictability in both probable and proven sepsis and are also convenient and affordable.

**Keywords:** Culture Proven Sepsis; Modified Haematological Sepsis Score; Neonatal Sepsis; Probable Sepsis; Red Cell Distribution Width.

## Introduction

Neonatal sepsis is an important cause of neonatal morbidity and mortality in developing countries with close to 2 million deaths every year [1]. It is characterized by clinical symptoms and signs, accompanied by bacteremia in the first 28 days of life [2-3]. According to World Health Organization (WHO), neonatal deaths contribute to 40% of all deaths in children below 5 years of age [4]. With 2.7 million deaths annually, global neonatal mortality rate (NMR) stands at 19/1000 live births [5]. India accounts for NMR of 27.7, 33% being due to neonatal infections [6-7]. Diagnosing sepsis in neonates may be difficult as the early signs may be subtle and different at different gestational ages. This variability in clinical presentation makes clinical diagnosis of sepsis difficult [8]. To facilitate the clinicians objective, which is to identify all possible sepsis cases and initiate early antibiotic therapy[9], laboratory investigations have considerable relevance [2]. With early diagnosis and treatment, neonates are not likely to experience long-term health problems; however, if early signs are missed, mortality increases [10].

Mortality from neonatal sepsis may be as high as 50% for infants who are not treated, reducing the case fatality rate to as low as

10% in cases promptly diagnosed and treated [11]. Hence, the timely diagnosis of neonatal sepsis is critical.

The definitive diagnosis of septicemia is made by a positive blood culture, which is considered as a Gold standard but it requires a minimum of 48-72 hours and yields a positive result in only 10-60% of cases [2]. Inability to adequately exclude the diagnosis of neonatal sepsis can result in unnecessary and prolonged exposure of the newborn to antibiotics. Hence various adjunctive tests like C-Reactive Protein (CRP), Micro-Erythrocyte Sedimentation Rate, Immature to Total neutrophil ratio (commonly called as sepsis screen tests) along with other haematological indices, acute phase reactants, protein markers, cytokine levels etc are being extensively studied for early diagnosis of sepsis, in order to guide the clinical management, of which, haematological parameters would have the advantage of being rapid, easy to perform and cost effective [8].

Haematological Sepsis Score (HSS): Rodwell et al [12] gave a haematological sepsis scoring system for early diagnosis of neonatal sepsis based on 7 haematological parameters and by assigning a score to each of the parameter where total score could be from 0-8. Score $\leq$2 suggests sepsis to be unlikely and $\geq$5 suggests very high probability of sepsis. Although the scoring is known for long, it is hardly used in routine clinical practice and researchers have suggested a need for a simplified and standardized interpretation of this score [9].

Modified Haematological Sepsis Score (HSS - M): Modified HSS developed by Krishnamurthy V et al [11] has good diagnostic accuracy with sensitivity and specificity of 84% and 82% respectively and it consists of only 6 parameters, immature neutrophils and immature to mature neutrophil ratio has been replaced by nucleated red blood cells (nRBCs), which may be elevated in stressful conditions. Neutropenia has been given a higher weightage in modified HSS as shown in table 1.

**Table-1: Scores given to parameters in HSS and Modified HSS (Table used with permission from Krishnamurthy V et al [11])**

| Parameter | Value | HSS | Modified HSS |
|---|---|---|---|
| Total leucocyte count | < 5000 | 1 | 2 |
| | >25000 (at birth) | 1 | 1 |
| | >30000(12-24 hrs) | 1 | 1 |
| | >21000(day 2 onwards) | 1 | 1 |
| | Normal | 0 | 0 |
| Total neutrophil count | No neutrophils | 2 | 2 |
| | Increased/Decreased | 1 | ½ |
| | Normal (1800-5400/$mm^3$) | 0 | 0 |
| Immature neutrophils | Increased | 1 | NA |
| | Not increased | 0 | NA |
| Immature : total neutrophil ratio (IT ratio) | >0.2 | 1 | 1 |
| | <0.2 | 0 | 0 |
| Immature: mature neutrophil ratio (IM ratio) | >0.3 | 1 | NA |
| | <0.3 | 0 | NA |
| Degenerative changes | Present | 1 | 1 |
| | Absent | 0 | 0 |
| Platelet count | <150000 | 1 | 1 |
| | >150000 | 0 | 0 |
| Nucleated RBC | >5% | NA | 1 |
| | <5% | NA | 0 |

Red Cell distribution width (RDW): Red cell distribution width is defined as a numerical measure of size variability of erythrocytes in circulation [13-14]. It is calculated by dividing standard deviation(SD) of red blood cell volume by the mean corpuscular volume (MCV) and multiplied by 100. Higher RDW levels are suggested to be associated with inflammatory process. It is a part of routine complete blood count and can be obtained quickly without any additional cost. Few studies have compared the use of RDW as a potential independent predictor of clinical outcome in adults with sepsis [15-16], as against pediatric sepsis and concluded that further studies are required in pediatric and neonatal population to establish the relation.

The current study aims to assess the predictive role of Modified Haematological Sepsis Scoring system and red cell distribution width in facilitating early diagnosis of neonatal sepsis, being faster and cheaper over most of the other available sepsis screening tests.

## Material and Methods

*Study design:* Cross sectional, descriptive study.

*Study setting:* Neonatal Intensive Care Unit (NICU) and postnatal wards of Father Muller Medical College Hospital (FMMCH), Karnataka State, India.

*Source of data:* Clinical assessment, diagnosis and management details of neonates with sepsis were noted and maternal details were collected from maternal case sheets, using a pre validated, structured proforma.

*Study period:* From January 2018, after obtaining approval from Institutional Ethical committee (Ref No. FMMC/FMIEC/4400/2017) till December 2022 (5 years).

*Sample size:*

$$n \geq \frac{Z^2_{1-\alpha/2} \times p(1-p)}{d^2}$$

With p=14% [17] which was the incidence of neonatal sepsis in a multicenter cohort study done in Delhi, with power of 80%, alpha at 0.05 and allowable error of 5%, n=186, rounded off to 200 and consecutive sampling was done till sample size was reached.

*Inclusion criteria:* Neonates with probable diagnosis of sepsis, term or preterm neonates with risk factors or clinical suspicion of sepsis were included.

*Exclusion criteria:* Neonates with congenital malformations, suspected congenital infections like TORCH, all suspected cases of pathological jaundice other than sepsis, neonates with suspected inborn errors of metabolism, neonates who had undergone exchange transfusion, suspected haemolytic diseases in neonates, those neonates who have had more than one episode of nosocomial infection, neonates with lethal conditions on palliative care, neonates in whom maternal case sheets/ risk factors could not be obtained were excluded.

*Study technique:* After application of the above criteria and obtaining written informed consent from the parents, blood samples were collected within 6 hours of admission in the post natal wards or NICU from neonates with suspected sepsis, under complete aseptic precautions by peripheral venepuncture and used for the following:

(i) Sample in Ethylene diamine tetra acetic acid (EDTA) containing vacutainer was used to do a complete blood count using a Coulter machine (BC-750). There after total and differential leucocyte count, platelet count, as well as total, mature and immature Polymorphonuclear neutrophil (PMN) count was calculated using Cell counter.
(ii) Red cell distribution width (RDW) was assessed from the same sample.
(iii) Peripheral smear was prepared, stained with Leishmann stain and examined under oil immersion lens of light microscope at a magnification of 1000 x. Also degenerative changes in neutrophils like cytoplasmic vacuolations, toxic granulations, presence of Dohle bodies were noted. Number of nucleated red blood cells per 100 white blood cells was counted.

Based on clinical and laboratory parameters sepsis was broadly classified under 2 categories in our study for analysis purpose:

1) *Proven sepsis* [11]: Blood culture positive in the presence of clinical features suggestive of sepsis and/or presence of risk factors for sepsis.
2) *Probable sepsis (Clinical sepsis)* [11]: Presence of clinical features and risk factors with sepsis screen positive but blood culture negative. As per our NICU protocol we took probable sepsis to be C-Reactive Protein (CRP) $\geq$ 6 mg/L [18].

HSS-M and RDW values obtained were evaluated against blood culture report in proven sepsis and against CRP in probable sepsis.

*Normal values of laboratory variables considered in our study:*

- HSS-M : $\leq$2 – sepsis unlikely; 3-4 – sepsis possible; $\geq$5 – sepsis very likely [11].
- *RDW :* Term neonates – 15.5-20% , preterm neonates– 15.5-23% [19].
- *CRP :* <6 mg/L [18].

*Statistical analysis:* Data was entered in Microsoft Excel 2007 and analyzed using SPSS version 23.0 (IBM Corp., Armonk, NY). Categorical data was analyzed by Chi square test, summary statistics being presented as Odds ratio with 95% confidence interval. Sensitivity, specificity was computed for HSS-M and RDW against blood culture and CRP, an ROC curve was plotted to determine the optimal cut off point with maximal sensitivity and specificity. Diagnostic ability of the score was assessed using area under the curve with 95% Confidence interval. p value <0.05 was considered statistically significant. Descriptive data was presented as tables and figures as appropriate.

## Results

In our study, 200 neonates were included, out of which 120 were male babies (60%) and 80 were female babies (40%). Among the mothers, 134 (67%) were multiparous, 104 (52%) were residents of rural areas and 145 (72.5%) belonged to lower middle class families. Only 70 (35%) mothers received education beyond high school and were employed in earning jobs. Of the 200 babies, majority (138 i.e. 69%) were delivered by vaginal route.

Out of the 200 neonates, 143 (71.5%) had early onset sepsis and 57 (28.5%) had late onset sepsis. Only 27 (13.5%) cases of neonatal sepsis were culture proven. Among the maternal risk factors, pre labor rupture of membranes (PROM) was found to be the most common (16.5%), whereas prematurity (33%) and low birth weight (30%) were found to be the commonest neonatal risk factors. Respiratory distress in the form of tachypnea and chest wall retractions was found to be the most common manifestation (93.5%) of neonatal sepsis in our study, as shown in table 2.

**Table-2: Risk factors and clinical presentation of neonatal sepsis**

| S. no | Risk factors and clinical presentation | Number (N) * | % |
|---|---|---|---|
| 1. | **Maternal risk factors** | | |
| | a. PROM | 33 | 16.5 |
| | b. Pregnancy induced hypertension | 18 | 9 |
| | c. Maternal infections including chorioamnionitis | 14 | 7 |
| | d. Preterm labor | 13 | 6.5 |
| | e. Gestational diabetes | 4 | 2 |
| | f. Meconium stained amniotic fluid | 4 | 2 |
| 2. | **Neonatal risk factors** | | |
| | a. Prematurity | 66 | 33 |
| | b. Low birth weight | 60 | 30 |
| | c. Invasive management in NICU | 14 | 7 |
| | d. Congenital heart disease | 5 | 2.5 |
| | e. Delayed enteral feeding | 5 | 2.5 |
| | f. Birth asphyxia | 2 | 1 |

| S. no | Risk factors and clinical presentation | Number (N) * | % |
|---|---|---|---|
| 3. | **Clinical features** | | |
| | a. Respiratory distress | 187 | 93.5 |
| | b. Feed intolerance | 33 | 16.5 |
| | c. Jaundice | 22 | 11 |
| | d. Metabolic abnormalities | 19 | 9.5 |
| | e. Poor activity | 10 | 5 |
| | f. Seizures | 7 | 3.5 |
| 4. | **Type of sepsis** | | |
| | a. Culture proven | 27 | 13.5 |
| | b. Probable sepsis | 173 | 86.5 |
| 5. | **Time of onset of sepsis** | | |
| | a. Early onset sepsis | 143 | 71.5 |
| | b. Late onset sepsis | 57 | 28.5 |
| *N=200 | | | |

In our study, culture proven sepsis was 7 times more likely when latency period following PROM was more than 24 hours and this was statistically significant (p=0.01). Other risk factors for culture proven sepsis included Caesarean sections, presence of maternal infections in peri-partum period, preterm gestation, low birth weight, need for resuscitation and invasive procedures in neonates with a highly significant p value (p<0.001).

Neonatal sepsis cases were 10 times more likely to be culture proven in case of late onset sepsis as compared to early onset sepsis, in our study. It was observed that prophylactic antibiotics given to mother in peri-partum period had no protective role in our study. In fact, high statistical significance (p<0.001) was seen with culture proven neonatal sepsis cases being diagnosed in babies born to mothers who had received prophylactic antibiotics, as shown in table 3.

**Table-3: Association of risk factors with culture proven neonatal sepsis**

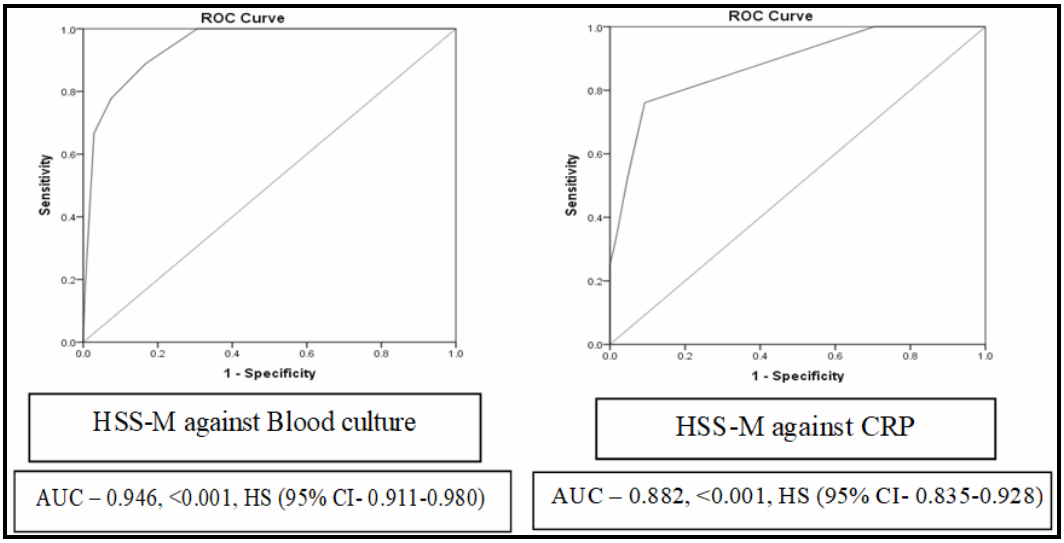
| Risk factors | Type of neonatal sepsis | | Odds ratio (95% C.I.) | P value |
|---|---|---|---|---|
| | Probable | Proven | | |
| **Latency period from PROM*** | | | | |
| ≥ 24 hours | 8 (47.1%) | 9 (52.9%) | 7.87 (1.35-45.83) | 0.01, sig |
| <24 hours | 14 (87.5%) | 2 (12.5%) | | |
| **Gestation**** | | | | |
| Preterm | 50 (70.4%) | 21 (29.6%) | 8.61 (3.28-22.59) | <0.001, HS |
| Term | 123 (95.3%) | 6 (4.7%) | | |
| **Mode of delivery**** | | | | |
| Caesarean section | 43 (69.4%) | 19 (30.6%) | 7.18 (2.93-17.57) | <0.001, HS |
| Vaginal | 130 (94.2%) | 8 (5.8%) | | |
| **Maternal infections**** | | | | |
| Present | 22 (66.7%) | 11 (33.3%) | 4.71 (1.94-11.47) | 0.001, HS |
| Absent | 151 (90%) | 16 (10%) | | |
| **Prophylactic antibiotic usage in mother**** | | | | |
| Yes | 67 (75.3%) | 22(24.7%) | 6.96 (2.51-19.26) | <0.001, HS |
| No | 106 (95.5%) | 5 (4.5%) | | |
| **Birth weight**** | | | | |
| Low | 40 (66.7%) | 20 (33.3%) | 9.5 (3.74-24.08) | <0.001, HS |
| Normal | 133 (95%) | 7 (5%) | | |

| Risk factors | Type of neonatal sepsis | | Odds ratio (95% C.I.) | P value |
|---|---|---|---|---|
| | Probable | Proven | | |
| **Need for resuscitation at birth**** | | | | |
| Present | 30 (63.8%) | 17 (36.2%) | 8.1 (3.37-19.43) | <0.001, HS |
| Absent | 143 (93.5%) | 10 (6.5%) | | |
| **Neonatal invasive procedures**** | | | | |
| Present | 22 (53.7%) | 19 (46.3%) | 16.3 (6.37-41.7) | <0.001, HS |
| Absent | 151 (95%) | 8 (5%) | | |
| **Time of onset of sepsis**** | | | | |
| Late onset | 37 (64.9%) | 20 (35.1%) | 10.5 (4.12-26.7) | <0.001, HS |
| Early onset | 136 (95.1%) | 7 (4.9%) | | |
| *N=33, **N=200 | | | | |

Modified haematological sepsis score (HSS-M) showed excellent diagnostic predictability [Area under curve (AUC) – 0.946)] when compared against blood culture in neonatal sepsis and this was highly statistically significant (p<0.001). Based on validity scores and value judgment, HSS- cut off score of 3 was derived and it was noted that all 27 culture proven sepsis cases in our study had a HSS-M score of ≥ 3. Hence HSS-M was found to be a highly sensitive screening test with a sensitivity of 100%, specificity of 69%, positive predictive value of 34% and negative predictive value of 100% when compared with blood culture.

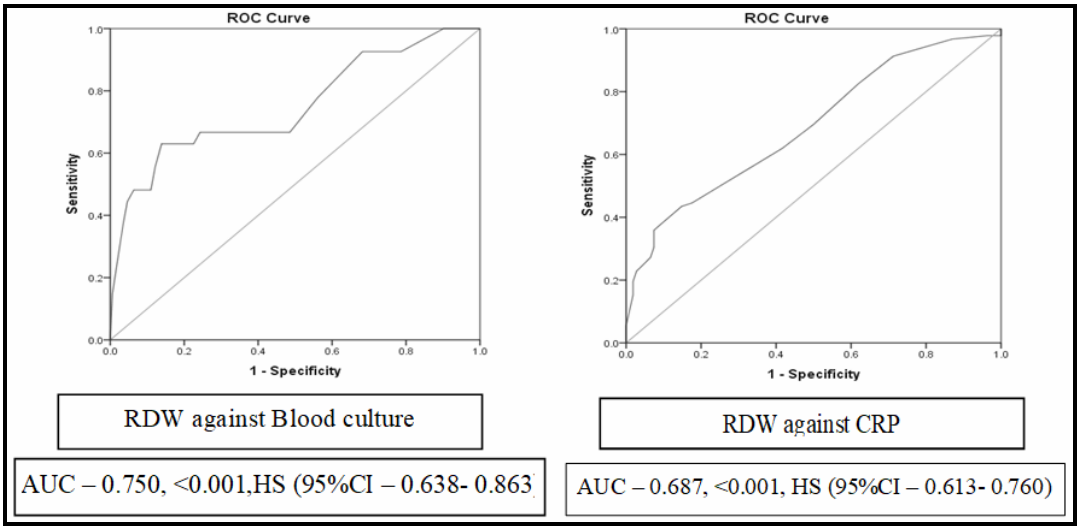
With AUC of 0.882, HSS-M was found to be a better diagnostic test when compared to C-Reactive protein (CRP) with a high specificity of 91%, sensitivity of 76%, positive predictive value of 88% and negative predictive value of 82% and this was highly statistically significant (p<0.001) as shown in figure 1.

**Fig-1:** ROC Curves of HSS-M against blood culture and CRP




Red cell distribution width (RDW) showed good diagnostic predictability (AUC-0.75) when compared against blood culture in neonatal sepsis and this was highly statistically significant (p<0.001). Based on validity scores and value judgment, RDW cut off value of 18.5 was derived and among the 27 culture proven sepsis cases, 21 were found to have a RDW value of ≥ 18.5. Hence RDW was found to have a sensitivity of 78%, specificity of 44%, positive predictive value of 18% and a high negative predictive value of 93% when evaluated against blood culture. AUC was 0.687 for comparison of RDW against CRP and this was highly statistically significant (p<0.001) with a fair sensitivity of 70%, specificity of 50%, positive predictive value of 54% and negative predictive value of 66%, as shown in figure 2.

**Fig-2:** ROC Curves of RDW against blood culture and CRP




## Discussion

In our study, 200 neonates with sepsis were included. Out of these, 120 (60%) were male babies, with an overall male : female ratio of 1.5 : 1 which is close to the ones seen in several other studies [20-22]. We also captured data regarding socio economic status in our study, which is an important factor influencing development of infections. 145 (72.5%) were below lower middle class on Modified Kuppuswamy socio economic status scale. This factor has not been included in most studies available on neonatal sepsis. In our study, early onset sepsis was more common and seen in 143 (71.5%) neonates which is comparable with majority of the studies on this topic [17, 21, 23]. However late onset sepsis was more common in few studies [3, 11].

Among the 200 neonates with sepsis in our study, only 27 (13%) were culture proven, remaining were probable sepsis cases. The percentage of yield of blood culture growth in our study was low and similar to several studies [11, 23-24]. However most of the other studies [2, 21, 25] had a better yield (more than 30%). The most probable reason for this could be taking a single culture, very early, soon after suspecting sepsis and not following it up with repeated cultures from multiple sites. In our study, the most common maternal risk factor was PROM (16.5%) which was similar to several other studies [20, 26]. Around 138 (69%) mothers delivered by vaginal route in our study, which is similar to several other studies [17, 20]. However it is in disagreement with few studies [3, 24] where the rates of Caesarean sections were much higher. Culture proven sepsis was 7 times more likely in babies born by Caesarean section in our study which is in disagreement with a study done by Sriram R [21] where it was more common in babies born by vaginal delivery. This may be an incidental finding because there were several other maternal and neonatal factors that led to the baby being delivered by Caesarean section and these factors could have individually contributed to the development of sepsis in the neonates. In our study, culture proven neonatal sepsis was 4 times more common when there was maternal infection, similar to a study done by Seaward PGR et al [27].

Prematurity (33%) and low birth weight (30%) were the most common neonatal risk factors for sepsis in our study which is similar to several other Indian studies [8, 17, 21, 24]. The most common manifestation of neonatal sepsis was respiratory distress (93.5%), which is in agreement with several studies [3, 23, 24]. However the percentage of septic neonates presenting with respiratory distress was very high in our study as compared to these studies. The objective of our study was to determine the role of Modified Haematological Sepsis Score and red cell distribution width in early diagnosis of neonatal sepsis by comparing these against blood culture and CRP.

Rodwell [12] had designed a haematological sepsis score as a rapid and simple diagnostic test, but it had low specificity. Krishnamurthy V et al [11] in their study gave a modified version of the original haematological sepsis score with better specificity and likelihood ratios, thereby facilitating its use in early diagnosis of neonatal sepsis. There are no studies that have used this modified scoring system. We used it in our study and observed that, at a cut off score of 3, sensitivity and specificity in diagnosing neonatal sepsis was 100% and 70% respectively when compared against blood culture, making HSS- M an excellent screening test for neonatal sepsis. Although our study showed 100% sensitivity, the specificity was slightly lower compared to the above study, likely reason for which could have been that we directly used the total score and compared it against blood culture and CRP. This was the drawback of our study, as we did not evaluate each individual parameter of the score as done in most other studies using the original Haematological Sepsis Score [2,9].

Comparison of haematological sepsis score with CRP showed 76% sensitivity and 90% specificity, proving to be a better diagnostic test rather than a screening test as compared to CRP. This is in agreement with 2 other Indian studies [8, 22]. We obtained the HSS-M reports within few hours of sample collection, whereas final blood culture reports were available after a minimum of 48-72 hours in our set up. This was found to be a major advantage with the use of HSS-M as it could be used in predicting sepsis early, followed by prompt and judicious use of antibiotics. Role of red cell distribution width in sepsis has been studied in several adult studies [15-16]. However very few studies were available for studying its role in pediatric and neonatal subjects. In our study it was noted that RDW showed a fair diagnostic predictability when compared against blood culture. At a cut off of 18.5, it had a sensitivity of 77% and low specificity (43%). When compared against CRP, it was noted to have 70% sensitivity and 50 % specificity which was similar to a study done by Chen J et al [28]. Our findings were also comparable with a study done by Cosar H et al [19] in Turkey where they concluded that RDW and CRP can be used in combination for the diagnosis of neonatal sepsis.

*Limitations:* It was a single centre study with a small sample size and we did not evaluate each and every individual component of the modified haematological sepsis score separately.

## Conclusion

Modified Haematological Sepsis Score and red cell distribution width showed good diagnostic predictability when compared against the conventionally used investigations like CRP and blood culture, in diagnosing neonatal sepsis, with HSS-M proving to be an excellent screening test in early diagnosis of neonatal sepsis. Hence we conclude that HSS-M and RDW have a good predictive role in early diagnosis of neonatal sepsis and can replace the existing conventional methods due to the ease, convenience and affordability of these tests. However conducting a multicentre study with a larger sample size and detailed evaluation of individual parameters of the score might add on to the diagnostic value of this study.

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