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Investigation of Clinical and Laboratory Symptoms in Rapid Diagnosis of Neonatal Sepsis

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Abstract

Objectives: Neonatal sepsis is associated with significant mortality and morbidity, and its rapid diagnosis remains a clinical challenge due to the non-specific nature of its symptoms. The hematological scoring system (HSS) is a low-cost, rapid diagnostic tool based on a complete blood count (CBC). This study aimed to investigate clinical and laboratory findings and to evaluate the diagnostic accuracy of HSS in the rapid diagnosis of neonatal sepsis in a tertiary care center.

Materials and Methods: This cross-sectional-analytical study was conducted on 80 neonates (aged <28 days) with suspected sepsis admitted to Tabriz children's hospital. Based on blood culture results, infants were divided into two groups: definite sepsis (n=3) and non-sepsis (n=77). Clinical symptoms and laboratory parameters (including CBC and CRP) were recorded, and the HSS score was calculated for each infant. The diagnostic accuracy of HSS was evaluated using receiver operating characteristic (ROC) analysis, and indices such as sensitivity and specificity were calculated.

Results: The mean gestational age and birth weight were significantly lower in the sepsis group (P<0.001). Gram-positive cocci, specifically *Staphylococcus aureus* (Coagulase-positive), were the most common causative agents (n=2, 66.7%), followed by *Klebsiella pneumoniae* (n=1, 33.3%). Among clinical signs, lethargy, hypothermia, and cyanosis were significantly more frequent in the sepsis group (P<0.05). CRP, I/T ratio, and HSS scores were significantly higher, and platelet counts were lower in the sepsis group (P<0.001). The ROC curve for HSS showed an area under the curve (AUC) of 0.88. At the optimal cut-off point (≥4), sensitivity was 100% (95% CI: 43.9–100), specificity 83.1%, positive predictive value (PPV) 15.8%, and negative predictive value (NPV) 100%.

Conclusions: In this cohort with a low prevalence (3.75%) of culture-proven sepsis, the HSS demonstrated high diagnostic accuracy, particularly with a perfect sensitivity and NPV within our limited sample, effectively ruling out sepsis when the score is low. Incorporating this simple, inexpensive tool into clinical assessment may improve decision-making regarding antibiotic initiation and reduce unnecessary antibiotic exposure. However, the precision of sensitivity and PPV estimates is limited by the small number of sepsis-positive cases.

Keywords: Neonatal sepsis, Rapid diagnosis, Hematological scoring system, Laboratory markers, Clinical signs

Introduction

Neonatal sepsis is a life-threatening medical emergency characterized by a systemic inflammatory response to infection and is associated with significant mortality and neurodevelopmental morbidity (1). This condition poses a greater risk to preterm and low-birth-weight infants due to their immature immune systems. Every year, an estimated 2.4 million neonates die, with infections like sepsis accounting for a substantial proportion, especially in low- and middle-income countries (2). Early and accurate diagnosis plays a pivotal role in improving clinical outcomes (3).

However, diagnosing sepsis in neonates remains challenging. Early clinical signs—such as lethargy, poor feeding, temperature instability, and respiratory distress—are often non-specific and overlap with many other neonatal conditions (4). Moreover, the gold standard for diagnosis, a positive blood culture, requires at least 24 to 48 hours, and its sensitivity can be affected by prior antibiotic exposure (5). This diagnostic delay may lead to either late initiation of therapy or unnecessary empirical broad-spectrum antibiotic use, which carries risks such as antimicrobial resistance and increased healthcare costs (5,6).

In response, rapid and accessible laboratory markers have gained attention. Hematological parameters from a complete blood count (CBC) and inflammatory markers like C-reactive protein (CRP) are routinely used (3). However, none of these markers alone offers ideal sensitivity and specificity (7). Consequently, combined scoring systems that integrate multiple parameters have been proposed as adjunctive tools (8). The hematological scoring system (HSS), based on simple CBC and peripheral smear parameters, is one such tool designed to quantify the likelihood of sepsis rapidly and at low cost (8,9). Previous studies have reported variable performance of HSS, likely due to differences in demographic characteristics and local microbial patterns (7,9,10).

Beyond diagnostics, knowledge of the local microbiological profile and antibiotic susceptibility

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patterns is crucial for selecting appropriate empirical therapy (11,12). Given the continuous changes in microbial epidemiology and resistance patterns, regular updating of this information is essential (13).

This study was designed with the overarching aim of investigating clinical and laboratory findings in the rapid diagnosis of neonatal sepsis, with specific objectives to determine the local microbiological profile, identify common clinical and laboratory signs, and evaluate the diagnostic accuracy of HSS in a tertiary pediatric care center.

Materials and Methods

Study Design and Population

This cross-sectional analytical study was conducted over 16 months (2024) at the Children's Educational and Treatment Hospital in Tabriz. The study was reported according to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines (14). The study population consisted of all neonates under 28 days of age who were admitted to the neonatal intensive care unit (NICU) or neonatal ward with an initial suspicion of neonatal sepsis.

Inclusion and Exclusion Criteria

Inclusion criteria were: (a) age less than 28 days, (b) presence of at least two clinical signs suggestive of sepsis (such as lethargy, poor feeding, temperature instability, respiratory distress, cyanosis), (c) the attending physician's decision to perform a full sepsis workup (including blood culture and CBC), and (d) written informed consent obtained from parents or legal guardians. Exclusion criteria included: (a) major known congenital anomalies, (b) receipt of broad-spectrum antibiotics for more than 24 hours before sampling (except for perinatal prophylaxis), (c) parental refusal to participate, and (d) incomplete essential data.

Sampling Method and Sample Size

A consecutive sampling method was used to enroll eligible neonates. The sample size was calculated using a diagnostic study formula, assuming an initial sensitivity of 85% for the HSS, a 95% confidence level, and a desired precision of 0.08. The estimated sample size was approximately 80 neonates. After accounting for potential attrition, 80 neonates were included in the final analysis. It is acknowledged that this calculation did not incorporate an assumed disease prevalence, resulting in a low number of positive cases (n=3), which limits the precision of performance estimates for the sepsis group.

Data Collection and Variables

After obtaining ethical committee approval (code: IR.TBZMED.FMD.REC.1404.181), data were collected using a structured checklist. Demographic information (gestational age, birth weight, sex, Apgar score), clinical signs present at admission, and laboratory results—including CBC with white blood cell differential, CRP level, and blood culture results—were recorded. Blood cultures were performed using standard methods in both aerobic and anaerobic bottles, with sample volumes appropriate for neonates. Two independent pediatric infectious disease specialists reviewed all positive blood cultures to distinguish true pathogens from probable contaminants, based on clinical correlation, time-to-positivity, and the presence of the organism in multiple cultures if repeated. Microorganism identification and antibiotic susceptibility testing were performed according to CLSI guidelines.

Hematological Scoring System

The HSS score was calculated for each neonate based on seven hematological parameters as originally described by Rodwell et al (8). The specific criteria and thresholds for abnormality for each parameter are detailed in Table S1. In brief, one point was assigned for each abnormal parameter, except for an abnormal mature polymorphonuclear leukocytes count, which received two points. The total score ranged from 0 to 9.

Group Definitions

Neonates were classified into two groups based on final blood culture results:

- Definite sepsis group: neonates with clinical signs and a positive blood culture.
- Non-sepsis group: neonates with suspected clinical signs but a negative blood culture, in whom sepsis was ruled out.

Statistical Analysis

Data were analyzed using SPSS software version 26. Descriptive statistics (mean ± standard deviation, median and interquartile range, frequency and percentage) were used to summarize baseline characteristics. Continuous variables were compared between the two groups using the independent t-test or Mann–Whitney U test, depending on data distribution. Given the small sample size in the sepsis group, non-parametric tests were emphasized where applicable. Categorical variables were compared using the chi-square test or Fisher's exact test. The diagnostic performance of HSS and other markers was evaluated using receiver operating characteristic (ROC) curve analysis, with calculation of the area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). A P value <0.05 was considered statistically significant. The promising AUC (0.88) should be interpreted with caution due to the potential for overfitting in a small sample with few positive events.

Results

Demographic and Baseline Characteristics

A total of 80 neonates with suspected sepsis were enrolled

in the study. Based on blood culture results as the gold standard, 3 neonates (3.75%) were classified into the definite sepsis group (culture-positive) and 77 neonates (96.25%) into the non-sepsis group (culture-negative).

The mean (± SD) age at presentation was 5.8 ± 4.3 days (range: 1–25 days). The gender distribution was nearly equal (55.0% male). Regarding gestational age, 25 neonates (31.3%) were preterm and 55 (68.7%) were term. The mean birth weight was 2800 ± 920 grams. Statistically significant differences were observed between the two groups for gestational age, birth weight, and Apgar score at 5 minutes ($P < 0.05$ for all). The baseline characteristics of the study population are presented in Table 1.

Clinical Signs

The frequency of clinical signs at presentation, stratified by group, is shown in Table 2.

Performance of Individual Hematological Parameters

The diagnostic performance of each of the seven hematological parameters of the HSS and that of CRP are presented in Table 3. Given the very small number of positive cases, estimates of sensitivity and PPV should be interpreted with caution. Specificity, NPV, and Accuracy are more stable estimates in this context.

As shown in Table 3, many parameters, including CRP, I/T (immature neutrophil to total neutrophil) ratio, and white blood cell (WBC) count, showed 100% sensitivity in this small cohort, but with correspondingly low PPV. The I/M (immature neutrophil to mature neutrophil) ratio and platelet count demonstrated the highest specificity (93.5% and 90.9%, respectively). The I/T ratio showed an excellent balance with 100% sensitivity and 85.7% specificity.

Performance of the Hematological Scoring System

The HSS score for each neonate ranged from 0 to 9. The distribution of scores differed markedly between the two groups. All three neonates in the definite sepsis group had HSS scores ≥4, while the majority of the non-sepsis group had scores <4. The mean HSS score was significantly

Table 1. Baseline Characteristics of the Study Population (n=80)

Characteristic	Total (n=80)	Definite Sepsis (n=3)	Non-Sepsis (n=77)	*P* value
Gestational age (wk), Mean ± SD	37.2 ± 3.5	32.0 ± 2.6	37.4 ± 3.3	<0.001
Preterm, n (%)	25 (31.3)	3 (100)	22 (28.6)	0.014
Birth weight (g), Mean ± SD	2800 ± 920	1580 ± 320	2860 ± 900	<0.001
Male gender, n (%)	44 (55.0)	1 (33.3)	43 (55.8)	0.618
Apgar score at 5 min, Mean ± SD	8.5 ± 1.2	7.0 ± 1.0	8.6 ± 1.2	0.023
Age at presentation (days), Mean ± SD	5.8 ± 4.3	6.3 ± 3.2	5.8 ± 4.4	0.830

Note: Continuous variables were compared using the Mann–Whitney U test. Categorical variables were compared using Fisher›s exact test because of the small number of culture-positive cases. Statistically significant ($P < 0.05$).

Table 2. Frequency of clinical signs in neonates with suspected sepsis

Clinical Sign	Definite Sepsis (n=3), n (%)	Non-Sepsis (n=77), n (%)	*P* value
Lethargy	3 (100)	18 (23.4)	0.011*
Poor feeding	2 (66.7)	35 (45.5)	0.596
Temperature instability	2 (66.7)	29 (37.7)	0.307
Respiratory distress	2 (66.7)	40 (51.9)	0.624
Hypothermia	3 (100)	12 (15.6)	0.005*
Cyanosis	2 (66.7)	10 (13.0)	0.045*

*All *P* values were calculated using Fisher›s exact test because of the small sample size and expected cell counts. $P < 0.05$ was considered statistically significant.

Table 3. Diagnostic Performance of Individual Hematological Parameters and CRP in Neonatal Sepsis (n=80)

Parameter	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)
WBC count	100	68.8	10.3	100	70.0
Platelet count	66.7	90.9	20.0	98.6	90.0
Total PMN count	100	44.2	5.6	100	46.3
Immature PMN count	100	63.6	8.3	100	65.0
I/T ratio (>0.2)	100	85.7	18.8	100	86.3
I/M ratio	66.7	93.5	28.6	98.7	92.5
Degenerative changes in PMN	100	55.8	6.7	100	57.5
CRP (≥10 mg/L)	100	71.4	11.1	100	72.5

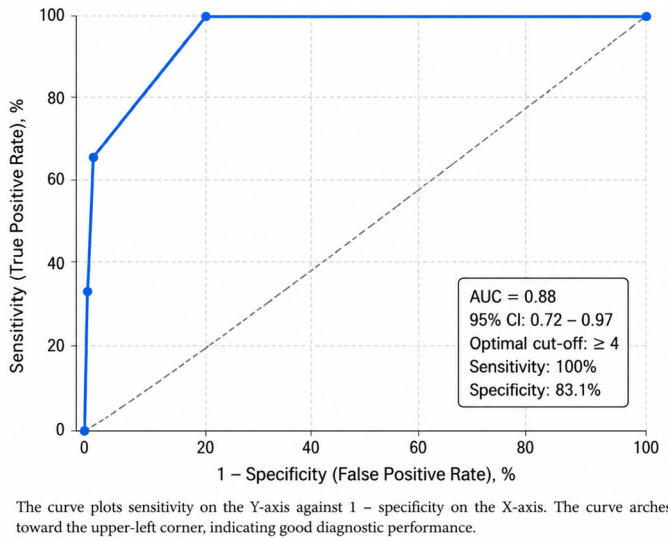

Figure 1. Receiver operating characteristic (ROC) curve of the Hematological Scoring System (HSS) for the diagnosis of culture-proven neonatal sepsis. The ROC curve demonstrates good diagnostic performance of the HSS, with an area under the curve (AUC) of 0.88 (95% CI: 0.72–0.97). An HSS cut-off value of ≥4 provided the optimal diagnostic performance, yielding 100% sensitivity, 83.1% specificity, 15.8% positive predictive value (PPV), and 100% negative predictive value (NPV) in the study population.

higher in the definite sepsis group than in the non-sepsis group ($P < 0.001$).

To determine the optimal cut-off and evaluate the overall performance of HSS, an ROC curve analysis was performed (Figure 1).

The area under the curve (AUC) for HSS was 0.88 (95% CI: 0.72–0.97), indicating very good diagnostic accuracy.

As detailed in Table 4, a score of 4 was identified as the optimal cut-off. At this threshold, HSS demonstrated perfect sensitivity (100%) and perfect negative predictive value (100%) in this cohort. This means that no neonate with a negative blood culture had an HSS score of 4 or higher that was incorrectly flagged, and all culture-positive cases were identified. The specificity was robust at 83.1%. The low PPV (15.8%) is expected, given the very low prevalence (3.75%) of culture-positive sepsis in the study sample, and highlights that a positive HSS score should prompt further investigation rather than be used alone for diagnosis.

Table 4. Diagnostic Indices of the Hematological Scoring System at the Optimal Cut-off Point

Parameter	Value (%)	95% CI
Optimal cut-off	≥4	-
Sensitivity	100	43.9–100
Specificity	83.1	73.2–90.0
PPV	15.8	4.8–39.0
NPV	100	94.9–100
Positive likelihood ratio (LR+)	5.9	3.7–9.5
Negative likelihood ratio (LR-)	0.00	-
Overall accuracy	83.8	74.0–90.5

Microbiological Profile

Among the 3 neonates with definite sepsis, the causative organisms were:

- *Staphylococcus aureus* (Coagulase-positive): 2 cases (66.7%) (both deemed true pathogens after review)
- *Klebsiella pneumoniae*: 1 case (33.3%)

Antibiotic Susceptibility Pattern

The two *S. aureus* isolates were both susceptible to oxacillin (MSSA) and vancomycin. The single *K. pneumoniae* isolate was susceptible to cefotaxime, gentamicin, and meropenem, but resistant to ampicillin.

Discussion

This study aimed to evaluate the performance of the HSS in the rapid diagnosis of neonatal sepsis at a tertiary care teaching hospital. In our cohort with a very low incidence of culture-proven sepsis (3.75%), HSS demonstrated outstanding rule-out utility. With a cut-off score of ≥4, it achieved perfect sensitivity (100%) and a perfect negative predictive value (100%) in this limited sample, meaning no cases of sepsis were missed, and a score below 4 reliably excluded bacteremia.

The perfect sensitivity and NPV observed, though based on only three cases, although in a small sepsis group, align with the core strength of HSS reported in larger studies: its high negative predictive value (7-9). This makes it a potentially valuable tool for "ruling out" sepsis in low-probability or borderline cases. Clinicians can use a low HSS score (<4) to support withholding or delaying empirical antibiotics, opting instead for close monitoring. This practice directly supports antimicrobial stewardship by reducing unnecessary antibiotic exposure, a critical concern in neonatology (5,6).

The specificity of HSS in our study was 83.1%, which is commendable and consistent with previous reports ranging from 80% to 96% (7,9). The low positive predictive value (15.8%) is a direct statistical consequence of the very low disease prevalence in our sample (Bayes' theorem). It is a common finding in screening tests when the condition is rare. For example, even with a test having 100% sensitivity and 83% specificity, a disease prevalence of 3.75% yields a PPV of approximately 18%, closely matching our finding. It reinforces that a positive HSS score (≥4) is not diagnostic of sepsis but should be interpreted as a strong indicator to continue the diagnostic workup and consider empirical therapy pending culture results, especially in the presence of concerning clinical signs.

Analysis of individual markers in this small cohort showed several (CRP, I/T Ratio, WBC) with 100% sensitivity but poor PPV. The I/M ratio and platelet count showed high specificity (>90%), consistent with literature portraying them as good "rule-in" parameters when abnormal (9). The integration of these complementary parameters within the HSS likely contributes to its balanced performance.

The microbiological profile in our limited positive cohort showed a predominance of gram-positive cocci (*S. aureus*), differing from many studies reporting gram-negative predominance in NICU settings (11,15). This underscores the importance of local surveillance. Both MSSA isolates and the single *K. pneumoniae* isolate were susceptible to commonly used antibiotics in neonates (oxacillin, cefotaxime, gentamicin), which is reassuring for the current empirical protocol at our center (16).

Given the small number of positive cases, our findings regarding the microbiological profile should be considered preliminary. The isolation of *S. aureus* in two of three cases differs from studies reporting gram-negative predominance in NICU settings (11,17) and underscores the necessity of continuous local surveillance rather than indicating a definitive shift in epidemiology.

Study Limitations

This study has several important limitations. First, the very low number of culture-positive sepsis cases (n=3) limits the precision of sensitivity and PPV estimates and precludes meaningful subgroup analysis. Second, its single-center design may affect generalizability. Third, some advanced biomarkers, like procalcitonin, were not available for comparison. Finally, while we employed criteria to minimize misclassification, the possibility of blood culture contamination, especially for coagulase-positive staphylococci, cannot be entirely ruled out.

Conclusion and Recommendations

In summary, in our setting with a low incidence of confirmed sepsis, the HSS proved to be a <mark>potentially useful rule-out tool in this preliminary evaluation, perfectly identifying all sepsis cases and reliably excluding sepsis when the score was low (<4) in our cohort. Its simplicity and low cost make it a candidate for routine use.

We recommend:

- Incorporating the calculation of the HSS score into the initial assessment protocol for neonates suspected of sepsis.
- Revising empirical sepsis treatment protocols at the center based on updated local microbiological profiles and resistance patterns.
- Conducting future multi-center studies with larger sample sizes and higher disease prevalence to validate the performance of HSS and more precisely define its role within comprehensive diagnostic algorithms. Such studies could also explore the incremental value of combining HSS with CRP or other biomarkers using multivariate models and investigate integrating these scores with emerging point-of-care technologies or machine learning approaches.

Authors' Contribution

Conceptualization: Majid Mahallei, Mirhadi Mussavi, Yousef Tavakolifar.
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Supervision: Majid Mahallei, Mirhadi Mussavi, Yousef Tavakolifar.
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Visualization: Mirhadi Mussavi, Yousef Tavakolifar.
Writing–original draft: Majid Mahallei, Mirhadi Mussavi, Yousef Tavakolifar.
Writing–review & editing: Majid Mahallei, Mirhadi Mussavi, Yousef Tavakolifar.

Conflict of Interests

None.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding

Ethical Issues

This study was approved by the ethics committee of Tabriz University of Medical Sciences (Approval No.: IR.TBZMED.FMD.REC.1404.181)

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Declaration of AI-assisted Tools in the Writing Procedure

The authors used Deepseek AI tool to refine grammar points and language style. Subsequently, they thoroughly reviewed and edited the content as necessary, assuming full responsibility for the publication's content.

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Supplementary files

Supplementary file 1 contains Table S1.

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