Original Article

RDW-SD -A more sensitive marker of anisocytosis than RDW-CV in patients with macrocytosis

Harsha M Dangare[1], Janice Jaison[1], PS Murgod[2]

[1]Department of Pathology, MAEER MIT Pune's MIMER Medical College and Dr BSTR Hospital, Talegaon Dabhade, Pune, Maharashtra, India
[2]Department of Pathology, KAHER'S JGMM Medical College, Hubbali, India

ABSTRACT

Background: Anisocytosis is a variation in RBC size. This can be conventionally observed on peripheral blood smear microscopy and also by red cell distribution width, a RBC parameter obtained on automated hematology analyzers. Red cell distribution width is considered a quantitative measure of anisocytosis and does not carry subjective variation associated with microscopy and it is reported as RDW-CV % and RDW-SD. Both these values are calculated differently. Although it is one of the most frequently used hemogram parameters for anisocytosis, it is not well understood. This study aimed to compare the sensitivity of RDW-CV and RDW-SD as markers of anisocytosis in macrocytosis.

Material and methods: This was a cross-sectional prospective observational study conducted in a laboratory of a tertiary care hospital in western India. The sample size of 138 was calculated at a precision 0.1 and 90% confidence interval. 138 blood samples each from patients with macrocytosis and normocytosis control group were analysed.

Results: The range for RDW-CV & RDW-SD was 12.59%-15.13% & & 40.05fl-47.81fl respectively in our population. Gender-wise distribution showed 72% male & 28% female. The Z score value of 4.12 (>1.96 is significant) reflected a significant difference in anisocytosis detection using RDW-SD compared to RDW-CV in macrocytosis.

Conclusions: RDW-SD is a more sensitive marker of RBC anisocytosis than RDW-CV in macrocytosis.

Correspondence:
Dr. H.M. Dangare, MD
Associate Professor, Department of Pathology
MAEER MIT Pune's MIMER Medical College and Dr BSTR Hospital,
Talegaon Dabhade, Pune, Maharashtra, India
ORCID ID: 0000-0002-6347-9130
Email: dr.harshahj@gmail.com

INTRODUCTION

According to mean corpuscular volume (MCV) values, morphologically RBCS are divided into microcytic, normocytic, and macrocytic.[1,2]Anisocytosis is a variation in RBC size which is conventionally observed on microscopy of peripheral blood smear.[3] The first quantitative assessment of variation was described by a British pathologist Cecil Price-Jones in 1910, who postulated that such variation could be helpful in the diagnosis of anemia. The Red cell distribution width (RDW) was first measured by the automated cell analyzer "Coulter counter Model S-Plus II", which was expressed as the coefficient of variation.[4]

The earliest parameter provided by the hematology analysis instruments to measure red cell variations was the RDW-CV.[5,6]

The RDW is the most commonly reported index on the variation or degree of anisocytosis in red cell volume. The impedance and flow cytometric counters provide this parameter, which is directly calculated from the RBC histogram.[7,8] More recently RDW-SD that is standard deviation as a marker of RBC anisocytosis was introduced first by Beckman coulter LH 780 hematology analyser (Beckman Coulter Inc, Brea CA) followed by analysers like Sysmex (Kobe, Japan), Beckman Coulter (Brea, USA) and Mindray (Shenzhen, China).[7,9]

RDW is considered a quantitative measure of anisocytosis and it does not carry subjective variation associated with microscopy. Although the RDW is one of the most studied and frequently used parameters of anisocytosis in CBC, it is not well understood. RDW values obtained from the cell counters are RDW-CV and RDW-SD. These values are calculated differently. RDW-CV is calculated mathematically as coefficient of variation as follows:

RDW (CV) %= 1SD/MCVx100%.[10,11]

RDW-CV covers 68.26% of the distribution area of the RBC curve. As it is a ratio, any changes in 1SD (width) or MCV will influence the results. Any proportionate increase or decrease of SD and MCV will produce a normal value & vice versa.
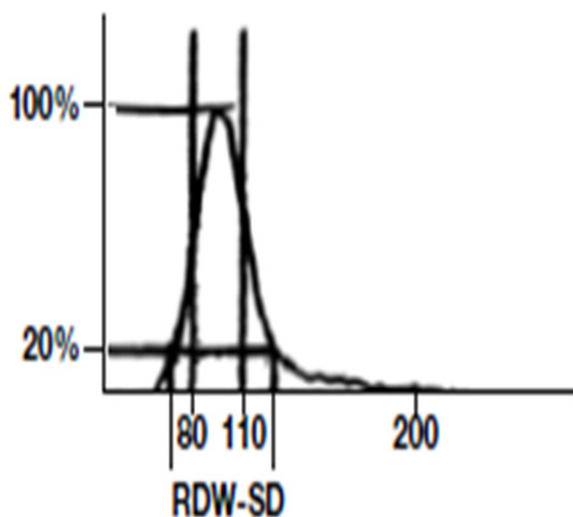
Figure 1: RBC histogram showing RDW-CV and SD.

In contrast, RDW-SD is a direct measure of RDW which is taken at the 20% frequency level of the RBC histogram which gives the actual picture of RBC anisocytosis.[11] RDW-CV is dependent on MCV, so it is higher in microcytic RBCs and could be normal in macrocytic RBCs as the denominator (MCV) is increased.[10] Another limitation of RDW-CV is that the readings are taken at one standard deviation (1SD) and

thus do not consider extreme values.[9] Both these limitations can be overcome by the use of RDW-SD in macrocytic blood pictures. Although both values are given by cell counters without any extra cost, only RDW-CV is routinely used for analysis and mentioned in reports as a marker of anisocytosis. Many studies are showing the importance of RDW-CV in microcytic anemia and other conditions.[12-17] A study by Łochowski M, on the Prognostic value of (RDW-SD) in patients operated on due to non-small cell lung cancer highlights the importance of RDW-SD in prognosis. A study by Constantino et al & Carpoaral et al highlights the importance of RDW-SD and histogram interpretation in conjunction with RBC indices.[10,11]

The RDW-CV is used popularly as a marker of RBC anisocytosis, but the inconsistencies in the estimation of RBC anisocytosis using RDW-CV alone raise the need for this study. Both RDW-CV % RDW-SD have their role in the estimation of anisocytosis depending on the MCV values. We propose that RDW-SD is a more sensitive marker of anisocytosis than RDW-CV in Macrocytosis. This study aimed to compare the sensitivity of RDW-CV and RDW-SD as markers of anisocytosis in macrocytosis.

MATERIALS AND METHODS

This was a cross-sectional, prospective, observational study conducted in the central clinical laboratory of a tertiary care hospital in western India. The sample size of 138 was calculated at a precision 0.1 and 90% confidence interval. 138 samples each from patients with macrocytosis and normocytosis control group, based on MCV were analysed. Patients having MCV>100fl, adults>18 years for the study group, and control group MCV between 80-100fl and Hb>13gm/dl were included in the study. Patients with a history of recent blood transfusion or haematinics supplementation were excluded. The study variables included MCV, RDW-CV, RDW-SD, histograms, Peripheral blood smear findings, age, and sex of the patients.

Peripheral venous blood (2 mL) sample was collected in an EDTA vacutainer and was processed on Haematology Analyzer Mindray BC 6000. Study Variables like MCV, RDW-CV, and RDW-SD were recorded for each sample. A peripheral blood smear was prepared and stained by Leishman stain using standard protocol for each sample & microscopic examination was done by a pathologist for the presence of anisocytosis. Anisocytosis was categorized using the ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features.[19]

The mean value of RDW-CV & RDW-SD was calculated from the normocytic control group. Normal range was calculated for RDW-CV & RDW-SD using the mean value & standard deviation of the normocytic group. Using the above cut-off RDW-CV > 15.1% and RDW-SD > 47.8fl, analysis of the data from macrocytosis patients was done

for the presence of anisocytosis. Sensitivity, specificity, and positive & negative predictive values were calculated for both RDW-CV &RDW-SD in comparison with peripheral blood smear findings. Z score was also calculated to compare both parameters using SPSS software.

This study was approved by the institutional ethics review board (IEC/2021/766) which confirms that the study complies with all ethical regulations. Patient consent was taken for including the data in the study.

RESULTS

Age distribution ranged from 18 to 85 years with a predominant age group between 18-40 years followed by 41-60 years. The gender-wise distribution shows male predominance that is 72% male and 28% female patients. On comparison of RDW-CV & RDW-SD with the peripheral blood smear findings it showed 100% co-relation between RDW-SD and Peripheral blood smear findings, whereas RDW-CV is less co-related. (Table 1) The normal range of RDW-CV was 12.59-15.13% & for RDW-SD it was 40.05-47.81fl for our machine (MINDRAY BC 6000) and

our population with a mean value of 13.86% & 43.93, with a Standard deviation of 1.27 & 3.88 respectively. Z score calculated was 9.1 which shows RDW-SD is a significantly superior marker of anisocytosis as compared to RD-SD in macrocytosis. (Table 2,3)

Table 1: The comparison of RDW-CV & SD with PBS microscopy

Methods	Anisocytosis Present	Anisocytosis Absent	Total
RDW-CV>15.13%	85(62.7%)	53	138
RDW-SD>47.81fl	137(100%)	1	138
PBS Anisocytosis	137	1	138

Table 2: Markers of diagnostic accuracy

	RDW-CV	RDW-SD
Sensitivity	62.7%	100
Specificity	100	100
PPV	98.8	100
NPV	1.92	100

Table 3: Mean values of MCV, RDW-CV & RDW-SD as per severity of anaemia

HB levels	No. of patients	MCV(fl) (mean)	RDW-CV% (mean)	RDW-SD(fl) (mean)
Hb<7gm%(Severe)	24(17.4%)	111.61	23.13	95.11
Hb 7-9.9 gm%(moderate)	30(21.7%)	109.73	18.58	73.82
Hb 10-11.9 gm%(mild)	35(25.4%)	106.23	17.06	65.62
Hb>12gm%	49(35.5%)	106.5	14.71	57.03
Total	138			

DISCUSSION

Anisocytosis is a variation in the size of RBCs.[4] Depending on the presence of anisocytosis that is the presence of a heterogenous or homogenous population the underlying condition causing macrocytosis varies. This highlights the importance of determination of the presence of RBC anisocytosis.

MCV is an average value obtained from the RBC histogram and it is a measure of central tendency; thus, it does not reflect on the anisocytosis of the RBC populations. Macrocytosis which is MCV more than 100fl is a relatively common finding, with prevalence ranging from 1.7% to 3.6%.[21,22,23] Conditions causing macrocytosis without anisocytosis are aplastic anemia, Chronic liver disease, chemotherapy/antivirals, and alcoholism.[20,24]Macrocytosis is a common presentation of alcoholism, with or without liver disease, and is considered one of the most common causes of homogenous macrocytosis.[20,25] Conditions causing macrocytosis with anisocytosis are Folate/B12 deficiency, Immune hemolytic anemia, Cytotoxic chemotherapy, Chronic liver disease, MDS.[20,24]

RDW is considered a quantitative measure of anisocytosis and does not carry subjective variation associated with microscopy. Thus, RDW helps to determine the degree & severity of anisocytosis as they are the measures of dispersion of data around the mean and act as an objective marker of anisocytosis. The RDW is a frequently studied and commonly used CBC parameter, but it is not well understood. RDW values are expressed as RDW-CV & RDW-SD on automated hematology cell counters. Both these values are calculated differently.

CV is the ratio of 1SD and MCV. As it is a ratio any increase or decrease of MCV value and one standard deviation (1SD) value can affect the result of RDW-CV. For example, in patients with microcytosis, RDW-CV results will be high as the denominator that is MCV is decreased, whereas in macrocytosis RDW-CV value could be normal as the denominator that is MCV is increased.

Also, the limitation of RDW-CV is the readings are taken at (1SD) level so covers only 68% RBC population and does not consider extreme values. Thus, does not give the real picture of anisocytosis. Despite the above limitations, RDW-CV is a more useful marker for low normocytic &

microcytic blood pictures where MCV is low, it is useful in the diagnosis of early Iron deficiency anemia treatment response of iron deficiency.

SD is a direct measure of RBC anisocytosis taken at a 20% frequency level of RBC histogram, covering the majority of the RBC population on both sides of the mean. This gives the actual picture of RBC anisocytosis.

Vitamin B12 deficiency is the most common cause of heterogenous macrocytosis and anemia, other causes being hemolytic anemia, cytotoxic chemotherapy, and MDS which are rare. The reason for anisocytosis in vitamin B12 deficiency is the presence of many fragmented RBCs, target cells, produced during the process of ineffective erythropoiesis and macro-ovalocytes leading to poikilocytosis which can

interfere with anisocytosis estimation. So, in vitamin B12 deficiency we have macrocytes, macro-ovalocytes at one end, and fragmented RBCs at the other end in the RBC histogram, which leads to widening of the histogram and anisocytosis on PBS.

The findings of RDW-SD as a more sensitive and specific parameter in the determination of anisocytosis in macrocytosis have been highlighted by the present study and other studies by Carporal et al, Constatino et al and Rajalakshmi Birur Rajashekar et al.[10,11,24]

A study by Rajalakshmi Birur Rajashekar et al have studied RDW-CV & RDW-SD in vitamin B12 deficiency patients retrospectively showing the RDW-SD as a more sensitive marker in the diagnosis of Vitamin B12 deficiency.[24]

Table 4: Comparison with other studies

	Present Study		Carporal et al.[11]		Rajalakshmi BirurRajashekar et al.[22]	
	RDW-CV- n(%)	RDW-SD-n(%)	RDW-CV- n(%)	RDW-SD-n(%)	RDW-CV- n(%)	RDW-SD-n(%)
Sample size	138 samples		806 samples		100 samples	
TP	85(62)	126(91.3)	30(49.2)	55(90.2)	-	-
TN	1(100)	12(8.6)	1(1.6)	3(4.9)	-	-
FP	0(0)	0(0)	2(3.3)	0(0)	-	-
FN	52(38)	0(0)	28(45.9)	3(4.9)	-	-
Total	138(100)	138(100)	61(100)	61(100)	-	-
Sensitivity	61.59%	100%	51.70%	90.20%	81%	95%
Specificity	100%	100%	33.30%	100%	68%	31%
PPV	100%	100%	93.80%	100%	91%	85%
NPV	1.8%	100%	3%	50%	46%10	60%

RDW-SD is a more sensitive marker than RDW-CV in macrocytic blood pictures, especially in cases with normal hemoglobin where RDW-CV is completely normal. It is important to diagnose early vitamin B12 deficiency as it leads to irreversible neurological damage.[26]

In big labs with high sample loads where it's not possible to screen peripheral blood smear of each patient and labs where a full-time pathologist is not available, in such places the quantitative markers of anisocytosis such as RDW play an important role. Although everyone is aware of the role of RDW-CV, many budding pathologists, pathology residents, and lab technicians are not aware of RDW-SD and its role in the interpretation of CBC. This article will help to increase awareness of the RDW-SD, a simple CBC parameter that is freely available, and its role in macrocytosis.

Limitations: The sample size is small as we have done this as a pilot study & need to study larger samples. The Vitamin B12 levels were not checked and follow-up of patients was not done.

CONCLUSIONS

RDW-SD is a more sensitive marker than RDW-CV in the macrocytic blood picture, especially in cases with normal hemoglobin where RDW-CV is completely normal and also in neonates in whom physiologically MCV is high. So, we recommend including RDW-SD in all the CBC reports and during the interpretation of CBC in macrocytosis.

A larger sample size study can be carried out along with the serum Vitamin B12 levels and investigations for other underlying conditions. As newborn babies have high MCV physiologically, RDW-SD can also be a useful marker for RBC anisocytosis which may predict underlying sepsis or hemolysis in them.

Conflicts of interest: None

REFERENCES

1. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. World Health Organization; 2011.

2. Bessman JD, Gilmer Jr PR, Gardner FH. Improved classification of anemias by MCV and RDW. American journal of clinical

pathology. 1983 Sep 1;80(3):322-6. Bessman JD, Gilmer Jr PR, Gardner FH. Improved classification of anemias by MCV and RDW. American journal of clinical pathology. 1983;80(3):322-6. Crossref

3. Lippi G, Mattiuzzi C, Cervellin G. Learning more and spending less with neglected laboratory parameters: the paradigmatic case of red blood cell distribution width. Acta Biomed. 201616;87(3):323-8. Website

4. Price- Jones C. The variation in size of red cells. Brit Med J.1910;02:4418.

5. Beckman Coulter LH. 780 On Line IBO72641. Miami Lakes: Beckman Coulter Education Center. 2007. (Cited on: 12 February 2023). Available from: Website

6. Rowan RA, Fraser C, Gray JH, Mdonald G. The Coulter Counter Model S Plus-the shape of things to come. Clinical & Laboratory Haematology. 1979;1(1):29-40. Crossref

7. Coulter WH. High speed automatic blood cell counter and cell size analyzer. In Proceedings of the National Electronics Conference 1956;12: 1034). Website

8. CELL D. Sapphire TM System Operator Manual. Abbott Park, IL: Abbott Laboratories. 2005. (Cited on: 12 February 2023). Available from: Website

9. Groner W, Simson E, Groner W, Simson E. History of cell counting. Groner w, Simson E. Practical guide to modern hematology analyzers. Chichester: John Wiley & Sons Ltd. 1995:1-9

10. Constantino BT. Red cell distribution width, revisited. Laboratory Medicine. 2013 May 1;44(2):e2-9. Crossref

11. Caporal FA, Comar SR. Evaluation of RDW-CV, RDW-SD, and MATH-1SD for the detection of erythrocyte anisocytosis observed by optical microscopy. JornalBrasileiro de Patologia e Medicina Laboratorial. 2013;49(5):324-31. Crossref

12. Qurtom HA, Al-Saleh QA, Lubani MM, et al. The value of red cell distribution width in the diagnosis of anaemia in children. European journal of pediatrics. 1989;148(8):745-8. Crossref

13. Ahmad J. Diagnostic Accuracy of Red Cell Distribution Width in Mild Iron Deficiency Anaemia in Women of Childbearing Age. Journal of Rawalpindi Medical College. 2016;20(4):309-11. Website

14. Sultana GS, Haque SA, Sultana T, et al. Role of red cell distribution width (RDW) in the detection of iron deficiency anaemia in pregnancy within the first 20 weeks of gestation. Bangladesh Medical Research Council Bulletin. 2011;37(3):102-5. Crossref

15. Aulakh R, Sohi I, Singh T, Kakkar N. Red cell distribution width (RDW) in the diagnosis of iron deficiency with microcytic hypochromic anemia. The Indian Journal of Pediatrics. 2009;76(3):265-8. Crossref

16. Baqar MS, Khurshid M, Molla A. Does red blood cell distribution width (RDW) improve evaluation of microcytic anaemias?. Journal of Pakistan Medical Association. 1993;43(8):149. Website

17. Fava C, Cattazzo F, Hu ZD, et al. The role of red blood cell distribution width (RDW) in cardiovascular risk assessment: useful or hype?. Annals of translational medicine. 2019;7(20). Crossref

18. Łochowski M, Chałubińska-Fendler J, Łochowska B, et al. Prognostic value of red blood cell distribution width-standard deviation (RDW-SD) in patients operated on due to non-small cell lung cancer. Journal of Thoracic Disease. 2020;12(3):773. Crossref

19. Palmer L, Briggs C, McFadden S, et al. ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. International journal of laboratory hematology. 2015;37(3):287-303 Crossref

20. Brigden ML. A systematic approach to macrocytosis: Sorting out the causes. Postgraduate medicine. 1995;97(5):171-86. Crossref

21. Davidson RJ, Hamilton PJ. High mean red cell volume: its incidence and significance in routine haematology. Journal of clinical pathology. 1978;31(5):493-8. Crossref

22. Breedveld FC, Bieger R, van Wermeskerken RK. The clinical significance of macrocytosis. Acta MedicaScandinavica. 1981;209(1-6):319-22. Crossref

23. Colon-Otero, G., Menke, D., & Hook, C. C. A practical approach to the differential diagnosis and evaluation of the adult patient with macrocytic anemia. The Medical Clinics of North America. 1992;76(3), 581-97. Crossref

24. RajaShekar RB, Patel S, KulKaRni P. Discriminant functions in the diagnosis of vitamin B12 deficiency anemia, the value of RDW-SD: An analytical study. National Journal of Laboratory Medicine. 2017;6(1): PO01-6 Website

25. Savage DG, Ogundipe A, Lindenbaum J, et al. Etiology and diagnostic evaluation of macrocytosis. The American journal of the medical sciences. 2000;319(6):343-52. Crossref

26. Herrmann W, Obeid R. Causes and early diagnosis of vitamin B12 deficiency. DeutschesÄrzteblatt International. 2008;105(40):680. Crossref