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Diagnostic Accuracy of Haematological Scoring System (HSS) in Neonatal Sepsis: A Facility Based Cross-Sectional Study

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Diagnostic accuracy, Haematological scoring System, C-reactive protein, Neonatal sepsis

ABSTRACT:

Objectives: The aim of the present study was to determine the diagnostic accuracy of Haematological scoring System (HSS) in neonatal sepsis. **Methods:** This was a laboratory based cross-sectional study conducted in the Department of Pathology, Vinayaka Missions Medical College & Hospital, Karaikal, Puducherry, India (tertiary healthcare facility) in south India between January 2023 and August 2023 among neonates presenting with features suggestive of sepsis (n = 145). **Results:** More than half (57.9%) the neonates were males; and 55.2% were between 1 and 7 days of age. The distribution of normal vaginal and caesarean delivery was 42.1% and 57.9% respectively. More than half (54.5%) the neonates were born with birthweight ranging between 1500 and 2499 grams; and 10.3% were born with birthweight less than 1500 grams. In terms of gestational age, 53.1% neonates included were born preterm. Refusal to feed was the most common (58.6%) presenting complaint. The results of peripheral smear showed that 91.7% neonates had neutrophilia, 37.9% had degenerative neutrophilic changes, 17.2% had thrombocytopenia, 13.8% had leucocytosis, 11.0% had nucleated RBCs (>5/100 WBCs) and 2.1% had leukopenia. One in four samples were positive for c-reactive protein (26.9%). Importantly, the number of samples found positive in blood culture was 30.3%. The results showed that the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of c-reactive protein in comparison with blood culture results were 54.5%, 85.1%, 61.5% and 81.1% respectively. The diagnostic accuracy of haematological scoring system was assessed with two cutoff points – with 5 as cutoff, the sensitivity was 13.6%, specificity was 100%, PPV was 100% and NPV was 72.7; and with 4 as cutoff, the sensitivity, specificity, PPV, and NPV were 90.9%, 62.4%, 51.3% and 94.0% respectively. **Conclusion:** The HSS, with adjustable cutoff points, allows for a tailored diagnostic approach, balancing sensitivity and specificity based on clinical priorities.

Introduction

Neonatal sepsis is characterized by a positive blood culture within the first month of life and is more prevalent in low- and middle-income countries.(1) Despite advancements in antibiotic treatment and care, the mortality rate associated with neonatal sepsis ranges from 13 to 60%.(2-4) Although this condition is life-threatening, early diagnosis enables effective treatment. Unfortunately, identifying early warning signs is challenging, as they often overlap with symptoms of non-infective causes. The ambiguity of these signs complicates the establishment of an early clinical diagnosis. Initiating antibiotic therapy based on clinical suspicion may lead to overtreatment, fostering the

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emergence of multi-drug resistant organisms and placing a financial burden on underprivileged parents.(5, 6)

While blood culture remains the 'gold standard' for septicaemia diagnosis, its accuracy is questioned due to spurious positive results and negative cultures in fatal bacterial infections.(7) The positive yield of blood culture varies widely, and its time-consuming nature requires well-equipped laboratories, which are lacking in many community hospitals.(8) In cases where blood and other sterile site cultures are negative but clinical signs persist, the term "clinical" sepsis may be considered. It's important to note that a positive blood culture is not mandatory for meeting the consensus definition of sepsis in adults and children.(9) Thus, there is a demand for a cost-effective, easily administered test with quick report availability. An ideal diagnostic test for neonatal sepsis should exhibit maximum sensitivity and specificity.

Recent investigations have explored highly sensitive and specific inflammatory markers for diagnosing neonatal sepsis.(10) Despite their accuracy, these markers are sophisticated and expensive, making them impractical for developing countries. Various affordable and reliable laboratory tests, such as the complete blood count (CBC) with neutrophil parameters and C-reactive protein (CRP), have been assessed for systemic infection diagnosis in neonates.(11) Against this background, the aim of the present study was to determine the diagnostic accuracy of Haematological scoring System (HSS) in neonatal sepsis.

Methods

This was a laboratory based cross-sectional study conducted in the Department of Pathology, Vinayaka Missions Medical College & Hospital, Karaikal, Puducherry, India (tertiary healthcare facility) in south India between January 2023 and August 2023. The study was approved by the Institutional Human Ethics Committee (IHEC). After obtaining necessary approvals from the Dean, Medical Superintendent, and the Head of Medical records department (MRD), the electronic medical records and laboratory information system were accessed. The study included the haematological parameters of neonates presenting with features suggestive of sepsis (including fever, lethargy, poor feeding, need for supplemental oxygen and low APGAR score), and neonates with history of maternal infections (including maternal intrapartum fever (more than 38ºC), premature rupture of membrane before 37 weeks), prolonged rupture of membrane (for more than 12 hours, and maternal urinary tract infection). Additionally, the haematological parameters of neonates with suspected sepsis were included. However, neonates with major congenital anomalies, inborn errors of metabolism, administration of antibiotics prior to admission, respiratory distress syndrome (RDS), neonates of mothers with pregnancy induced hypertension (PIH) and asphyxia were excluded after detailed perinatal history and clinical examination.

We resorted to complete enumeration of all cases of neonatal sepsis (in accordance to prespecified inclusion and exclusion criteria) during the study period and therefore, sample size was not estimated. A purpose predesigned, semi structured, pretested questionnaire was used to collect data on sociodemographic characteristics, birth information, presenting complaints, significant clinical history, relevant general physical examination findings and probable diagnosis. Blood sample was collected (aseptically in EDTA vacutainers by peripheral venipuncture (3ml)) – complete blood count was done using SYSMEX (parameters included were total red cell count, total leucocyte count (WBCs), differential count, haemoglobin concentration, packed cell volume, mean corpuscular haemoglobin, platelet count, mean platelet volume, and platelet distribution width). Leishman-stained peripheral smears (heel prick peripheral smear) were examined by counting for 200 WBCs (looked for nucleated RBCs, differential counts, absolute neutrophil count, immature neutrophils, toxic granulations and/or Dohle bodies and/or vacuolation in neutrophils and degenerative neutrophils). C-reactive protein (CRP, using latex slide agglutination test method) and blood culture was done. The findings were assessed using Haematological Scoring System developed by Rodwell et al.(12)

The data obtained was manually entered in Microsoft Excel and analysed using Software for Statistics and Data Science (Stata) v16. Descriptive analysis was presented using numbers and percentages for categorical variables; mean and standard deviation or median and interquartile range for continuous variables. Chi square test of significance (two-sided) was used for categorical variables to test for association; in situations where

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expected values in the cells were less than five in 20% of the cells Fisher's exact test (two-sided) was used. To test for association between continuous variables independent "t" tests (two-sided) was applied. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated to assess the validity of tests. Statistical significance was considered at $p<0.05$.

Results

In the present study, a total of 145 neonates with features suggestive of sepsis were reported between January 2023 and August 2023. More than half (57.9%) were males; and 55.2% were between 1 and 7 days of age. The proportion of neonates less than 1 day was 11.0% and between 7 to 28 days was 33.8%. The distribution of normal vaginal and caesarean delivery was 42.1% and 57.9% respectively. Regarding the maternal risk factors, it was found that mothers of 38.6% neonates had history of prematurity, 24.1% had prelabour rupture of membranes, and 18.6% had other risk factors including pregnancy induced hypertension, gestational diabetes mellitus etc.

More than one-third neonates (35.2%) were born with normal birthweight (more than or equal to 2500 grams). However, 54.5% neonates were born with birthweight ranging between 1500 and 2499 grams; and 10.3% were born with birthweight less than 1500 grams. In terms of gestational age, 53.1% neonates included were born preterm. Refusal to feed was the most common (58.6%) presenting complaint. This was followed by respiratory distress (31.7%), reduced movements (22.8%), fever (4.8%) and jaundice (0.7%), in that order.

Descriptive analysis of laboratory investigations: The results of peripheral smear showed that 91.7% neonates had neutrophilia, 37.9% had degenerative neutrophilic changes, 17.2% had thrombocytopenia, 13.8% had leucocytosis, 11.0% had nucleated RBCs (>5/100 WBCs) and 2.1% had leukopenia. One in four samples were positive for c-reactive protein (26.9%). Importantly, the number of samples found positive in blood culture was 30.3%.

Diagnostic accuracy: The results showed that the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of c-reactive protein in comparison with blood culture results were 54.5%, 85.1%, 61.5% and 81.1% respectively. The diagnostic accuracy of haematological scoring system was assessed with two cutoff points – with 5 as cutoff, the sensitivity was 13.6%, specificity was 100%, PPV was 100% and NPV was 72.7; and with 4 as cutoff, the sensitivity, specificity, PPV, and NPV were 90.9%, 62.4%, 51.3% and 94.0% respectively.

Discussion

Neonatal sepsis is a critical health concern, contributing significantly to neonatal morbidity and mortality worldwide. Early and accurate diagnosis is essential for timely intervention and improved outcomes. This study aimed to assess the diagnostic accuracy of the Haematological Scoring System (HSS) in neonatal sepsis, focusing on a cohort of 145 neonates with features suggestive of sepsis. The results showed that the majority of neonates were males (57.9%), and a substantial proportion fell within the 1 to 7 days age range (55.2%). This age distribution is consistent with previous studies highlighting the vulnerability of neonates during the early postnatal period.(13) The nearly equal distribution of delivery methods (42.1% normal vaginal delivery, 57.9% caesarean section) underscores the importance of considering various perinatal factors in the context of neonatal sepsis.(14) Understanding maternal risk factors is crucial for identifying neonates at higher risk of sepsis. In this study, a noteworthy 38.6% of neonates had mothers with a history of prematurity, emphasizing the association between maternal and neonatal health outcomes.(15) Additionally, 24.1% had prelabour rupture of membranes, a known risk factor for neonatal infection.(16, 17) Other risk factors, including pregnancy-induced hypertension and gestational diabetes mellitus, were present in 18.6% of cases, highlighting the multifactorial nature of neonatal sepsis risk.(18, 19) The findings have several clinical implications. The higher proportion of neonates with features suggestive of sepsis within the first week of life aligns with the known vulnerability of neonates during the immediate postnatal period.(20) The prevalence of caesarean section delivery in this population suggests the need for heightened surveillance and preventive strategies in such cases, given the potential association between caesarean section and neonatal morbidity.(21, 22) The association between maternal risk factors and neonatal sepsis reinforces the importance of maternal

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health in preventing neonatal infections. Addressing prematurity and managing conditions such as gestational diabetes and hypertension could potentially contribute to reducing the incidence of neonatal sepsis.(23, 24)

Low birthweight and preterm birth significantly contribute to neonatal morbidity and mortality, with potential long-term health implications. The study revealed a concerning distribution of birthweights among the neonates. While more than one-third (35.2%) had normal birth weight (≥2500 grams), a substantial 54.5% were born with birth weights ranging between 1500 and 2499 grams, and 10.3% had birth weights less than 1500 grams. This distribution is consistent with the known association between low birthweight and adverse neonatal outcomes, including an increased risk of infections.(25, 26) The high percentage of neonates with birthweights below 2500 grams underscores the vulnerability of this population. More than half (53.1%) of the neonates included in the study were born preterm. Preterm birth is a major risk factor for neonatal sepsis, as premature infants often have underdeveloped immune systems, making them more susceptible to infection.(27, 28) The high prevalence of preterm births in this cohort emphasizes the need for targeted interventions and close monitoring of preterm neonates to prevent and manage sepsis.(29)

Refusal to feed emerged as the most common presenting complaint, reported in 58.6% of cases. Refusal to feed can be indicative of various underlying issues, including sepsis, and underscores the importance of vigilant monitoring of neonates.(30) Respiratory distress (31.7%) and reduced movements (22.8%) were also notable complaints. These symptoms align with the clinical presentation of neonatal sepsis, highlighting the importance of early recognition and intervention.(31) The lower frequency of fever and jaundice in this cohort suggests that these symptoms may not be as prominent in neonatal sepsis cases identified through this study.(32) The findings have important clinical implications for the management of neonatal sepsis. The high prevalence of low birthweight and preterm birth emphasizes the need for targeted antenatal care strategies to identify and manage at-risk pregnancies. Interventions to improve birth outcomes, such as maternal nutrition programs and prenatal care, may contribute to reducing the incidence of neonatal sepsis.(33)

The haematological parameters and biomarkers discussed in this study provide crucial insights into the pathophysiology and diagnostic indicators of neonatal sepsis. Recognizing these markers is essential for early and accurate identification of sepsis, facilitating prompt intervention to improve neonatal outcomes. The peripheral smear results revealed a predominant presence of neutrophilia in 91.7% of the neonates. Neutrophilia, characterized by an elevated neutrophil count, is a common response to infection and inflammation, reflecting the activation of the immune system.(34) Concurrently, 37.9% exhibited degenerative neutrophilic changes, indicating the severity of the infection and potential tissue involvement.(35) Thrombocytopenia (17.2%) and leucocytosis (13.8%) further contribute to the haematological profile indicative of an inflammatory response. The presence of nucleated red blood cells (RBCs) in 11.0% of cases is noteworthy, as it can be associated with bone marrow stress and may indicate a severe systemic response.(36) Additionally, leukopenia was observed in 2.1% of neonates, suggesting possible suppression of the immune response. CRP, an acute-phase reactant produced by the liver, serves as a valuable biomarker for inflammation. In this study, 26.9% of neonates had elevated CRP levels, indicating an inflammatory response.(37) The utility of CRP in neonatal sepsis is well-established, and elevated levels are indicative of systemic inflammation, prompting further investigation and intervention.(38)

The most critical diagnostic measure for confirming sepsis is blood culture. In this study, 30.3% of blood cultures were positive for bacterial growth. Positive blood cultures underscore the presence of a bacterial infection in the bloodstream, validating the clinical suspicion of sepsis.(39) However, it's essential to interpret blood culture results judiciously, considering potential contaminants and ensuring that proper sampling techniques are employed. The combined analysis of peripheral smear findings, CRP levels, and blood culture results provides a comprehensive understanding of the haematological and biochemical changes associated with neonatal sepsis. Neutrophilia, degenerative neutrophilic changes, and positive blood cultures collectively point towards a systemic bacterial infection. Thrombocytopenia, leucocytosis, and the presence of nucleated RBCs further highlight the severity of the inflammatory response. Elevated CRP levels serve

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as a valuable adjunct to clinical and haematological assessments, providing a quick and sensitive indicator of ongoing inflammation. The integration of these diagnostic measures enhances the precision of sepsis diagnosis and aids in timely and targeted therapeutic interventions.

Assessing the diagnostic accuracy of biomarkers and scoring systems is pivotal in refining strategies for the early identification of neonatal sepsis. The evaluation of CRP in comparison with blood culture results revealed a sensitivity of 54.5%, indicating its ability to correctly identify true-positive cases. The specificity of 85.1% suggests a high rate of true-negative identifications. The positive predictive value (PPV) of 61.5% reflects the probability that a positive CRP result corresponds to an actual positive blood culture. The negative predictive value (NPV) of 81.1% signifies the likelihood that a negative CRP result accurately indicates the absence of sepsis. These findings align with the existing literature, emphasizing CRP's role as a valuable acute-phase reactant in the diagnosis of neonatal sepsis.(40) However, the moderate sensitivity indicates that reliance solely on CRP may result in missed cases, emphasizing the need for a multifaceted diagnostic approach.(41)

The HSS, assessed with two cutoff points, demonstrated distinct performance characteristics. With a cutoff of 5, the HSS exhibited high specificity (100%), ensuring accurate identification of true-negative cases. However, the sensitivity at this cutoff was notably low (13.6%), indicating a risk of false-negative results. At a cutoff of 4, the HSS displayed improved sensitivity (90.9%) while sacrificing specificity (62.4%). The high sensitivity indicates the HSS's efficacy in correctly identifying true-positive cases, reducing the likelihood of false negatives. The NPV of 94.0% at this cutoff underscores the HSS's ability to reliably exclude the presence of sepsis when negative. The diagnostic accuracy of CRP and HSS has direct implications for clinical decision-making. CRP, while widely used, exhibits limitations in sensitivity, potentially leading to false-negative results. Clinicians should interpret CRP results judiciously and consider complementary diagnostic measures, especially in cases with clinical suspicion of sepsis.(42) The HSS, with its adjustable cutoff points, allows for a tailored approach. While a higher cutoff maximizes specificity, a lower cutoff enhances sensitivity. Clinicians must balance the trade-off between sensitivity and specificity based on the clinical context, emphasizing the importance of individualized patient care.(43)

Conclusion

This study provides a comprehensive exploration of diagnostic parameters and scoring systems for neonatal sepsis. The distribution of birthweight and gestational age underscores the vulnerability of neonates, with a substantial proportion exhibiting low birthweight and preterm birth. Maternal risk factors, including prematurity and prelabour rupture of membranes, further contribute to the intricate landscape of neonatal sepsis risk. Haematological parameters, as revealed by peripheral smear results, offer crucial insights into the inflammatory response, with neutrophilia, degenerative neutrophilic changes, and thrombocytopenia indicative of systemic infection. The presence of nucleated red blood cells and leukopenia adds depth to the understanding of the haematological profile associated with neonatal sepsis.

The assessment of biomarkers, specifically c-reactive protein (CRP), and the Haematological Scoring System (HSS), provides valuable diagnostic tools. While CRP demonstrates moderate sensitivity and high specificity, its reliance alone may lead to missed cases. The HSS, with adjustable cutoff points, allows for a tailored diagnostic approach, balancing sensitivity and specificity based on clinical priorities. The study's clinical implications emphasize the importance of a multidimensional diagnostic strategy, integrating clinical assessments, haematological parameters, and biomarkers to enhance the precision of neonatal sepsis diagnosis. These findings contribute to the ongoing efforts to refine diagnostic algorithms and improve therapeutic interventions for neonates at risk of or presenting with sepsis.

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Table 1: Haematological Scoring System

Criteria	Abnormality	Score
Total WBC count	≤5,000/µl OR ≥25,000 at birth OR	1

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	≥30,000 at 12 to 24 hours OR ≥21,000 from Day 2 onwards	
Total PMN count	No mature PMN seen	2
	Increased or decreased	1
Immature PMN count	Increased	1
I:T PMN ratio	Increased	1
I:M PMN ratio	≥0.3	1
Degenerative changes in PMN	Toxic granules or cytoplasmic vacuoles	1
Platelet count	≤150,000/µl	1
The normal values are, Total PMN count between 1800 to 5400; Immature PMN count – 600; Immature : Total PMN ratio – 0.120; Immature : Mature PMN ratio ≥0.3 **Interpretation:** Scores less than or equal to 2, sepsis is unlikely; scores 3 or 4, sepsis is possible; and scores more than to equal to 5, sepsis or infection is very likely		

Table 2: Characteristics of study participants

		Number N = 145	**Percentage**
		n	**%**
Gender	Male	84	57.9
	Female	61	42.1
Age (in days)	Less than 1	16	11.0
	1 to 7	80	55.2
	More than 7	49	33.8
Mode of delivery	Normal	61	42.1
	Caesarean	84	57.9
Maternal risk factors, history of (numbers are not mutually exclusive)	Prematurity	56	38.6
	PROM	35	24.1
	Others	27	18.6
Birthweight (in grams)	Less than 1500	15	10.3
	1500 to 2499	79	54.5
	More than 2500	51	35.2
Gestational age	Preterm	77	53.1
	Term	68	46.9
Chief complaints	Refusal to feed	85	58.6
	Respiratory distress	46	31.7
	Reduced movement	33	22.8
	Fever	7	4.8
	Jaundice	1	0.7
	Others	12	8.3
PROM, Prelabour rupture of membranes			

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Table 2: Haematological parameters in neonatal sepsis

		Number N = 145	**Percentage**
		n	**%**
Peripheral smear abnormalities (numbers are not mutually exclusive)	Neutrophilia	133	91.7
	Degenerative changes	55	37.9
	Thrombocytopenia	25	17.2
	Leucocytosis	20	13.8
	Nucleated RBCs (>5/100 WBCs)	16	11.0
	Leukopenia	3	2.1
C-reactive protein	Positive	39	26.9
	Negative	106	73.1
Culture	Positive	44	30.3
	Negative	101	69.7
RBC, Red blood cells; WBC, White blood cells			

Table 3: Diagnostic accuracy of CRP and Haematological scoring system

		Culture positive N = 44	**Culture negative N = 101**	**Total N = 145**	**Diagnostic accuracy**	**P value**
		n (%)	**n (%)**	**n (%)**		
C-reactive protein	Positive	24 (54.5)	15 (14.9)	39 (26.9)	Sensitivity = 54.5 Specificity = 85.1 PPV = 61.5 NPV = 81.1	<0.001*
	Negative	20 (45.5)	86 (85.1)	106 (73.1)		
Haematological score	≥5	6 (13.6)	0 (0.0)	6 (4.1)	Sensitivity = 13.6 Specificity = 100 PPV = 100 NPV = 72.7	<0.001*
	<5	38 (86.4)	101 (100)	139 (95.9)		
Haematological score	≥4	40 (90.9)	38 (37.6)	78 (53.8)	Sensitivity = 90.9 Specificity = 62.4 PPV = 51.3 NPV = 94.0	<0.001*
	<4	4 (9.1)	63 (62.4)	67 (46.2)		
*Statistically significant at p<0.05						