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Evaluation of RDW-CV, RDW-SD, and MATH-1SD for the detection of erythrocyte anisocytosis observed by optical microscopy

Avaliação de RDW-CV, RDW-SD e MATH-1SD na detecção da anisocitose dos eritrócitos visualizados na microscopia óptica

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ABSTRACT

Introduction and objective: To evaluate the performance of red cell distribution width reported statistically as coefficient of variation (RDW-CV), standard deviation (RDW-SD), and mathematical deduction of 1 standard deviation (SD) around mean corpuscular volume (MATH-1SD) in identifying anisocytosis in automated blood counts when compared with the manual quantification of erythrocyte anisocytosis in peripheral blood smears. **Material and methods:** 806 routine samples obtained from the hematology laboratory of Hospital de Clínicas da Universidade Federal do Paraná (HC-UFPR) were analyzed. Performance evaluations were carried out by dividing samples into microcytic, normocytic and macrocytic mean corpuscular volume (MCV). For each MCV range, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and efficiency were calculated. In addition, the Youden index (Y) was obtained and a comparative analysis with receiver operating characteristic (ROC) curves was done to evaluate the performance of RDW-SD, RDW-CV, and MATH-1SD on different MCV ranges. **Results and discussion:** RDW-CV had the best sensitivity (86.8%) and efficiency (86.8%) in detecting anisocytosis in microcytic MCV ranges. RDW-SD and MATH-1SD were more sensitive and efficient in normocytic (82.9% and 83.3%; 92.1% and 92.3%, respectively) and macrocytic (90.2% and 90.2%; 95.1% and 95.1%, respectively) MCV ranges. A ROC curve analysis indicated that RDW-CV was more efficient in detecting anisocytosis in microcytic MCV ranges ($p < 0.05$ vs. RDW-SD and MATH-1SD). In normocytic and macrocytic MCV ranges, RDW-SD and MATH-1SD showed similar efficiency in detecting anisocytosis ($p < 0.05$ vs. RDW-CV). **Conclusion:** RDW-SD, RDW-CV, and MATH-1SD deliver different performances in detecting blood smear anisocytosis according to MCV values. They are parameters that complement each other and should be used together to identify erythrocyte size heterogeneity.

Key words: laboratory automation; RDW-CV; RDW-SD; mean corpuscular volume; cellular analysis.

INTRODUCTION

Since Antonie van Leeuwenhoek's discovery of human red blood cells in 1674, many researchers have reported observations about the mean diameter of erythrocytes. However, the exact diameter was first measured by James Jurin in 1718, and the biconcave disc shape was observed by William Hewson in 1773. The first quantitative assessment of variation in erythrocyte diameter was described in 1910 by the British pathologist Cecil Price-Jones, who postulated that such a variation could be useful to diagnose anemia[17, 20, 24, 25].

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The electrical impedance method, developed by Wallace Henry Coulter in 1949 and employed on the Model A Coulter cell counter in 1956, suggested that cell size and cell counts could be determined simultaneously, because the magnitude of the electrical impulse was proportional to cell volume. As this and other new technologies were created and improved, routine counts became faster and more accurate. Besides, new parameters were made available, such as the red cell distribution width (RDW), which correlates with the degree of homogeneity/heterogeneity of erythrocyte size and is equivalent to anisocytosis in blood smears. RDW was firstly measured by the analyser Coulter Counter Model S-Plus II, being expressed as coefficient of variation (RDW-CV) and, more recently, as standard deviation (RDW-SD), especially in analysers Sysmex (Kobe, Japan), Beckman Coulter (Brea, USA) and Mindray (Shenzhen, China)[11, 12, 15].

Anisocytosis is a medical term meaning the size variation among microscopically observed erythrocytes in a blood smear. It frequently, but not always, correlates with RDW. Samples with high presence of alterations in erythrocyte shape indirectly affect the automated determination of RDW. Other factors that make spurious RDW alterations are long-term sample storage, erythrocyte agglutinins, hyperglicemia, erythrocyte fragmentation, high lymphocyte counts, the presence of giant platelets, platelet aggregates and intense microcytosis[3, 14, 22]. Since this last fator affects principally RDW-CV, because it is inversely proportional to MCV, Walters *et al.*[29] suggested using the parameter MATH-1SD, which represents the mathematical deduction of 1 SD around MCV.

The microscopic classification of erythrocyte size variation is generally described, in semiquantitative terms, as mild, moderate or severe, or graded from 1+ to 4+. When appropriately carried out, consistently and systematically, it helps reach a correct diagnosis, at least in some cases. Significant anisocytosis may be observed in several clinical conditions, including situations with intense production of reticulocytes, megaloblastic anemia, blood transfusions, and serious iron deficiency anemia. The microscopic assessment of anisocytosis may be affected by visual limitations of the human eye, as described by Weber-Fechner's Law, and by the possible occurrence of different hemoglobin concentrations in erythrocytes. These concentrations alter the flattening effect of these cells in the glass of microscope slides[13, 16, 21, 29].

Although the automated quantification of anisocytosis suggested by RDW presents advantages, visual inspection of a properly prepared and stained blood smear is still an important action to search for significant hematologic alterations, both for clinical conduct and for comparing and assessing values delivered by hematology analysers. With this in mind, this work was aimed at assessing the performance of RDW-CV, RDW-SD and MATH-1SD in identifying erythrocyte anisocytosis in automated blood counts, when compared to the manual method of microscope slides, in situations of low, normal and high MCV.

MATERIAL AND METHODS

Study site and sample preparation

The investigation was conducted at the hematology laboratory of the Support and Diagnosis Unit of Hospital de Clínicas da UFPR (HC-UFPR), after approval by the local ethics committee. Representing two consecutive days of laboratory routine, 806 whole blood samples were used. The samples were collected in vials containing dipotassium ethylenediaminetetraacetic acid ($EDTA\text{-}K_2$) (1.8 mg/ml) (Vacutainer - Becton, Dickinson and Company, USA) and were analysed within three hours after collection, using hematology analysers XE-2100D or XT-2000i (both Sysmex Corporation, Japan). For each sample, a blood smear was prepared and stained by means of the slide maker/stainer SP-1000i (Sysmex Corporation, Japan) in no more than three hours after collection. Samples containing low-volume whole blood were manually prepared by means of the wedge-spread film. The staining technique employed in both the automated and the manual method was that of May Grünwald & Giemsa[19].

Determination of RDW-CV, MATH-1SD and RDW-SD

RDW-CV is calculated from the erythrocyte volume distribution histogram. It represents the coefficient of variation of erythrocyte volume around MCV. It is calculated as follows: RDW-CV (%) = 1 SD (femtoliters [fl])/MCV (fl) × 100, where 1 SD = 1 SD in relation to MCV, which is obtained at a height of 68.2% above the base of the erythrocyte volume distribution histogram. MATH-1SD is a parameter that represents the mathematical deduction of 1 SD around MCV and is calculated as follows: MATH-1SD (fl) = RDW-CV (%) × MCV (fl)/100. RDW-SD is determined from the width of erythrocyte volume distribution curve at level 20% above baseline and is expressed in femtoliters[29]. The **Figure** shows the obtainment of these parameters.

Manual method for quantification of erythrocyte anisocytosis

Erythrocyte size was compared to the nucleus of normal small lymphocytes. Erythrocytes with smaller (microcytes) and larger (macrocytes) diameter than the nucleus of normal small lymphocytes were counted in 10 microscopic fields at 1,000× magnification, where erythrocytes were uniformly distributed.

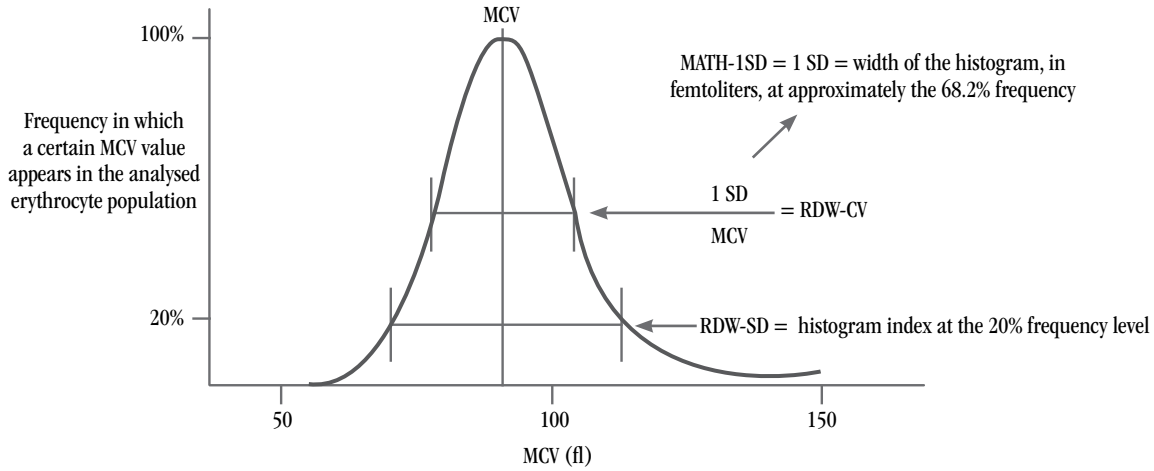

FIGURE – *Obtainment of RDW-CV, MATH-1SD and RDW-SD from erythrocyte volume distribution histogram (1 SD)*

RDW-CV: coefficient of variation of red cell distribution width; RDW-SD: standard deviation of red cell distribution width; SD: standard deviation; MCV: mean corpuscular volume.

After that, the average number of microcytes and macrocytes per field was calculated. These values were added, and anisocytosis was quantified according to **Table 1**.

TABLE 1 – Criteria for erythrocyte anisocytosis quantification in blood smears

Anisocytosis quantification	1+	2+	3+
Condition	5%-25% of microcytes and macrocytes	25.1%-50% of microcytes and macrocytes	> 50% of microcytes and macrocytes
200 erythrocytes per field	10 to 50 cells	51 to 100 cells	> 100 cells
150 erythrocytes per field	8 to 37 cells	39 to 75 cells	> 75 cells
100 erythrocytes per field	5 to 25 cells	26 to 50 cells	> 50 cells

Adapted from Gulati (2009)[15] and O'Connor (1984)[20].
1+: one cross (+); 2+: two crosses (++); 3+: three crosses (+++).

Sample classification criteria

First, reference ranges for RDW-CV, MATH-1SD and RDW-SD were calculated in 221 samples of normal subjects, who underwent regular routine examinations, according to the guidelines recommended in document C28-A3 of the Clinical and Laboratory Standards Institute (CLSI)[7]. The obtained values were: RDW-CV (11.7% to 14.3%); MATH-1SD (10.4 fl to 12.7 fl) and RDW-SD (38 fl to 46.1 fl). Therefore, the cut-off points or screening criteria adopted to consider a sample with likely significant heterogeneity of erythrocyte volume were RDW-CV > 14.3%; MATH-1SD > 12.7 fl and RDW-SD > 46.1 fl. If a sample was considered positive for one of these screening criteria and in the manual quantification of erythrocyte anisocytosis it contained a significant number of microcytes and macrocytes (Table 1), the sample was classified as true positive (TP). If a sample was positive for any screening criterion and contained no relevant anisocytosis in the microscope counterpart, the sample was classified as false positive (FP). If a sample was negative for any screening criterion and contained any significant anisocytosis in the microscopic analysis, it was classified as false negative (FN). Finally, if a sample was negative for any screening criterion and did not present any relevant findind on the slide, the sample was classified as true negative (TN)[2, 26].

Considerations

Hematologic quality assurance and quality control procedures were followed to ensure good operating conditions for the hematology analysers employed in this study. All adjustments and settings of hematology analysers were provided by the manufacturer's technical and scientific assistance service. Manual microscopic classifications of erythrocyte anisocytosis were performed using an optical microscope Olympus BX-41 (Olympus Corporation, Japan) with magnification of 1,000×. Erythrocyte size may be microscopically determined by comparing their diameters to those of lymphocyte nuclei. Normal erythrocytes are approximately the same size as the nucleus of a small lymphocyte[28]. Performance evaluations were carried out by categorizing samples into microcytic (MCV < 80), normocytic (80 ≤ MCV ≤ 99) and macrocytic (MCV > 99), in an effort to examine the behavior of parameters in different MCV ranges[6].

Statistical analyses

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and efficiency for parameters RDW-CV, RDW-SD and MATH-1SD in relation to the reference manual method were calculated as follows: sensitivity (%) = (TP/TP + FN) × 100; specificity (%) = (TN/TN + FP) × 100; NPV (%) = (TN/TN + FN) × 100; PPV (%) = (TP/TP + FP) × 100; and efficiency (%) = (TP + TN/TP + FP + FN + TN) × 100[25]. ROC curves, as well as statistical comparisons of ROC curves, were performed by program MedCalc® version 7.3.0.1 (MedCalc Software, Belgium). The Youden index (Y) was calculated by the formula: Y = sensitivity + (specificity - 1)[6, 27].

RESULTS

Among the 806 samples used in this work, 395 (49%) were considered positive and 411 (51%), negative for the presence of anisocytosis by the manual microscopic method. The results obtained in the calculation of RDW-CV, RDW-SD and MATH-1SD are displayed on **Table 2**. Samples were divided, according to MCV ranges, into microcytic (MCV < 80), normocytic (80 ≤ MCV ≤ 99) and macrocytic (MCV > 99). RDW-CV had the highest sensitivity when it detected anisocytosis in microcytic samples (86.8%). For normocytic and macrocytic samples, RDW-SD and MATH-1SD showed similar sensitivity values, higher than those of RDW-CV (82.9% and 83.3% in normocytosis and 90.2%-90.2% in macrocytosis, respectively). RDW-CV presented NPV of 72.2% in microcytic samples, whereas RDW-SD and MATH-1SD presented the same value of 37.5%. This indicates that the use of RDW-CV in evaluation of microcytosis increases the probability of not finding anisocytosis with a value below cut-off point (≤ 14.3%).

The Youden index is a function of sensitivity and specificity and ranges between 0 and 1, with 1 indicating the best relationship between sensitivity and the false-positive fraction, and 0, the worst. **Table 3** shows the statistical analyses among ROC curves of RDW-CV, RDW-SD and MATH-1SD. In microcytosis, RDW-CV presented statistical difference from RDW-SD and MATH-1SD to reveal anisocytosis ($p < 0.05$); in normocytosis and macrocytosis, RDW-SD and MATH-1SD presented statistical difference from RDW-CV to reveal anisocytosis ($p < 0.05$). However, RDW-SD and MATH-1SD presented no statistical difference to show anisocytosis in microcytosis, normocytosis and macrocytosis.

DISCUSSION

Erythrocyte morphology is traditionally assessed in blood smears. To this end, they are first examined in relation to staining quality and to uniform cell distribution on the microscope slide. After selection of an adequate microscopic field, erythrocytes are examined taking into account size, deviations in shape, color or hemoglobin content, and the presence of inclusions and precursor cells. Anisocytosis is one of the most frequent erythrocyte anomaly, a non-specific change in severe forms of anemia, yet no conclusion may be drawn about its origin. Although modern hematology analysers supply information on erythrocytes, there are still morphological abnormalities critical for the diagnosis of anemia which are only observed in the microscopic analysis of peripheral blood[1, 23].

Little advantage has been taken of the erythrocyte volume distribution histogram, which may provide useful information in monitoring reliability of results generated by analysers, investigating the potential causes of erroneous automated results and reaching a presumptive diagnosis. For example, the presence of fragmented erythrocyte or erythrocyte agglutination, which could not be identified without the microscopic examination of peripheral blood, may be presumably detected on the red cell histogram. Similarly, in patients with iron deficiency anemia or megaloblastic anemia in treatment, sequential histograms may early evidence the gradual appearence of a new erythrocyte population[9].

A marked anisocytosis is almost always accompanied by an increase in RDW, however, the other way around is not always true, particularly when accompanied by significant poikilocytosis. RDW is considered a quantitative, not subjective, measure of anisocytosis viewed on complete blood count, however it is only proportional to anisocytosis if erythrocyte volume has a Gaussian distribution. If volume distribution is falsely biased to left or right of the histogram curve, RDW may not be an adequate marker of anisocytosis and must be superseded by microscopic examination. Although there are several factors that cause spurious changes in RDW, as already mentioned, recognising the type of modification provoked in the pattern of erythrocyte volume distribution curve may help identify the causes, despite RDW value[3, 10]. According to Constantino[9], the presence of a bump on the right side of the histogram generally corresponds to reticulocytosis, and a tail on the far right of the histogram correlates with erythrocyte agglutination. A displacement to the left in the erythrocyte histogram means microcytosis; to the right, macrocytosis. Bimodal red cell histograms are normally associated with blood transfusions, response to the treatment of deficiency-related anemias, cold agglutinin and sideroblastic anemias[9].

TABLE 2 – Evaluation of RDW-CV, RDW-SD, and MATH-1SD in microcytic, normocytic and macrocytic MCV

Microcytic samples (MCV < 80)						
Classifications	RDW-CV		RDW-SD		MATH-1SD	
	n	%	*n*	%	*n*	%
TP	33	62.3	13	24.5	13	24.5
TN	13	24.5	15	28.3	15	28.3
FP	2	3.8	0	0	0	0
FN	5	9.4	25	47.2	25	47.2
Total	53	100	53	100	53	100
Sensitivity	86.8%		34.2%		34.2%	
Specificity	86.7%		100%		100%	
PPV	94.3%		100%		100%	
NPV	72.2%		37.5%		37.5%	
Efficiency	86.8%		52.8%		52.8%	
Youden index	0.735		0.342		0.342	
Normocytic samples (80 ≤ MCV ≤ 99)						
Classifications	RDW-CV		RDW-SD		MATH-1SD	
	n	%	*n*	%	*n*	%
TP	228	32.9	248	35.8	249	36
TN	391	56.5	389	56.2	390	56.4
FP	2	0.3	4	0.6	3	0.4
FN	71	10.3	51	7.4	50	7.2
Total	692	100	692	100	692	100
Sensitivity	76.3%		82.9%		83.3%	
Specificity	99.5%		99%		99.2%	
PPV	99.1%		98.4%		98.8%	
NPV	84.6%		88.4%		88.6%	
Efficiency	89.5%		92.1%		92.3%	
Youden index	0.758		0.819		0.825	
Macrocytic samples (MCV > 99)						
Classifications	RDW-CV		RDW-SD		MATH-1SD	
	n	%	*n*	%	*n*	%
TP	30	49.2	55	90.2	55	90.2
TN	1	1.6	3	4.9	3	4.9
FP	2	3.3	0	0	0	0
FN	28	45.9	3	4.9	3	4.9
Total	61	100	61	100	61	100
Sensitivity	51.7%		90.2%		90.2%	
Specificity	33.3%		100%		100%	
PPV	93.8%		100%		100%	
NPV	3.4%		50%		50%	
Efficiency	50.8%		95.1%		95.1%	
Youden index	0.194		0.902		0.902	

MCV: mean corpuscular volume; RDW-CV: coefficient of variation of red cell distribution width; RDW-SD: standard deviation of red cell distribution width; MATH-1SD: mathematical deduction of 1 standard deviation (SD) around MCV; TP: true positive; TN: true negative; FP: false positive; FN: false negative; PPV: positive predictive value; NPV: negative predictive value.

TABLE 3 – Statistical analyses among ROC curves of RDW-CV, RDW-SD and MATH-1SD

Comparison among ROC curves (MCV < 80)			
Indices	**RDW-CV and RDW-SD**	**RDW-CV and MATH-1SD**	**RDW-SD and MATH-1SD**
Difference among areas	0.196	0.196	0
Standard deviation	0.087	0.087	0.082
95% confidence interval	0.026-0.367	0.026-0.367	-0.162-0.162
Significance	$p < 0.05$	$p < 0.05$	$p = 1.00$
Prevalence of positive samples: 71.7% ($n = 53$)			
Comparison among ROC curves (80 ≤ MCV ≤ 99)			
Indices	**RDW-CV and RDW-SD**	**RDW-CV and MATH-1SD**	**RDW-SD and MATH-1SD**
Difference among areas	0.034	0.031	0.003
Standard deviation	0.009	0.01	0.006
95% confidence interval	0.015-0.052	0.01-0.051	-0.008-0.014
Significance	$p < 0.05$	$p < 0.05$	$p = 0.597$
Prevalence of positive samples: 43.2% ($n = 692$)			
Comparison among ROC curves (MCV > 99)			
Indices	**RDW-CV and RDW-SD**	**RDW-CV and MATH-1SD**	**RDW-SD and MATH-1SD**
Difference among areas	0.399	0.399	0
Standard deviation	0.177	0.177	0.026
95% confidence interval	0.053-0.745	0.053-0.745	-0.051-0.051
Significance	$p < 0.05$	$p < 0.05$	$p = 1.00$
Prevalence of positive samples: 98.3% ($n = 59$)			

ROC: receiver operating characteristic curve; MCV: mean corpuscular volume; RDW-CV: coefficient of variation of red cell distribution width; RDW-SD: standard deviation of red cell distribution width; MATH-1SD: mathematical deduction of 1 standard deviation (SD) around MCV.

RDW within reference ranges indicates that erythrocytes follow a pattern of size distribution that approaches the normal of a population of individuals. This suggests the presence of a homogeneous cell population, but not necessarily that all cells have normal size. So, it is important to make it clear that a normal RDW does not exclude the presence of a significant amount of cells that are much larger than the majority cell population. It does not mean that the majority erythrocyte population is normal either. There is a natural desire to use the several possible combinations of MCV and RDW to conduct to possible diagnoses of anemias, but this practice may produce errors and must never substitute more specific laboratory investigations, including the analysis of peripheral blood slides[3, 4, 13].

RDW reference intervals, calculated for healthy individuals, differ when obtained by analysers of different manufacturers, and sometimes, even on different models of the same manufacturer. This may be explained by the fact that analysers use different algorithms to analyse cell distribution. These algorithms are essential to eliminate extreme values, normally due to artifacts. Any consideration about the clinical use of RDW must be evaluated, preferably by comparison with reference ranges established for each hematology analyser model[18].

The present study evaluated the performance of RDW-CV, RDW-SD and MATH-1SD in detecting anisocytosis on peripheral blood smear. These parameters were provided by Sysmex® hematology analysers. In order to obtain consistency in manual classifications, a standardized system of erythrocyte anisocytosis quantification in blood smears was created (Table 1). There are several classification systems, yet there is not the best one. In order to follow good clinical and laboratory practices and the recommendations of laboratory accreditation agencies, it is important to keep consistency in the chosen system and ensure that all professionals in the laboratory use it as the standard. The classification system has clinical importance in some cases of abnormal findings such as, for example, three crosses (3+) of microcytes for patients with iron deficiency anemia, and 3+ of dacrocytes for patients with megaloblastic myelofibrosis[16].

Considering the RDW-CV formula (Figure), 1 SD reflects the size variation of erythrocyte volumes around MCV. As 1 SD is divided by MCV, RDW-CV presents an inversely proportional

relationship to it. Besides, when dividing 1 SD by MCV, there is a tendency towards a falsely normal coefficient of variation (RDW-CV), that is, RDW-CV may be normal, but erythrocytes may be microcytic or macrocytic on the slide[29]. In this study, this was particularly observed in macrocytic MCV, perhaps because the higher the MCV, the more likely the RDW-CV is within reference ranges. In microcytic MCV (Tables 2 and 3), RDW-CV presented higher sensitivity and relative efficiency to detect anisocytosis when compared to the manual method. Perhaps this performance is due to the fact that microcytic MCV highlight RDW-CV values, what may, however, have caused decrease in specificity in relation to RDW-SD and MATH-1SD.

RDW-SD, as well as MATH-1SD, is not affected by MCV. It differentiates from this because it is the direct measurement, in femtoliters, of the curve variation at the 20% level above baseline of the erythrocyte volume distribution histogram, instead of the 68.2% height in the case of MATH-1SD. The 20% height was chosen because at this level a greater size variation occurs among the erythrocytes of an individual[29]. In relation to RDW-CV, RDW-SD obtained better efficiency and better relationship between sensitivity and the false positive fraction in identifying anisocytosis, when compared to the manual method, in normocytic and macrocytic MCV ranges.

MATH-1SD was used in an attempt to eliminate the dependence of RDW-CV on the average size of erythrocytes, what was confirmed by the presented results. However, MATH-1SD obtained a performance similar to that of RDW-SD when it indicated anisocytosis in microcytic, normocytic and macrocytic MCV ranges (p = 1, 0.597 and 1, respectively). Thus, since MATH-1SD is not provided by hematology analysers, it is of limited usefulness.

We can conclude that RDW-CV, RDW-SD and MATH-1SD must be used in association, so as to help identifying the heterogeneity of erythrocyte size. Along with good assessment of the histogram shape, they may lead to an excellent morphologic analysis of erythrocytes.

At last, by grouping the obtained data, we suggested cut-off points for each parameter studied in this article, giving them a scale of crosses to be routinely used in hematology laboratories (**Table 4**).

TABLE 4 – Criteria for quantification of erythrocyte anisocytosis in peripheral blood smears

Parameters	Quantification of erythrocyte anisocytosis in peripheral blood smears		
	1+	2+	3+
RDW-CV (%)	15.5-19	19.1-24	> 24
RDW-SD (fl)	47-62	62.1-75	> 75
MATH-1SD (fl)	12.8-16	16.1-19.7	> 19.7

1+: one cross (+); 2+: two crosses (++); 3+: three crosses (+++); RDW-CV: coefficient of variation of red cell distribution width; RDW-SD: standard deviation of red cell distribution width; MATH-1SD: mathematical deduction of 1 standard deviation (SD) around MCV.

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RESUMO

Introdução e objetivo: *Avaliar o desempenho do* red cell distribution width *expresso em coeficiente de variação (RDW-CV) e desvio padrão (RDW-SD) e da dedução matemática de 1 desvio padrão (DP) ao redor do volume corpuscular médio (MATH-1SD) ao identificar anisocitose nos hemogramas automatizados, quando comparados com o método manual de quantificação da anisocitose eritrocitária em lâmina.* ***Material e métodos:*** *Foram analisadas 806 amostras obtidas da rotina laboratorial da Seção de Hematologia do Hospital de Clínicas da Universidade Federal do Paraná (HC-UFPR). As avaliações de desempenho foram realizadas dividindo-se as amostras em volume corpuscular médio (VCM) microcítico, VCM normocítico e VCM macrocítico. Para cada faixa de VCM, sensibilidade, especificidade, valor preditivo positivo (VPP), valor preditivo negativo (VPN) e eficiência foram determinados. Além disso, o índice de Youden foi calculado e uma análise comparativa de curvas de características de operação do receptor (curvas ROC [*receiver operating characteristic*]) foi realizada para verificar o desempenho de RDW-CV, RDW-SD e MATH-1SD em diferentes faixas de VCM.* ***Resultados e discussão:*** *O RDW-CV obteve a melhor sensibilidade (86,8%) e eficiência (86,8%) ao detectar anisocitose em faixas de VCM microcítico. O RDW-SD e o MATH 1SD foram mais sensíveis e eficientes em VCM normocítico (82,9% e 83,3%; 92,1% e 92,3%, respectivamente) e macrocítico (90,2% e 90,2%; 95,1% e 95,1%, respectivamente). A comparação de curvas ROC demonstrou que o RDW-CV foi mais eficiente ao detectar anisocitose em VCM microcítico (*$p < 0,05$ *vs. RDW-SD e MATH-1SD). Em VCM normocítico e macrocítico, o RDW-SD e o MATH-1SD mostraram eficiência semelhante ao*

*detectar anisocitose (*p < *0,05 vs. RDW-CV).* **Conclusão:** *RDW-CV, RDW-SD e MATH-1SD possuem desempenhos diferentes ao detectar anisocitose em lâmina conforme a faixa de VCM. São parâmetros que se complementam e que devem ser utilizados em conjunto na identificação de heterogeneidade dos tamanhos eritrocitários.*

Unitermos: *automação laboratorial; RDW-CV; RDW-SD; volume corpuscular médio; análise celular.*

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