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Diagnostic Accuracy of Red Cell Distribution Width in Diagnosing Early-Onset Neonatal Sepsis in Term Newborns

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ABSTRACT

Inflammation in neonatal sepsis triggers cytokine-driven disruption of erythropoiesis, producing a mix of immature and damaged red cells. **Objectives:** To determine the diagnostic accuracy of RDW for early onset neonatal sepsis (EONS) in term newborns, taking culture-proven EONS as the gold standard. **Methods:** This prospective validation study was conducted at the Department of Neonatology of Children's Hospital, Institute of Child Health, Multan. A total of 147 term neonates with suspected EONS were enrolled consecutively. Neonates with asphyxia, meconium aspiration, major congenital malformations, or hemolytic disease were excluded. Clinical and laboratory data, including RDW, were collected. Blood, urine, and cerebrospinal fluid cultures were performed as per CLSI guidelines. EONS was confirmed by positive blood culture. A cutoff value of RDW ≥17% was used for labelling EONS. Data were analyzed using SPSS version 25.0, and the diagnostic accuracy of RDW was calculated, taking culture-proven neonatal sepsis as the reference standard. **Results:** The mean postnatal age was 3.7±1.4 days. The mean RDW was 16.9 ± 1.9%. RDW of ≥17% was observed in 54.4% of the neonates. Culture confirmed EONS was diagnosed in 59.9%. RDW showed sensitivity of 84.1% (95% CI: 74.8-91.0%), specificity of 89.8% (95% CI: 79.2-96.2%), positive predictive value of 92.5%, negative predictive value of 79.1%, and diagnostic accuracy of 86.4%. The area under the ROC curve was 0.87 (95% CI: 0.81 – 0.93, p<0.001). **Conclusions:** RDW ≥17% demonstrated high diagnostic accuracy as an early predictor of culture-confirmed EONS in term neonates.

INTRODUCTION

The terminology "neonatal sepsis," which refers to the systemic illnesses that affect neonates during the initial twenty-eight days of life, includes bloodstream infections (BSIs) or septicemia, pneumonia, meningitis, urinary tract infections, and bone/joint infections [1]. The condition, if manifested in the initial 72 hours of life, is known as Early Onset Sepsis (EOS), while sepsis that is developed after that time is known as Late Onset Sepsis (LOS) [2]. Neonatal sepsis occurs in conjunction with or as a consequence of a suspected or proven infective process [3]. Blood cultures have a long turnaround time and only identify sepsis in one-third of suspected infants, despite being the gold standard for diagnosing neonatal sepsis [4]. The result may be negative if the test is performed before the increase in C-Reactive Protein, which happens only 6 to 8 hours after the infection begins [5]. Microscopic analysis of the degree of anisocytosis is connected with the Red Cell Distribution Width (RDW), a quantitative measure of red cell volume variability [6]. A larger RDW indicates anisocytosis, while a normal RDW indicates the absence of anisocytosis. RDW can be described as the coefficient of variation (CV) of red cell volume measurements in percentage. RDW shows if

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the size of the red cell volume is constant. As the RDW rises, so does the volume heterogeneity and the irregularity in red blood cell size [7]. Because proinflammatory cytokines affect the synthesis of red blood cells, RDW is elevated in sepsis [8]. Research has shown that infection and inflammation increase RDW. Deka A *et al.* examined 100 babies, 50 of whom had sepsis and the other 50 of whom were healthy. Fifty percent of babies had neonatal sepsis. They found that newborn sepsis could be diagnosed with 93.5% accuracy, 86% sensitivity, and 87% specificity at an RDW cut-off level of 17.25% [9]. Similarly, 110 neonates (55 with EOS and 55 controls) were investigated by Nargis *et al.* They concluded that, using Youden's index, the critical cutoff point of RDW was 18.55, with a diagnostic accuracy of 94.45%, a sensitivity of 94.55%, and a specificity of 96.36% for the diagnosis of EOS [10]. Cytokine-induced inflammation suppresses erythropoietin activity and disturbs iron metabolism, leading to the production of red cells of variable sizes (anisocytosis). This variability increases RDW, which reflects underlying inflammatory stress and impaired erythropoiesis.

A timely diagnosis of neonatal sepsis is essential to its enhanced outcome since it is a frequent cause of morbidity and death among infants. There is limited research on RDW in newborns and its relation to neonatal sepsis. To diagnose and treat newborn sepsis as early as possible, RDW, as an effective predictor, shall be used to guide the treatment of pediatricians and neonatologists. The research will aid in generating evidence among septic neonates who are admitted to our facility. It will lead to the use of RDW as a helpful predictive tool to treat and diagnose neonatal sepsis as early as possible by treating doctors. This study aimed to find the diagnostic value of RDW in neonatal sepsis.

METHODS

This prospective validation study was performed at the Department of Neonatology in Children's Hospital (CH) and The Institute of Child Health (ICH), Multan, from 1st January 2024 to 30th June 2024 after approval from the Institutional Ethics Review Committee (2148 CH&ICH Multan). A total of 147 term neonates, admitted within 7 days of postnatal life due to suspected early onset neonatal sepsis, were consecutively included in the study after informed consent was provided by the parents. Neonates with a history of perinatal asphyxia, meconium aspiration, major congenital malformation, and ABO / RH – isoimmunization were excluded from the study. Baseline characteristics of neonates, including postnatal age, gestational age, gender, mode of delivery, and weight on admission (kg), were recorded. Through aseptic technique, five millilitres of venous blood were drawn on admission for complete blood counts and red cell distribution width. CBC analysis was performed on an autoanalyzer (Model: Sysmex XN-2000, features: 3-part differential) in the hematology section. All neonates had blood and urine cultures obtained on admission before the first dose of antibiotics as per hospital protocol. Based on clinical manifestations and indications, neonates were subjected to chest x-rays (Model: Toshiba Rotanode, Type: Floor-mounted digital radiography systems) and cerebrospinal fluid examination and cultures. CSF examination and culture were performed after lumbar puncture, where cerebrospinal fluid is aseptically collected from the L3–L4 or L4–L5 interspace using a sterile spinal needle. The fluid was divided into sterile tubes for biochemical, cytological, and microbiological analysis. For culture, a portion was inoculated immediately onto blood and chocolate agar and incubated in the laboratory to identify bacterial growth and sensitivity. All cultures were performed in the microbiology section as per The Clinical & Laboratory Standards Institute (CLSI) guidelines. Rephrase and divide into small sentences so that the meaning becomes clear. Neonates ≤7 days of age were evaluated for early-onset sepsis if they had at least one maternal risk factor. These included maternal fever (T ≥101 °F) within 72 hours before delivery, foul-smelling liquor, or rupture of membranes for more than 24 hours. Clinical suspicion was raised when a neonate exhibited three or more features: temperature instability (>100.5 °F or <96 °F), weak cry, poor feeding, lethargy, capillary refill time >3 seconds, decreased tone, reduced neonatal reflexes, apnea, respiratory rate >60/min, or heart rate <80 or >160/min. EONS was confirmed if a pathogen was detected from the blood, urine, or cerebrospinal fluid in a newborn with suspicion of EONS. The sample size was calculated through one sample sensitivity and specificity formula through online software https://wnarifin.github.io/ssc/sssnsp.html. The sample size required was 147 neonates based on 86% sensitivity and 87% specificity of RDW, 50 percent prevalence of EONS, 8 percent precision, and 95 percent confidence [10]. The data were analyzed with the help of SPSS version 25.0. The Shapiro-Wilk test was used to identify numerical data as normally distributed. The descriptive statistics were performed as mean, standard deviation, and frequency (percentages) of numerical and categorical variables, respectively. RDW (%) cut-off value of 17 or more, as reported in literature previously, was taken to classify EONS as positive or negative. Based on culture-positive EONS as a reference standard, the diagnostic accuracy of the RDW was computed, including the area under the receiver operating characteristic curve and 95% confidence interval.

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RESULTS

The mean postnatal age on presentation was 3.7 ± 1.4 days, and the mean gestational age on delivery was 37.9 ± 0.7 weeks. The participants included 74 (50.3%) male and 80 (54.4%) were born through cesarean section. The mean weight on admission was 2.8 ± 0.1 kg. The mean red cell distribution width (RDW) was 16.9 ± 1.9%. At cut off value of ≥ 17% RDW, the early neonatal sepsis (EONS) was labelled in 80 (54.4%). The diagnosis of definitive EONS was made in 88 (59.9%) of the suspected neonates (Table 1).

Table 1: Characteristics of Term Neonates Presenting with Suspected Early Neonatal Sepsis (n=147)

Variables	Mean ± SD, n (%)
Age	
Days	3.7 ± 1.4
Gestational Age	
Weeks	37.9 ± 0.7
Gender	
Male	74 (50.3%)
Female	73 (49.7%)
Weight (kg)	
On Admission	2.8 ± 0.1
Mode of Delivery	
SVD	67 (45.6%)
Cesarean Section	80 (54.4%)
Red Cell Distribution Width (%)	16.9 ± 1.9
EONS using RDW (≥17%)	
Yes	80 (54.4%)
Definitive EONS	
Yes	88 (59.9%)

*EONS – Early onset neonatal sepsis

Culture positive early onset neonatal sepsis was diagnosed in 88 (59.8%), and using RDW EONS was diagnosed in 80 (54.4%) of the neonates. Taking definitive EONS as reference standard, the sensitivity, specificity, positive and negative predictive values (PPV and NPV) and accuracy of RDW were 84.1% (95% CI: 74.8% to 91.0%), 89.8% (95% CI: 79.2% to 96.2%), 92.5% (95% CI: 85.2% to 96.4%), 79.1% (95% CI: 69.9% to 86.1%) and 86.4% (95% CI: 79.8% to 91.5%) respectively (Table 2).

Table 2: Diagnostic Accuracy of RDW for EONS Taking Definitive EONS as Gold Standard (n=147)

EONS Using RDW	Definitive EONS		p-value
	Yes	No	
Yes (≥17%)	74	6	<0.001
No (<17%)	14	53	
Sensitivity	84.1% (95% CI: 74.8% to 91.0%)		–
Specificity	89.8% (95% CI: 79.2% to 96.2%)		
PPV	92.5% (95% CI: 85.2% to 96.4%)		
NPV	79.1% (95% CI: 69.9% to 86.1%)		
Accuracy	86.4% (95% CI: 79.8% to 91.5%)		

Clopper-Pearson exact confidence intervals reported. RDW had an area under the receiver operating characteristic curve of 0.87 (95% CI: 0.81 – 0.93), for the diagnosis of EONS, taking culture-confirmed neonatal sepsis as the gold standard. Using Youden's index, the RDW optimal cut-off was 17.25 with a sensitivity of 84.1 and a specificity of 91.5%. Area Under Curve: 0.87 (95% CI: 0.81 – 0.93), p-value<0.001 (Figure 1).

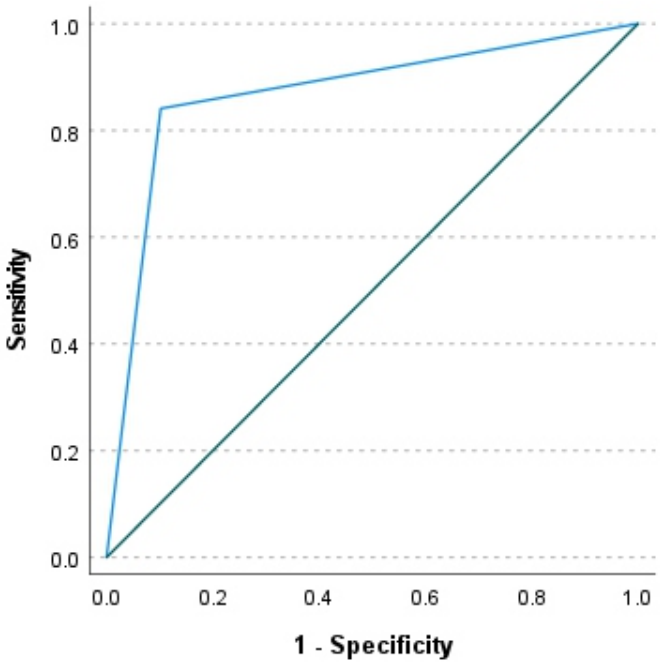

Figure 1: RDW Receiver Operating Characteristic Curve for Diagnosis of EONS, Taking Definitive EONS as Gold Standard

DISCUSSION

Red cell distribution width was examined in this work as a potential early, accessible, and affordable biomarker for the detection of newborn sepsis. We observed that the mean postnatal age on presentation was 3.7 ± 1.4 days, and 50.3% were male. With its high death rate, neonatal sepsis continues to be difficult for neonatal healthcare professionals to diagnose and treat. Early detection of neonatal septicemia reduces mortality rates by enabling the prompt initiation of antibiotic therapy and preventing the needless treatment of a baby who is not sick. Due to a lack of early diagnosis and identification of high-risk cases, mortality rates are high in underdeveloped regions [11]. In research by Nargis *et al.* 67.27% of patients were male, and the mean age of the cases was 26.5±19 hours, while the mean age of the controls was 26 ± 12.3 hours [10]. Cosar *et al.* identified a male majority and reported that the mean age of the cases was 1.98 ± 0.9 days, whereas the mean age of the controls was 1.87 ± 0.92 days [12]. However, Saleh *et al.* found that there were more females than men [13]. In the present study, the mean red cell distribution width (RDW) was 16.9 ± 1.9%. In comparison to controls (16.23 ± 1.16%),

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Nargis *et al.* found that newborn sepsis cases had a mean RDW level of 21.31 ± 3.08% [10]. Chen *et al.* also discovered that the sepsis group had a considerably higher mean RDW level [14]. The reason for this is that inflammation raises the body's levels of neurohormones [15]. These neurotransmitters can promote the growth of red blood cells by secreting more erythropoietin (EPO), which raises the RDW value. Inflammatory substances can also impact the body's iron metabolism and marrow hematopoietic function [16]. In current study, taking definitive EONS as a reference standard, the sensitivity, specificity, PPV, NPV, and accuracy of RDW (≥17%) were 84.1%, 89.8%, 92.5%, 79.1% and 86.4%, respectively. The area under the curve for RDW was 0.87, indicating excellent discrimination. RDW was considerably greater in culture-confirmed newborn sepsis than controls (sensitivity 60%, specificity 88.3%, AUC ~0.80), according to 2021 retrospective cohort research from a tertiary care university hospital [17]. Their specificity patterns are similar to our findings, despite having a little lower sensitivity. The AUC in our study (0.87) was slightly higher than what they studied (0.80), indicating that RDW demonstrated better overall diagnostic performance for early-onset neonatal sepsis in our population. High value of RDW was associated with increasing sepsis severity (sepsis severe septic shock), according to a 2022 comparative study conducted in Egypt that found mean RDW levels were significantly greater in septic neonates compared to controls. Our high PPV and the hypothesis that higher RDW indicates more advanced disease are supported by this [18]. The pooled sensitivity and specificity of RDW for neonatal sepsis were 0.88 and 0.90, respectively, with an AUC of 0.95 in a meta-analysis of 15 trials involving more than 1,300 neonates [19]. These figures highlight the overall diagnostic strength of RDW and are in good agreement with our findings (sensitivity 0.84, specificity 0.90). The AUC in our study was slightly lower than the pooled AUC of 0.95 reported in this meta-analysis, indicating that while RDW showed excellent diagnostic accuracy in both, the combined data across multiple trials demonstrated even stronger discriminative performance than our single-center findings. Similar to our great PPV, a hospital-based study conducted in Sudan with term newborns with culture-proven sepsis found that the mean RDW was increased (~19.3%) in over 90% of cases, with a clear correlation with positive blood culture and CRP positivity [20]. RDW ≥20% was shown to be independently predictive of outcome and strongly linked with higher mortality ($p<0.003$) in another prospective observational study (India, ~251 septic neonates). This emphasizes the predictive, rather than only diagnostic, relevance of RDW [21]. Research indicates that, as compared to static RDW measurements, dynamic changes in RDW (rising from baseline) improve diagnosis accuracy in very low birth weight infants. Even though our study only used one RDW measurement, our accuracy rate of 86.4% indicates that baseline RDW still has a significant amount of diagnostic potential [22]. The mechanisms that underlie elevated RDW in infection states include inflammatory-mediated bone marrow failure, cytokine release, oxidative stress, and erythrocyte membrane instability—make RDW rise in conditions of sepsis [18]. A recurring concept in these recent international research studies is that high RDW is a marker for newborn sepsis that is both specific and sensitive enough to have both diagnostic accuracy and prognostic significance. RDW helps identify neonates at high risk for EONS early on by providing a quick and inexpensive measure that can be incorporated into CBC examinations. Our study's merits included utilizing strict inclusion criteria and enrolling only term neonates, which improved internal validity by lowering gestational age-related confounding variables. The reliability of the diagnostic accuracy estimates was increased by using a definitive reference standard, such as clinical and/or culture-proven EONS. The results were very pertinent to actual clinical practice because RDW is a standard and economical part of CBC, particularly in low-resource environments.

Some of the limitations of our study were that this was a single-center study, which limited the generalizability of research findings to other populations and healthcare settings. Only term newborns were included in the study; preterm infants, who are more likely to develop sepsis and may show distinct RDW dynamics, were not included. Future multicenter studies with larger and more diverse neonatal populations should include preterm infants, serial RDW measurements, and correlation with inflammatory markers (e.g., CRP, PCT) to further validate and refine the diagnostic utility of RDW in early-onset neonatal sepsis.

CONCLUSIONS

There were great sensitivity, specificity, and positive predictive value because red cell distribution width (RDW) showed high diagnostic accuracy in the diagnosis of early onset neonatal sepsis in term neonates. As a conveniently located and relatively inexpensive biomarker, RDW may act as a valuable adjuvant in the diagnosis of newborn sepsis in the early stages of this condition, particularly in settings where the availability of the latest diagnostic instruments is limited.

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Authors' Contribution

Conceptualization: MJI, AK
Methodology: MHR, MZS
Formal analysis: MZS, JR
Writing and Drafting: MJI, MHR, JR, AK
Review and Editing: MJI, MHR, MZS, JR, AK

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflicts of Interest

All the authors declare no conflict of interest.

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