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Diamla HP, Garcia RDJ. Red Cell Distribution Width and its Association with Neonatal Bacteremia: A Case-Control Study
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ORIGINAL ARTICLE

RED CELL DISTRIBUTION WIDTH AND ITS ASSOCIATION WITH NEONATAL BACTEREMIA: A CASE-CONTROL STUDY

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ABSTRACT

Background: Bacteremia is a major cause of prolonged hospital stay and mortality in neonates and its early diagnosis remains a challenge to pediatricians. Red cell distribution width (RDW) is a component of a complete blood count test which is accessible and inexpensive and has been reported to be a possible diagnostic marker for neonatal bacteremia. This study determined the association of RDW with neonatal bacteremia in term and preterm neonates**.**

Methodology: This is a retrospective case-control study of 26 bacteremic neonates as cases and 104 non-bacteremic neonates, either symptomatic or with risk factors for bacteremia, as controls. Included newborns were seen between January 1, 2010 to September 30, 2021. Laboratory data obtained were CBC, C-reactive protein and blood culture.

Results: RDW values between bacteremic and non-bacteremic neonates were not significantly different. There was an association between RDW and neonatal bacteremia at an RDW level of $\geq$ 16.1, where the likelihood of bacteremia was three times higher compared with lower RDW values. Significantly lower levels of hemoglobin, hematocrit, RBC count, WBC count, platelet count, MCH and MCHC, and a higher CRP level were seen among bacteremic neonates compared to those who were not. The median RDW for both term and preterm neonates was close to 16, with a narrow inter-quartile range at 1 and 2 for controls and cases, respectively. The range (minimum to maximum) of RDW values of bacteremic preterm neonates was more variable than those of term neonates. Using RDW to detect bacteremia, it had an equivocal discriminatory power or AUC of 0.6056. We found insufficient evidence to demonstrate a correlation between RDW and other CBC parameters, except for MCHC. For MCHC, the results suggest a very weak and indirect correlation.

Conclusion: RDW was not significantly different between bacteremic and non-bacteremic neonates, but there was a suggested association between RDW and bacteremia at an RDW level of $\geq$ 16.1, at which level there was a 3-fold risk for bacteremia.

KEYWORDS: *Red Cell Distribution Width (RDW), Neonatal Bacteremia, Case-Control Study*

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The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

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INTRODUCTION

Bacteremia is a major cause of prolonged hospital stay and mortality in neonates. It can present with nonspecific signs and symptoms; thus, early diagnosis remains a challenge to pediatricians. Neonatal bacteremia is defined as a systemic inflammatory syndrome, in the presence of a growth in the blood culture, occurring within the first 28 days of life.[1] This can progress to various complications, such as severe sepsis, septic shock, multi-organ failure and death. In the evaluation of neonatal bacteremia, various hematologic markers are commonly used, such as a complete blood count (CBC), C-reactive protein (CRP), procalcitonin, and blood culture. However, blood culture, the gold standard, may take many hours to days to grow, with the identification of the organism taking even longer. Previous studies showed a positivity rate of blood cultures at a range of 25% to 54%.[2] Therefore, a rapid and practical means of predicting for bacteremia in a neonate would be invaluable for clinicians.

Red cell distribution width (RDW), a component of the CBC, measures the variability of the red blood cell size. It has been widely used to identify and differentiate between hematologic diseases, such as iron deficiency anemia and thalassemia, among others. Lippi, et al., reported that RDW increases in cases of infection.[3] RDW has been proven to predict outcomes of various illnesses in adults; however, its use among neonates has not been well-studied.

Using RDW as an initial screening test for neonatal bacteremia is proposed. This study's main objective is to determine the association between RDW and neonatal bacteremia among term and preterm neonates. Specifically, this study aims to compare the RDW of neonates based on age of gestation and blood culture results; determine the correlation between RDW with CRP and CBC indices; determine the optimal cut-off point and discriminatory power of RDW in predicting for bacteremia; and lastly, identify the organisms that cause bacteremia.

METHODOLOGY

This research utilized a retrospective, case-control study design among admitted neonates in a private tertiary hospital, from January 1, 2010 to September 30, 2021. A minimum sample size of 125 (25 cases and 100 controls) preterm and term neonates, with both CBC and blood culture done within the first 28 days of life were required for this study, based on a level of significance of 5%, a 1:4 ratio of cases to control, and an AUC of 0.938. This assumed that the AUC is significantly different from a null hypothesis value of 0.80 (good). The computation for sample size was based on the study of Deka, et al.[4]

Newborns admitted to the neonatal intensive care unit (NICU) or to the wards were identified using records review. Case patients were neonates, who had documented bacteremia on blood culture, within the first 28 days of life, with age of gestation (AOG) at term (>37 weeks AOG) or preterm (29 0/7 to 36 6/7 weeks AOG), delivered via spontaneous vaginal or caesarean section at the study institution, or outside of the institution as long as the neonate was transferred within 28 days of life. The CBC should have been taken on the same day that the blood culture was drawn, and the CRP within 48 hours from the time that the blood culture was obtained, prior to the start of empiric antibiotic therapy.

Neonates with the following conditions were excluded, based on studies that these conditions might have an effect on the RDW: (1) congenital malformations, (2) chromosomal abnormalities, (3) congenital infections due to the TORCH complex, (4) metabolic disease, (5) Rh or ABO incompatibility, (6) already on antibiotics in whom no blood culture was done, (7) had blood transfusion before the sepsis evaluation was done, and (8) perinatal asphyxia.[5-7]

After cases (bacteremic neonates) were identified, gestational-age-matched non-bacteremic controls were obtained by simple random sampling, with a 1:4 ratio.

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Neonatal bacteremia was defined as having a growth of a clinically significant bacterial organism in a neonate's blood culture. The controls were non-bacteremic neonates who were (1) culture-negative but symptomatic for possible infection, with any 1 of the following: temperature irregularity, cardiopulmonary issues [i.e., tachypnea, apnea, grunting, desaturations, retractions, tachycardia in the absence of cardiac disease, and cyanosis], feeding difficulties [i.e. poor suck, refusal to feed, feeding intolerance], early jaundice before the 24th hour of life; and (2) culture-negative asymptomatic patients, whose mothers had conditions that put their neonates at risk for bacteremia, such as maternal fever within one week from delivery, meconium-stained amniotic fluid, prolonged rupture of membranes for more than 18 hours, or maternal urinary tract infection within two weeks before delivery.

Descriptive statistics were used to summarize the general and clinical characteristics of the participants. Shapiro-Wilks test was used to determine the normality distribution, while Levene's test was used to test the homogeneity of variance of continuous variables. Continuous quantitative data were presented as mean and standard deviation (SD) when the distribution was normal, while median and range (minimum and maximum) were used when there was a non-Gaussian distribution. Continuous variables that satisfied the assumption of normality but violated the variance homogeneity were compared using Welch's test. If both assumptions were violated, the non-parametric Mann-Whitney U test was used. Categorical data were analyzed using Chi-square test. Fisher's exact test was used when the expected percentages in the cells were less than 5%.

The relationship of RDW with different laboratory parameters was tested using Spearman's correlation. The correlation coefficient interpretation is as follows: 0.0–0.2, very weak; 0.2–0.4, weak; 0.4–0.6, moderate; 0.6–0.8, strong; 0.8–1.0, very strong.

Receiver operating characteristic (ROC) curves were constructed to determine the optimal cut-off value of RDW in predicting neonatal bacteremia. Sensitivity, specificity, likelihood ratio and their 95% confidence intervals were computed. Youden's J index was defined for all points along the ROC curve, and the maximum value of the index was used as a criterion for selecting the best cut-off point. The null hypothesis was rejected at 0.05 α-level of significance. STATA version 15.0 (Stata Corp SE, College Station, TX, USA) was used for data analysis.

Ethical Considerations

This study was administered in accordance with the Good Clinical Practice (GCP) training, which were completed by the investigators. This study went through IRB approval. A waiver of informed consent was allowed, and granted by the IRB, as the conduct of the study entailed only a medical records review. The identities and confidentiality of study participants were preserved using anonymized case report forms. The authors have no potential conflicts of interest to disclose.

RESULTS

There were 130 neonates included in this study: 26 bacteremic cases, and 104 non-bacteremic controls (Table 1). Among 37 eligible cases, the following were excluded: incomplete chart (n=4), poor APGAR score (n=4), history of antibiotic use (n= 2), and history of blood transfusion prior to blood drawing (n=1), yielding the 26 cases (see Figure 1.) Among 120 controls, 104 were selected by simple random sampling.

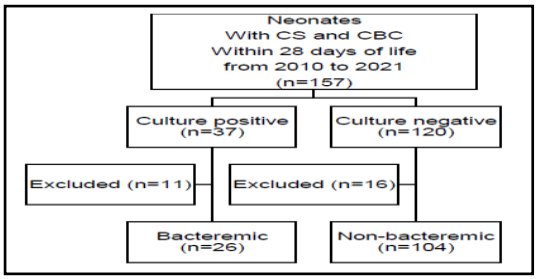

Figure 1. Methodology Flowchart

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Table 1. Neonatal characteristics, bacteremic versus non-bacteremic

Parameter	All (n=130)	Bacteremic (n=26)	Non-bacteremic (n=104)	p
	Median (Range); Frequency (%)			
Birthweight (grams)	2590 (795-4700)	2697.5 (1050-3600)	2570 (795-4700)	.903*
Sex				.722‡
Male	76 (58.46)	16 (61.54)	60 (57.69)	
Female	54 (41.54)	10 (38.46)	44 (42.31)	
APGAR	[n=121]		[n=97]	
1st min	9 (5-9)	9 (6-9)	9 (5-9)	.525*
5th min	9 (7-10)	9 (7-9)	9 (7-10)	.257‡
Delivery				.587‡
Cesarean section	81 (62.31)	15 (57.69)	66 (63.46)	
vaginal delivery	49 (37.69)	11 (42.31)	38 (36.54)	
Delivered				.593‡
Inborn	114 (87.69)	22 (84.62)	92 (88.46)	
Out born	16 (12.31)	4 (15.38)	12 (11.54)	
Indication for culture				<.001‡
Neonatal	79 (53.08)	23 (88.46)	46 (44.23)	
Perinatal	25 (19.23)	2 (7.69)	23 (22.12)	
Maternal	36 (27.69)	1 (3.85)	35 (33.65)	
Blood CS Day of life	1 (1-28)	4 (1-21)	1 (1-28)	<.001*
CBC day of life	1 (1-28)	4 (1-21)	1 (1-28)	<.001*
CRP day of life	1 (1-28); [n=126]	4 (1-21)	1 (1-28); [n=102]	<.001*

Statistical test used: * - Mann-Whitney U test; † - Fisher's Exact test; ‡ - Chi-square test

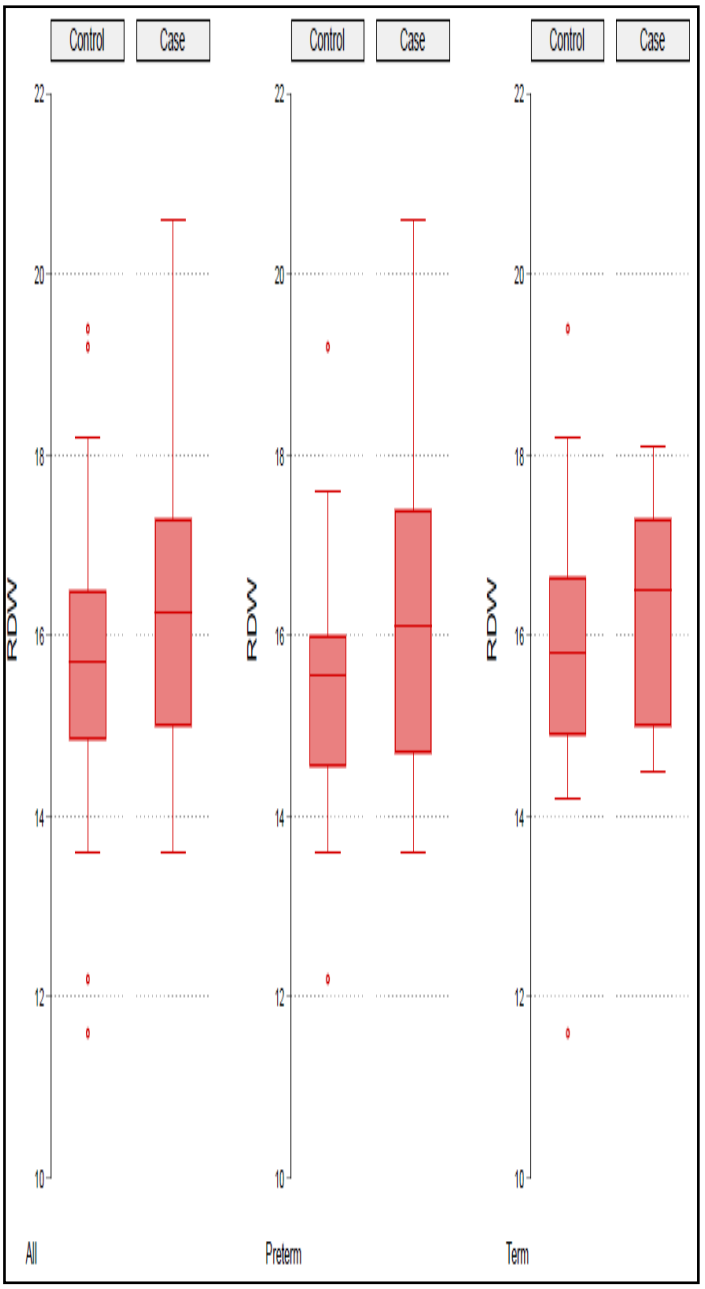

Figure 2. Boxplot of RDW between cases and controls; overall, preterm, and term

Overall, the median birth weight was 2.59 kilograms, 58% were male, 62% were delivered via caesarean section, and 12% were outborn. There were notable differences between the two groups in terms of indication for requesting for blood culture, 53% of which were due to neonatal causes. Blood culture, CBC and CRP were obtained at a significantly later day in the bacteremic group at a median of day 4, versus a median of day 1 for the control group.

The median RDW for both term and preterm neonates was close to 16, with a narrow inter-quartile range at 1 and 2 for controls and cases, respectively. The range (minimum to maximum) of RDW values for bacteremic preterm neonates was more variable than those of the term neonates, as demonstrated by the symmetric whiskers in the boxplot of term neonates.

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Table 2. CBC and CRP results of neonates, bacteremic versus non-bacteremic

Parameter	All (n=130)	Bacteremic (n=26)	Non-bacteremic (n=104)	*p*
	Median (Range); Mean ± SD; Frequency (%)			
RDW	15.75 (11.6-20.6)	16.25 (13.6-20.6)	15.7 (11.6-19.4)	.096*
Hemoglobin (g/L)	17.01 ± 2.9	14.81 ± 3.48	17.56 ± 2.46	**<.001**
Hematocrit	48.1 ± 7.89	42.5 ± 9.73	49.5 ± 6.71	**.002**
RBC (x 10^12/L)	4.76 ± 0.79	4.27 ± 0.98	4.89 ± 0.69	**.005**
WBC (10^9/L)	15.89 (1.51-48.06)	11.035 (1.51-35.31)	17.37 (4.85-48.06)	**.007***
Segmenter	61.5 (14-85)	59 (14-85)	62 (23-85)	.382*
Lymphocyte	27 (6-80)	26 (10-80)	27 (6-64)	.942*
Monocyte	10 (1-26)	10.5 (1-18)	10 (2-26)	.886*
Eosinophil	1 (0-9)	0.5 (0-4)	1 (0-9)	.118*
MCH (pg)	35.6 (27.7-41.6)	34.9 (27.7-41)	35.8 (30.1-41.6)	**.008***
MCHC	100.7 (81.1-192.4)	99.25 (81.1-119.7)	101.05 (88-192.4)	**.031***
MCV	35.33 ± 1.12	34.82 ± 1.17	35.46 ± 1.08	.139
Platelet	286500 (10000-2900000)	205000 (10000-521000)	304000 (33000-2900000)	**<.001***
CRP (mg/L)	2.6 (0.09-231.61)	17.995 (0.1-231.61)	1.85 (0.09-175.17)	**.002***
≤5	71 (56.35)	9 (37.5)	62 (60.78)	
>5	55 (43.65)	15 (62.5)	40 (39.22)	

Statistical test used: If with asterisk (*), Mann-Whitney U test. Otherwise, Welch's test.

There were significant differences between the two groups in the following: hemoglobin, hematocrit, RBC count, WBC count, MCH, MCHC and platelet count which were lower, and CRP which was higher among those with bacteremia versus non-bacteremia.

The median RDW values were not significantly different between the two groups.

Table 3. Blood CS growth results among neonates

Organism		Frequency	Proportion (%)
Gram positive	***Streptococcus agalactiae***	6	23
	Oxacillin-resistant *Staphylococcus epidermidis*	3	11.5
	Staphylococcus aureus	1	4
	Staphylococcus saprophyticus	1	4
	Micrococcus luteus	1	4
	Streptococcus sanguinis	1	4
Gram negative	***Escherichia coli***	3	11.5
	Klebsiella pneumoniae	2	7.7
	Pseudomonas stutzeri	2	7.7
	Serratia marcescens	2	7.7
	Salmonella enteritidis	1	4
	Acinetobacter baumanii complex	1	4
	Citrobacter koseri	1	4
	Enterobacter aerogenes	1	4

The most common blood isolates were *Streptococcus agalactiae* and oxacillin-resistant *staphylococcus epidermidis* for the gram-positive organisms, and *Escherichia coli* for the gram-negative organisms.

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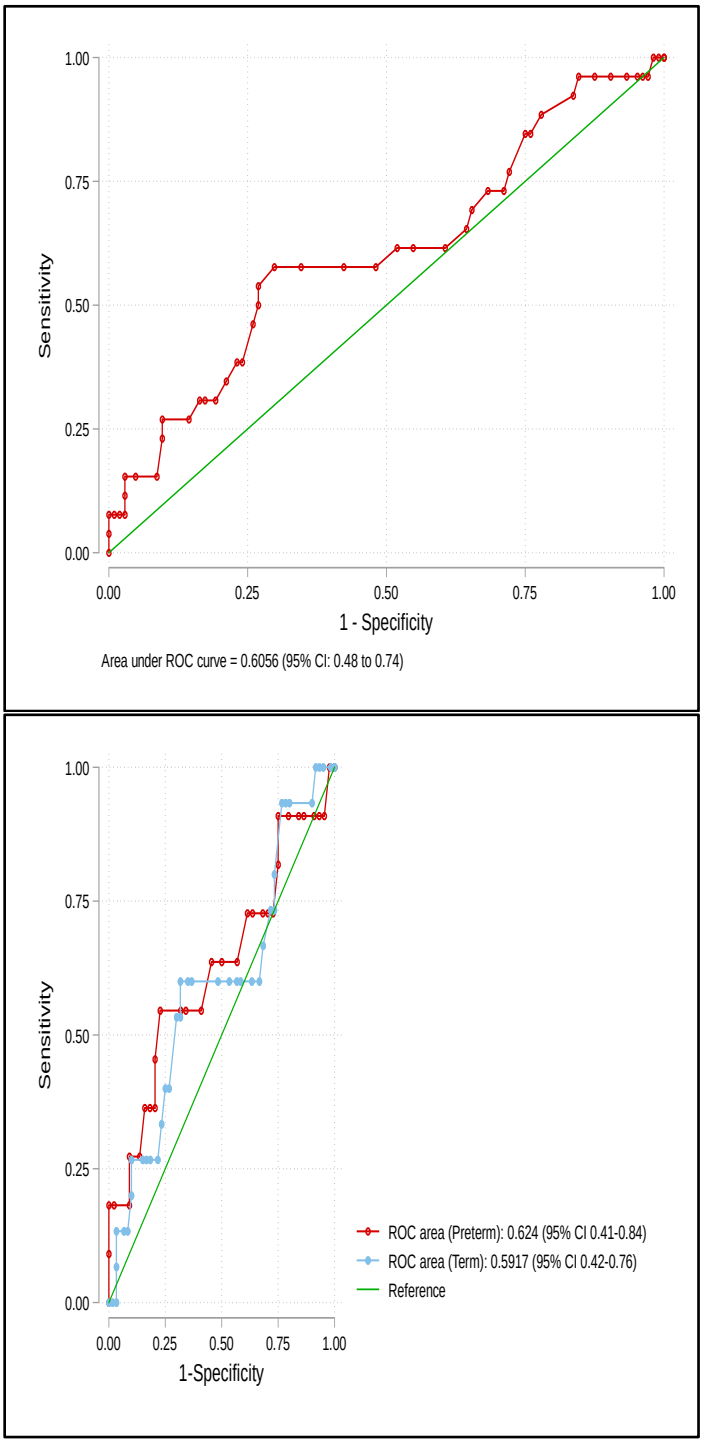

Figure 3. Receiver operating characteristic curve of RDW in predicting bacteremia

Using RDW to detect bacteremia, it had an equivocal discriminatory power or AUC of 0.6056 and 95% CI of 0.48 to 0.74.

Table 4. Diagnostic performance measure of RDW at different cutoff scores

Cutpoint	Sensitivity	Specificity	Correctly Classified	LR+	LR-	Youden's J index
(>= 15)	76.92%	27.88%	37.69%	1.0667	0.8276	4.80%
(>= 15.1)	73.08%	28.85%	37.69%	1.027	0.9333	1.93%
(>= 15.2)	73.08%	31.73%	40.00%	1.0704	0.8485	4.81%
(>= 15.3)	69.23%	34.62%	41.54%	1.0588	0.8889	3.85%
(>= 15.4)	65.38%	35.58%	41.54%	1.0149	0.973	0.96%
(>= 15.5)	61.54%	39.42%	43.85%	1.0159	0.9756	0.96%
(>= 15.6)	61.54%	45.19%	48.46%	1.1228	0.8511	6.73%
(>= 15.7)	61.54%	48.08%	50.77%	1.1852	0.8	9.62%
(>= 15.8)	57.69%	51.92%	53.08%	1.2	0.8148	9.61%
(>= 15.9)	57.69%	57.69%	57.69%	1.3636	0.7333	15.38%
(>= 16)	57.69%	65.38%	63.85%	1.6667	0.6471	23.07%
(>= 16.1)	57.69%	70.19%	67.69%	1.9355	0.6027	27.88%
(>= 16.2)	53.85%	73.08%	69.23%	2	0.6316	26.93%
(>= 16.3)	50.00%	73.08%	68.46%	1.8571	0.6842	23.08%
(>= 16.5)	46.15%	74.04%	68.46%	1.7778	0.7273	20.19%
(>= 16.6)	38.46%	75.96%	68.46%	1.6	0.8101	14.42%
(>= 16.7)	38.46%	76.92%	69.23%	1.6667	0.8	15.38%
(>= 16.8)	34.62%	78.85%	70.00%	1.6364	0.8293	13.47%
(>= 16.9)	30.77%	80.77%	70.77%	1.6	0.8571	11.54%
(>= 17)	30.77%	82.69%	72.31%	1.7778	0.8372	13.46%

Maximal cutpoint for both term and preterm neonates were based on the highest Youden's J index to maximize both specificity and sensitivity. The cut point with the highest Youden's J index was established at an RDW level of ≥16.1 (sensitivity 58%, specificity 70%, LR+ 1.94, LR- 0.60 and Youden's J index at 28%).

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Table 5. Association of RDW to bacteremia

Parameter		Odds Ratio (95% CI)	*p*
Overall (n=130)	RDW as continuous variable	1.403 (1.03-1.92)	**.034**
	RDW ≥ 16.1	3.211 (1.33-7.77)	**.010**
	RDW ≥ 16.1 *adjusted by birthweight*	3.238 (1.33-7.90)	**.010**
Preterm (n=55)	RDW as continuous variable	1.475 (0.97-2.25)	.070
	RDW ≥ 16.1	4.08 (1.03-16.23)	**.046**
	RDW ≥ 16.1 *adjusted by birthweight*	4.663 (1.11-19.58)	**.035**
Term (n=75)	RDW as continuous variable	1.313 (0.81-2.11)	.264
	RDW ≥ 16.2	3.237 (1.007-10.40)	**.049**
	RDW ≥ 16.2 *adjusted by birthweight*	3.325 (1.02-10.80)	**.046**

Our results suggest an association between RDW and bacteremia; overall, every unit increase of RDW corresponds with an increased odds of bacteremia by 1.4 times (95% CI 1.03-1.92, p = 0.034). Neonates with RDW ≥ 16.1 are three times as likely to have bacteremia, compared to those with lower RDW values, even after adjusting for birthweight. A similar association was seen even after the neonates were stratified by gestational age, into preterm and term groups.

Table 6.Correlation between RDW and laboratory values

Parameter	Correlation Coefficient	Interpretation	p
Hemoglobin (g/L)	0.084	Direct, very weak	.340
Hematocrit	0.129	Direct, very weak	.143
RBC (x 10^12/L)	0.138	Direct, very weak	.118
WBC (10^9/L)	0.068	Direct, very weak	.441
Segmenter	0.115	Direct, very weak	.191
Lymphocyte	-0.152	Indirect, very weak	.084
MCH (pg)	-0.098	Indirect, very weak	267
MCHC	-0.185	Indirect, very weak	.035
MCV	0.009	Direct, very weak	.922
Platelet	-0.074	Indirect, very weak	.405
CRP	0.167	Direct, very weak	.063

We found insufficient evidence to demonstrate a correlation between RDW and other CBC parameters, except for MCHC. For MCHC, the results suggest a very weak and indirect correlation (p = 0.035).

Table 7. Subgroup analysis for bacteremic neonates (n=26)

Parameter	Nonsurvivor (n=6)	Survivor (n=20)	cOR (95% CI)	*p*
RDW	15.65 (14.6-17.1)	16.4 (13.6-20.6)	0.767 (0.41-1.43)	.405
<16.1	3 (50)	8 (40)	Reference	-
≥ 16.1	3 (50)	12 (60)	0.68 (0.12-3.79)	.660

We found insufficient evidence to demonstrate an association between RDW and mortality among bacteremic neonates.

DISCUSSION

Neonatal bacteremia remains a diagnostic challenge to pediatricians. It has a high mortality rate, so that early diagnosis and appropriate treatment are critical. As previously mentioned, blood culture is the gold standard in diagnosing neonatal bacteremia. However, studies have shown that blood cultures have a low positivity yield, and the isolation and identification of an organism takes time.[2] Hence, another parameter for predicting bacteremia in a neonate is invaluable for pediatricians.

Red cell distribution width is part of a routine CBC test, which is readily available and cost-effective. Lanzkowsky wrote that RDW and mean corpuscular volume (MCV) are mainly used to determine the etiology of anemia.[6] Higher RDW values indicate an increase in the variations of RBC volume.[6] Jianping Chen, et al. found that aside from RDW's use as a tool to differentiate between various types of anemia, it can be increased during inflammation and oxidative stress.[8]

Although the mechanism of this increase in RDW during bacteremia is unknown, it has been proposed that an inflammatory process affects red cell production, as may occur in neonatal bacteremia.[9]

In our study, red cell distribution width values among term and preterm neonates, as well as those with bacteremia (16.2) and non-bacteremic controls (15.7), were not significantly different. These results are unlike that reported by Tonbul, et al., who prospectively studied RDW levels of 1,596 healthy newborns on the first day of life, and found that levels differed between term (16.6) and preterm newborns (17.9).[10] In the same study, it was speculated that increased erythropoiesis occurs in early gestational ages (GA), and their results showed that preterm neonates at 32-34 weeks GA had higher RDW values, compared to those of term neonates (37-42 weeks GA).[10] A negative correlation between RDW and GA may explain the broad range of RDW values in the bacteremic preterm neonate group, in contrast to the symmetric whiskers seen in the term neonates (Figure 2).[11]

A prospective observational study by Deka, et al. showed significantly different RDW values on day 1 of life, between the 50 septic neonates (18.6) and 50 well newborns (16.2).[4] Our study results may have varied from the above studies due to differences in population size, as the controls they used were healthy newborns, while our study used symptomatic newborns with no blood culture isolate; disease prevalence, and timing of blood sampling. Another possible reason for our findings as being different from the above studies may be the selection of cut-off levels for RDW. When the Youden J Index was applied to our study results to measure the RDW's ability to determine a balance between sensitivity and specificity, we found RDW level of ≥16.1 to be the optimal value to yield the highest J index. Other investigators may have used cut-off points based on a preference for either a more specific or sensitive test, instead of finding a good a balance between sensitivity and specificity.

This study found a significant association between RDW and neonatal bacteremia when an RDW of ≥ 16.1 was used as a cut-off point, at which a neonate was three times more likely to have bacteremia, compared to one with a lower RDW value, even after adjusting for birthweight, and after stratifying by gestational age. One study showed that an increased RDW is reflective of an inflammatory process triggered by hormones such as noradrenaline and angiotensin, which stimulate the production and proliferation of cells by erythrogenin (EPO), with the end-result of an increased RDW.[8] Ellahony, et al. also reported that in a septic patient, oxidative stress is increased, resulting in a decrease in the lifespan of circulating red blood cells (RBCs) which stimulate erythroid tissue to produce and release new RBCs from the bone marrow.[12]

Guo and Sun also reported an association between RDW and sepsis, but RDW was not found to be the sole predictor of neonatal sepsis based on the ROC analysis.[13] Guo's study showed that an elevated baseline RDW (17.9) in preterm infants was associated with sepsis (aOR 4.68).[13] As they followed the changes in the RDW during the hospital stay, they found a better association between RDW and sepsis, such that the ROC curve analysis of RDW at baseline, along with the RDW through the length of the hospital stay, showed an ROC curve analysis of 0.81; the sensitivity and specificity were 78.2 and 72.5%, respectively.[13] Our ROC analysis, on the other hand, showed an AUC of 0.605, which indicates RDW to be a poor predictor for neonatal bacteremia. An improvement on this result may be possible, if dynamic changes in RDW are recorded over the course of the hospitalization for neonates who may later develop bacteremia.

Our study found significantly higher C-reactive protein (CRP) levels and lower WBC and platelet counts among bacteremic neonates versus those who weren't. These findings were similar to findings in a review by Da Silva, et al., where CRP was shown to be a good diagnostic measure for neonatal sepsis with a sensitivity of 58-100%, and an NPV of 86-100%.[14]

Lippi, et al. reported CRP to have an AUC of 0.88, which indicates it to be a good diagnostic test for neonatal sepsis.[3] Thus, CRP was established as a specific diagnostic marker for neonatal bacteremia; however, CRP levels only begin to increase after 6 to 8 hours of infection, so that the level may be falsely test negative if done earlier.[14] CRP test is also expensive and not readily available in resource-limited settings, hence, its routine use locally may not be possible.

In our study, significantly lower platelet levels were seen among bacteremic neonates, which agrees with a local study of 100 NICU neonates by Mayuga, et al., where thrombocytopenia was significantly more common among septic babies, but with a sensitivity of 35% and negative predictive value (NPV) of 87%.[14,15] Hence, using platelet count as a sole diagnostic marker for bacteremia is not recommended and should be used in relation with other diagnostic parameters.

In our study, WBC count was notably lower in the bacteremic versus the non-bacteremic group. However, the median WBC count in the bacteremic group was still within normal limits for preterm and term neonates.[6] In the study by Mayuga, et al., no significant association between WBC and neonatal sepsis was reported.[15] Da Silva, et al. also reported that very high or very low WBC counts as sepsis indicators have a wide range of sensitivity at 17% to 90%, and specificity of 31% to 100%.[14] Most often, bacteremic babies will not present with marked leukocytosis nor leukopenia, and values may also be affected by other causes (e.g., metabolic factors, stress, etc.). Thus, using WBC count alone as a diagnostic marker for neonatal bacteremia is not recommended but may be used in conjunction with other diagnostic parameters.

Blood culture, CBC and CRP were obtained earlier in the non-bacteremic neonates (day 1) as compared to bacteremic neonates (day 4). Majority (55.8%) of the tests were requested due to neonatal and maternal reasons.

This early timing for the sepsis work-up is not unexpected as this is often done as a result of maternal risk factors and the early tachypnea commonly seen soon after birth, which may be due to a variety of reasons. Among the bacteremic neonates, however, the blood cultures and CBCs were done on the 4th day of life, often due to a clinical setback or observation in the neonate that may not have been evident at birth. The timing of these tests could have affected the results obtained in our study.

Our study showed MCH and MCHC to be significantly lower among bacteremic neonates. To the best of our knowledge, these findings have not been previously reported. Piagnerelli, et al. reported a decrease in the hematocrit, hemoglobin and RBC count among septic neonates; however, they found no association or significant differences for MCHC or MCH between septic and non-septic babies.[16] We report more gram-positive than gram-negative organisms in this study. In a local study by Meliton, gram-negative sepsis was more common in the study institution.[17] However, in our study, many gram-negative bacteremic cases were not included due to the inclusion and exclusion criteria (i.e., history of early antibiotic use, history of blood transfusion, and lack of a corresponding control group). Nevertheless, it is notable that there were six cases of *Streptococcus agalactiae* in our study. Among the 366 neonatal bacteremia in five local studies, there was only one previous report of a *Streptococcus agalactiae* blood culture growth, with that sole case coming from this study institution.[17-21]

In our study, a significant and positive correlation between RDW and MCHC in the neonatal bacteremia group was observed. This was in contrast to the study by Cosar, et al. which found a significant correlation between RDW and CRP.[4,22] In another retrospective study, data from 500 septic term neonates were obtained and compared RDW values with other diagnostic parameters for sepsis.[12] The study showed a positive correlation between RDW and CRP, WBC count, and a negative correlation between RDW and hemoglobin and platelet count.[12]

Our results may have varied from previous studies due to differences in the study population, disease prevalence and timing of blood sampling. Our study found no significant difference between RDW levels seen among bacteremic neonates who survived, versus those who died. This is in contrast to a prospective study that showed RDW levels of 251 septic neonates (19.9%) to be significantly higher than those of healthy controls (18.9%).[23] In another prospective study, 50 septic neonates were compared to a control group composed of healthy neonates; their results showed that an elevated RDW was associated with severe sepsis, septic shock and a higher mortality rate in the ICU.[24] The mechanism as to how an elevated RDW is associated with an increased severity of illness remains unknown, but factors such as augmented inflammation, leading to organ dysfunction, have been speculated.[23]

Our findings may have also varied from previous studies due to differences in the study population, wherein they included clinically and laboratory diagnosed septic neonates as cases, and healthy neonates as controls; while in our study, we included bacteremic neonates as cases and symptomatic non-bacteremic neonates as controls. An improvement in our findings may have been possible if the RDW values were correlated with severity of the disease (e.g., subgrouping cases as septic and septic shock); and if RDW were dynamically measured over time, from the baseline levels, through the different stages of bacteremia and sepsis.[12]

Being a retrospective study, a limitation of this study is that we could only test for an association between RDW and neonatal bacteremia, and no causal relationship could be made. The insufficient evidence to demonstrate a correlation between RDW and neonatal bacteremia, as well as between RDW with other factors, may be associated with a low prevalence of neonatal bacteremia in the study population, the selected cut-off points for RDW, and the choice of control group.

Another limitation in this study is that there is no serial monitoring of RDW levels of bacteremic patients after the administration of antibiotics was done. Our study was conducted in a single tertiary hospital; hence, results may not be generalized to the rest of the country.

CONCLUSION AND RECOMMENDATIONS

Early diagnosis of neonatal bacteremia is essential for clinicians to be able to provide prompt and appropriate intervention for bacteremic neonates. In this study, RDW, which is a part of a routine CBC test and is readily available, showed an association with neonatal bacteremia at an RDW level of $\geq$ 16.1 at which there was a three-fold risk for neonatal bacteremia. However, this study found that RDW was not significantly different between bacteremic and non-bacteremic neonates. Lastly, this study showed significantly lower levels of hemoglobin, hematocrit, RBC count, WBC count, platelet count, MCH and MCHC, and a higher CRP among bacteremic neonates versus non-bacteremic neonates.

We recommend a prospective cohort study with a larger population in future studies to be able to accurately evaluate RDW as diagnostic marker for neonatal bacteremia. Also, in future prospective cohort studies, the utility of MCH and MCHC as indicators for neonatal bacteremia may also be studied.

REFERENCES

1. Gomella T, Eyal FG, Bany-Mohammed F. Gomella's Neonatology: Management, Procedures, On-Call Problems, Diseases, and Drugs. 8th ed. USA: McGraw Hill; 2020. p.1175-1189
2. Kayange N, Kamugisha E, Mwizamholya DL, Jeremiah S, Mshana SE. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania. *BMC Pediatr* [Internet]. 2010 Jun [cited 2022 Mar 17];10:39. Available at: https://pubmed.ncbi.nlm.nih.gov/20525358/

3. Lippi G, Targher G, Montagnana M, Salvagno GL, Zoppini G, Guidi GC. Relation between red blood cell distribution width and inflammatory biomarkers in a large cohort of unselected outpatients. *Arch Pathol Lab Med* [Internet]. 2009 Apr [cited 2021 Feb 16];133(4):628-632. Available at: https://pubmed.ncbi.nlm.nih.gov/19391664/
4. Deka A, Aravind P. Red cell distribution width as a diagnostic marker in neonatal sepsis. *Int J Contemp Pediatr* [Internet]. 2020 Apr [cited 2021 Feb 16];7(4):820-825. Available at: https://www.ijpediatrics.com/index.php/ijcp/article/view/3161
5. Hansen AR, Soul JS. Perinatal Asphyxia and Hypoxic-Ischemic Encephalopathy. Eichenwald EC, Hansen AR, Martin CR, Stark AR, editors. Cloherty and Stark's manual of neonatal care. 8th ed. Philadelphia: Wolters Kluwer; 2017. p.791-811
6. Lanzkowsky P, Lipton J, Fish JD. Lanzkowsky's Manual of Pediatric Hematology and Oncology. 6th ed. London: Academic Press; 2016. p.32-41
7. Sarangi S, Acharya S. bleeding disorders in congenital syndromes. *Pediatrics* [Internet]. 2017 Feb [cited 2022 Mar 17]; 139 (2): e20154360. Available at: https://publications.aap.org/pediatrics/article-abstract/139/2/e20154360/60298/Bleeding-Disorders-in-Congenital-Syndromes?redirectedFrom=fulltext
8. Jianping C, Ling J, Tong Y. Clinical study of RDW and prognosis in sepsis new borns. *Biomed Res* [Internet]. 2014 Jul [cited 2021 Feb 16];25(4):576-579. Available at: https://www.biomedres.info/biomedical-research/clinical-study-of-rdw-and-prognosis-in-sepsis-new-borns.html
9. Yasar M, Gowda B. Red Cell Distribution Width as a marker for early onset neonatal sepsis – a cross sectional study. *Int J Sci Res* [Internet]. 2020 Apr [cited 2021 Feb 16];9(4):20-22. Available at: https://www.worldwidejournals.com/international-journal-of-scientific-research-(IJSR)/fileview/red-cell-distribution-width-as-a-marker-for-early-onset-neonatal-sepsis--a-cross-sectional study_April_2020_1585833234_1724995.
10. Tonbul A, Tayman C, Catal F, Kara S, Tatli MM. Red cell distribution width (RDW) in the newborn: normative data. *J Clin Lab Anal* [Internet]. 2011 Nov [cited 2021 Feb 16];25(6):422-425. Available at: https://pubmed.ncbi.nlm.nih.gov/22086796/
11. Garofoli F, Ciardelli L, Mazzucchelli I, Borghesi A, Angelini M, Bollani L, et al. The red cell distribution width (RDW): value and role in preterm, IUGR (intrauterine growth restricted), full-term infants. *Hematology* [Internet]. 2014 Sep [cited 2021 Feb 16];19(6):365-369. Available at: https://pubmed.ncbi.nlm.nih.gov/24225072/
12. Ellahony DM, El-Mekkawy MS, Farag MM. A study of red cell distribution width in neonatal sepsis. *Pediatr Emerg Care* [Internet]. 2020 Aug [cited 2021 Feb 16];36(8):378-383. Available at: https://pubmed.ncbi.nlm.nih.gov/29084071/
13. Guo BF, Sun SZ. Diagnostic accuracy of a dynamically increased red blood cell distribution width in very low birth weight infants with serious bacterial infection. *Ital J Pediatr* [Internet]. 2021 Feb [cited 2021 Feb 16];27:47(1):44. Available at: https://pubmed.ncbi.nlm.nih.gov/33640017/
14. Da Silva O, Ohlsson A, Kenyon C. Accuracy of leukocyte indices and C-reactive protein for diagnosis of neonatal sepsis: a critical review. *Pediatr Infect Dis J* [Internet]. 1995 May [cited 2021 Feb 16];14(5):362–366. Available at: https://www.ncbi.nlm.nih.gov/books/NBK66432/
15. Mayuga WA, Isleta PF. Clinical correlation of neonatal and maternal hematological parameters as predictors of neonatal sepsis. *PIDSP Journal.* 2005;9(2):36-43
16. Piagnerelli M, Boudjeltia KZ, Brohee D, Piro P, Carlier E, Vincent JL, et al. Alterations of red blood cell shape and sialic acid membrane content in septic patients. *Crit Care Med* [Internet]. 2003 Aug [cited 2021 Feb 16];31(8):2156-62. Available at: https://pubmed.ncbi.nlm.nih.gov/12973174/
17. Meliton-Ruiz AMV, Garcia RDJ. Outcome of current antibiotic regimens used for neonatal sepsis in a tertiary hospital. *PIDSP Journal.* 2018;19(2):51-59
18. Maramba-Lazarte C, Bunyi M, Gallardo E, Lim J, Lobo J, Aguilar C. Etiology of neonatal sepsis in five urban hospitals in the Philippines. *PIDSP Journal.* 2011;12(2):75-85
19. Imperial M, Mantaring III J. Risk Factors for Mortality in Service Neonatal Sepsis. *PIDSP Journal*. 2002;6(2):5-9
20. Ignacio R, Padilla C, Fabay X. Demographic profile and outcomes of potentially septic patients at Baguio general hospital (July 2004-June 2006). *PIDSP Journal.* 2012;13(1): 57-62
21. Maderal LAH, Cavan BCV. The clinical outcome and antibiotic sensitivity pattern of *Enterobacter spp*. Culture-positive neonates admitted at Cebu Doctors' University Hospital-Neonatal Intensive Care Unit (2005-2008). *PIDSP Journal.* 2012;13(2):22-29
22. Cosar H, Yilmaz O, Temur M, Ozun OP, Bulut Y. Relationship between early-onset neonatal sepsis and red blood cell distribution width (RDW). *J Hematol Thrombo Dis* [Internet]. 2017 [cited 2021 Feb 16];5(2):266. Available at: https://www.researchgate.net/publication/316949570_Relationship_between_Early-Onset_Neonatal_Sepsis_and_Red_Blood_Cell_Distribution_Width_RDW
23. Hu ZD, Lippi G, Montagnana M. Diagnostic and prognostic value of red blood cell distribution width in sepsis: A narrative review. *Clin Biochem* [Internet]. 2020 Mar [cited 2021 Feb 16];77:1-6. Available at: https://pubmed.ncbi.nlm.nih.gov/31935355/
24. Medhat A, Yasser T, Hesham H. Evaluation of neonatal sepsis and assessment of its severity by Red Cell Distribution Width indicator. *Egypt Community Med* [Internet]. 2017 Jul [cited 2021 Feb 16];35(3):21-32. Available at: https://jmscr.igmpublication.org/v7-i8/9%20jmscr.pdf

25. Saad Refaay MM, Refaat Hablas H, El-Karim Mohamed M, Abd El-Aziz AF. Significance of red cell distribution width as a predictor in neonatal sepsis. *Al-Azhar Journal of Ped* [Internet]. 2020 Jan [cited 2021 Feb 16];23(1):745-762. Available at: https://azjp.journals.ekb.eg/article_79327.html
26. Singh M, Sitaraman M, Choudhary R, Choudhary AS. Red blood cell distribution width as a marker of early onset neonatal sepsis: a hospital based analytical study. *J Med Sci Clin Res* [Internet]. 2019 Aug [cited 2021 Feb 16];7(8):59-65. Available at: https://jmscr.igmpublication.org/v7-i8/9%20jmscr.pdf
27. Kim CH, Park JT, Kim EJ, Han JH, Han JS, Choi JY, et al. An increase in red blood cell distribution width from baseline predicts mortality in patients with severe sepsis or septic shock. *Crit Care* [Internet]. 2013 Dec [cited 2021 Feb 16];17:R282. Available at: https://ccforum.biomedcentral.com/articles/10.1186/cc13145
28. Jo YH, Kim K, Lee JH, Kang C, Kim T, Park HM, et al. Red cell distribution width is a prognostic factor in severe sepsis and septic shock. *Am J Emerg Med* [Internet]. 2013 Mar [cited 2021 Feb 16];31(3):545-8. Available at: https://pubmed.ncbi.nlm.nih.gov/23380094/
29. Dogan P, Guney Varal I. Red cell distribution width as a predictor of late-onset gram-negative sepsis. *Pediatr Int* [Internet]. 2020 Mar [cited 2021 Feb 16];62(3):341-346. Available at: https://pubmed.ncbi.nlm.nih.gov/31880020/ DOI: 10.1111/ped.14123
30. Martin SL, Desai S, Nanavati R, Colah RB, Ghosh K, Mukherjee MB. Red cell distribution width and its association with mortality in neonatal sepsis. *J Matern Fetal Neonatal Med* [Internet]. 2019 Jun [cited 2021 Feb 16];32(12):1925-1930. Available at: https://pubmed.ncbi.nlm.nih.gov/29310472/ DOI: 10.1080/14767058.2017.1421932
31. Omer IM, Mohammed B. A study of red cell distribution width and neonatal sepsis at Soba University Hospital, Khartoum, Sudan. *Sudan J Paediatr* [Internet]. 2021 [cited 2021 Aug 12];21(1):42–47. Available at: https://pubmed.ncbi.nlm.nih.gov/33879942/
32. Bulut O, Akcakaya A, Bulut N, Ovali F. Elevated red cell distribution width as a useful marker in neonatal sepsis. *J Pediatr Hematol Oncol* [Internet]. 2021 Jul [cited 2021 Feb 16];1:43(5):180-185. Available at: https://pubmed.ncbi.nlm.nih.gov/33512870/
33. Christensen RD, Yaish HM, Henry E, Bennett ST. Red blood cell distribution width: reference intervals for neonates. *J Matern Fetal Neonatal Med* [Internet]. 2015 May [cited 2021 Feb 16];28(8):883-888. Available at: https://pubmed.ncbi.nlm.nih.gov/24968099/