J Bras Patol Med Lab. 2018 Aug; 54(4): 220-226.

10.5935/1676-2444.20180038

Review of peripheral blood smear slides: assessment of the compliance with criteria used by analysts in a laboratory of a public hospital in Bahia, Brazil

Revisão de lâminas hematológicas: avaliação da conformidade de critérios utilizados por analistas em um laboratório de um hospital público da Bahia, Brasil

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ABSTRACT

Introduction: Recommendations in the literature and publications of scientific societies have proposed classification systems and levels to report blood cell changes in the complete blood count (CBC). Aiming to standardize the results released in the clinical laboratory, the standardization in hematoscopy is an important action within the quality assurance, contributing for diagnosis and appropriate follow-up of patients. **Objective:** To verify the adequacy of the classification criteria of the blood smear changes adopted by the laboratory professionals at the General Hospital of Vitória da Conquista, Bahia, Brazil. **Material and method:** In the 12-week period, 159 slides of CBC were selected from 53 patients from the four intensive care units (ICUs) at the hospital, three slides per patient. The readings of the slides were performed by the analysts on duty and by two other quality analysts-control who used the criteria standardized by Palmer (2015), all analyses were blind and independent. The results were compared using the Cohen's *kappa* agreement tests between the analysts-control and between the quality control analysts and the analysts on duty. **Results:** For the analyzed parameters, there was moderate to excellent agreement among the quality analysts-control, and when they were compared to the analysts on duty, there was a slight to substantial agreement. **Discussion:** The lowest agreement occurred in the expression of poikilocytosis and lymphocytic atypia, which are not detected by the hemocytometer, and the analyst's technical capacity is required. **Conclusion:** The study evidenced the need of standardization of the criteria for hematoscopy used by analysts to obtain reproducible results that reflect quality and reliability by the prescribing physician. Therefore, it is necessary to invest in training the professionals involved in revision of blood smear slides.

Key words: hematology; microscopy; quality control.

INTRODUCTION

The complete blood count (CBC) is an exam of great importance in health screening, in the diseases diagnosis and follow-up. It consists of the quantitative and qualitative evaluation of the cellular elements of the blood[1].

Due to the modern instrumentation and technological sophistication of clinical laboratories, particularly those considered of medium and high complexity, for some time ago, the blood cell count have been predominantly performed by automated equipment or automated counters[2].

The hemocytometer available in the market present variation on the methodologies used for cell counting and differentiation, as well as differing in the runtime of the exam, the number of parameters analyzed and the methods of determination of hematimetric indices (directly or calculated determination)[3].

Several automated counting models issue flags when they detect changes in cell morphology, erythrocyte size, and presence of erythroblasts, immature granulocytes (IG), blasts and atypical lymphocytes. In such cases, a microscopic examination should be performed to allow the examination to be released, and when performed in a standardized and correct

First submission on 05/13/18; last submission on 05/13/18; accepted for publication on 07/10/18; published on 08/20/18

manner, it produces results consistent with the patients' clinical status[4].

The criteria for the blood smear slides review and differential counting performed by analyst depends on the ability of the equipment used to recognize abnormalities and to issue flags, as well as the characteristics of the population assisted, such as origin, age group and current clinical condition. In laboratories that assist patients coming from urgency and emergency care units or hospitalized patients, it becomes even more important to adopt specific criteria for slides reviewing, since these patients usually are those which have diseases or are in the process of diagnostic investigation.

Hematologic changes observed by microscopy are important for the diagnosis and should be notified in the laboratory examination report. Some are objects of quantification by the electronic counter itself, for example, the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and range of red cell distribution or red cell distribution width (RDW).

The current counters perform the counting and measurement of the erythrocytes by either impedance or optical method, incorporating the individual corpuscular volumes and generating the MCV. RDW is a by-product of the MCV electronic measurement used for the evaluation of erythrocytes volume heterogeneity. MCH is calculated by the ratio of hemoglobin to the number of red blood cells (RBC) and represents the mean amount of hemoglobin per erythrocyte[1].

Other changes, such as poikilocytosis (shape change in RBC), are not related to data provided by the hemocytometer or cannot be converted into numbers or percentages. However, it is essential that such changes are notified to the prescriber[1].

The evaluation of changes in cell morphology has been the subject of recommendations in the literature and regional publications of important scientific societies, such as the College of American Pathologists (CAP), the UK External Quality Assessment Services (UK NEQAS), the Japanese Society for Laboratory Hematology, in addition to the Royal College of Pathologists of Australasia Quality Assurance Program (RCPA QAP)[5].

Hematology textbooks and propositions of some laboratories present systems and classification levels for blood cell morphological changes. The magnitude of a change or the frequency of an abnormal element has been expressed numerically from 1+ to 4+, others textually, such as "slight", "mild", "moderate", "marked", "rare". There is still a proposal for the expression of abnormal findings only with the term "present"[1-6].

There is no evidence that a classification system is better, but standardization is an important action and has an impact on quality assurance of results, and is considered good laboratory practice and recommended by the accreditation services[6].

The Standardization Committee on the Hematological Morphology Nomenclature of the International Council of Standardization in Haematology (ICSH) published a guide using consensus opinions on the topic with the purpose of organizing a set of recommendations on nomenclature and for reporting abnormalities of red blood cells, leukocytes and platelets[5].

In Brazil, the laboratory quality assurance programs[7] have adopted these same criteria for nomenclature of morphological changes. However, there is still no consensus regarding the system and classification levels of some parameters obtained by automated hematology equipment, such as RDW, MCV and MCH, for example.

The absence of standardization may lead to mistakes or inconsistencies in the release of results and consequently in the clinical interpretation of them. Each laboratory should establish standardized procedures for the review of the slides and develop information and training policies to ensure the application of the established criteria[4].

In this context, the present study had as objective to compare the application of the classification criteria of hematological changes used by clinical analysts of the Laboratory of the Hospital Geral de Vitória da Conquista (HGVC), Bahia, Brazil. In order to allow the establishment of a standardized system with classification levels for blood count elements in order to be adopted and to discuss the need for standardization of the hematocrit abnormalities expression measured in blood count reports.

MATERIAL AND METHOD

This is a cross-sectional study conducted at the Clinical Analysis Laboratory of the HGVC. Data collection occurred in a 12-week period (April to June 2017). The hospital is a reference for urgency and trauma and meets the levels of attention of medium and high complexity. It has two adult intensive care units (ICUs), one pediatric ICU, one neonatal ICU, emergency department, surgical center, urgency and emergency care and pediatric ward, medical clinic and surgical clinic[8].

The sample consisted of 159 slides of CBC from 53 patients from the four ICUs of the hospital. The order of ICUs participation and patient selection are shown in the **Figure**.

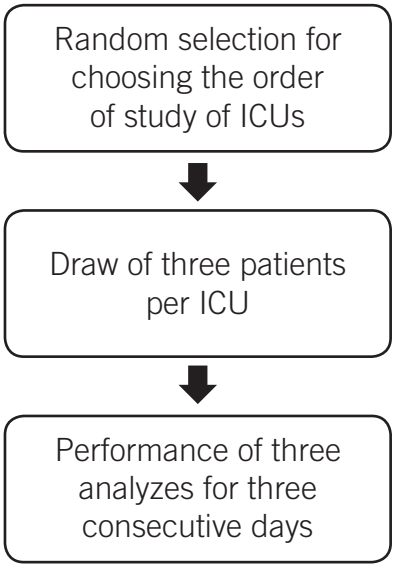

FIGURE – *Procedure for choosing ICU and patients*

ICU: intensive care unit.

Blood samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) and processed in Cell-Dyn Ruby® equipment by Abbott Diagnostics®. The slides were previously prepared and stained with Instant Prov® dye by Newprov® and were evaluated by the laboratory analyst on duty under the Nikon Eclipse E200® optical microscope and the results were released by the Complab Advanced® report system.

After the analyst on duty reading, the slides of the selected patients were stored. Then, two analysts [Pharmacists of the Multi-professional Residency Program in Emergency and Urgency with emphasis in Clinical Analyzes of the Universidade Federal da Bahia (UFBA)/HGVC] carried out the reading of these slides not knowing the result produced by the analyst on duty, using the parameters provided by the equipment and following the established criteria for the interpretative analysis of MCV, MCH, RDW, percentage of blasts/IG and lymphocytic atypia, described in **Table 1**. The readings were performed using double-blind criteria, without consulting the results of the analysts on duty and between the analysts-control.

The criteria defined for the classification of the peripheral blood cell morphology used in this study were adapted from Palmer (2015)[5], and the reference values were used according to the patient's age group[7], except for the RDW parameter, in which the normal range is defined by the manufacturer of the Cell Dyn Ruby® equipment (10.6%-13.2%).

TABLE 1 – Criteria defined for classification of changes in hematological morphology according to Palmer *et al*. (2015)[5]

Interpretation	Discrete	Moderate	Intense
Microcytosis	MCV: 7 points below the reference value	MCV: 7.1-14 points below the reference value	MCV: < 14.1 points below the reference value
Macrocytosis	MCV: 10 points above the reference value	MCV: 10.1-20 points above the reference value	MCV: > 20 points above the reference value
Hypochromia	MCH: points below the reference value	MCH: 4.1-8 points below the reference value	MCH: < 8 points below the reference value
Anisocytosis	RDW: 3% points above the reference value	RDW: 3.1%-6% points above the reference value	RDW: above 6%
Poikilocytosis	Schistocytes and sickle cells < 1%; other forms < 5%	Schistocytes and sickle cells 1%-2%; other forms 5%-20%	Schistocytes and sickle cells > 2%; other forms > 20%
Blast percentage/ IG; left-shift	They have a diameter of 10-14 µm with a non-segmented nucleus or showing rudimentary lobes attached by a thick filament. The cytoplasm is abundant, clear and contains fine and evenly distributed granulation. Performed counting of 100 cells. Classified "with left-shift" or "with no left-shift".		
Lymphocytic atypia	Abnormalities include increased cell size, nucleus immaturity with the presence of nucleolus and non-condensed chromatin, irregular nuclear outlines or lobulation, basophilia or vacuolization of the cytoplasm and irregular cell contour. Reported in percentage after counting 100 cells.		

IG: immature granulocytes; MCV: mean corpuscular volume; RDW: red cell distribution width.

For all slides, a differential count of at least 100 leukocytes was performed for quantification of blasts, IG and atypical lymphocytes. These values, in percentage, were used to calculate the *kappa* coefficient for left shift and lymphocyte atypia. For the classification of the left shift, the sum of blasts percentage and IG appropriated to the reference value by age group[7] was used; above this value, we classified as "with left shift"; and below the reference value, "with no left shift".

At the end of the analyst on duty and the analysts-control readings, data from all readings were tabulated in the Microsoft Excel 2010® software.

Statistical analyzes were performed for each parameter through interobserver agreement analysis. For the variables MCH,

poikilocytosis, left shift and lymphocytic atypia, Cohen's *kappa* was used. For MCV, RDW and lymphocytic atypia, we used *kappa* with quadratic weights (Fleiss-Cohen, 1973)[9] between analysts-control and between controls and analysts on duty. The strength of agreement was interpreted according to the criteria proposed by Landis and Koch (1977)[10] (**Table 2**).

The present study was carried out following all ethical principles indicated by the Resolution 466/2012 of the National Health Council, previously authorized by the Núcleo de Educaçção Permanente (NEP)-HGVC, and approved by the Research Ethics Committee of the Multi-disciplinary Health Institute of the UFBA, under CAEE protocol number: 66226017.0.0000.5556.

TABLE 2 – Classification of strength of agreement according to *Kappa* coefficient

0	Poor
0-0.2	Slight
0.21-0.4	Fair
0.41-0.6	Moderate
0.61-0.8	Substantial
0.81-1	Almost perfect

RESULTS

The CBC results of 53 patients were compared, of which 18 were from the Adult ICU I, 18 from the Adult ICU II, 11 from the pediatric ICU and nine from the neonatal ICU, since in the latter two units the patient flow and the frequency of requisition of exams were lower.

Three analysts, 159 slides each, reviewed them, totaling 477 readings. The agreement observed among the observers for the parameters evaluated is described in **Table 3**.

For the analyzed parameters, moderate to almost perfect agreement was observed between the analysts-control, and when compared with the analyst on duty, there was a slight to substantial agreement.

DISCUSSION

In this study the parameters MCV, MCH, RDW and poikilocytosis were considered for agreement analysis regarding the evaluation of RBC.

In the present study, we used the same result released by the analyzer by all the analysts and the same slide for hematoscopy. Thus, it was expected that there was an almost perfect agreement among all the analysts, which was only observed between the analysts-control for MCV and RDW analysis (*kappa* 0.98 and 0.85, respectively), which used the same standardized criteria when carrying out readings and classifications.

Between the analysts on duty and the analysts-control, lower agreement was observed in MCV, MCH and RDW evaluation, which reflects a lack of standardization regarding the range used to define these classifications.

Constantino (2015)[6] defines the range for MCV classification of every 10 fl below the reference value for microcytosis, using 10 fl for discrete macrocytosis and 15 fl for moderate macrocytosis. However, he recommends that laboratories set their own standard to be used by all professionals. In this study, we established the values extracted from Palmer *et al.* (2015)[5] recommendations.

For RDW, the reference value defined by the manufacturer of the Cell Dyn Ruby® equipment is similar to the range observed in the study of Vianna *et al*. (2014)[3] in the Brazilian population of the city of Curitiba, Paraná, Brazil.

Using MCV to classify anemia in micro-, normo- or macrocytic anemia is important, whereas RDW is useful in the differential diagnosis of anemia by hemoglobin synthesis, and MCH, in the diagnosis of hypochromic anemia[1]. Therefore, the lack of standardization in the reporting of MCV, MCH and RDW changes can lead to errors in the diagnosis of anemia and consequent damage to the correct diagnosis.

Poikilocytosis refers to abnormalities observed in the morphological characteristics of erythrocytes. There is a wide

TABLE 3 – Evaluation of the *kappa* index on hematological slides of patients admitted to the ICUs of the General Hospital of Vitória da Conquista, Bahia, Brazil

Parameter	Control I *vs.* control II	CI 95%	Control I *vs* analysts	CI 95%	Control II *vs.* analysts	CI 95%
MCV (macrocytosis or microcytosis)	0.98	-	0.61	-	0.63	-
MCH (hypochromia)	0.74	(0.4-1.08)	0.33	(-0.17-0.83)	0.49	(-0.11-1.1)
RDW (anisocytosis)	0.85	-	0.25	-	0.29	-
Poikilocytosis	0.59	(0.44-0.74)	0.07	(-0.16-0.31)	0.02	(-0.23-0.28)
Left-shift	0.72	(0.6-0.85)	0.41	(0.25-0.56)	0.57	(0.43-0.71)
Lymphocytic atypia	0.47	-	0.09	(-0.11-0.3)	0.2	-

Note: For the parameters MCV, RDW and lymphocitc atypia, we used Cohen's kappa *with quadratic weights (Fleiss-Cohen, 1973)[9].*

ICU: intensive care unit; CI: confidence interval; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; RDW: red cell distribution width.

variety of abnormal shape and in the description used to report them. The clinical significance of these changes also varies. The presence of schistocytes and sickle cells, for example, should be reported even when in small amounts. The quantification of abnormalities observed in peripheral blood provides the prescriber with valuable information about these findings[5].

For the evaluation of poikilocytosis in this study, only quantification was considered for the purpose of comparison among analysts, since the presence of codocytes or erythrocytes on target was reported in the hemoglobin reports. According to the criteria used, the codocytes are characterized by being thin cells that have a surface larger than the globular volume and an area stained in the center of the light halo[5]. The presence of codocytes is a frequent alteration in the routine of the laboratory studied, especially in CBC of newborns and patients with liver disease, and may be associated with hemoglobinopathies and thalassemias.

There was moderate agreement between the analysts-control (*kappa*: 0.59) and slight agreement between these and the analysts on duty (*kappa*: 0.07 and 0.02), evidencing the difficulty of standardization in the evaluation of a parameter that depends exclusively on the microscopy expert reading. Although haemocytometers emit alerts on morphology, some abnormalities in the blood count, such as poikilocytes, are not perceived by the technology, thus depending on the analyst for their detection and expression in the report.

The evaluation of agreement among analysts has been object of study in other areas. A study carried out in a laboratory of pathological anatomy analyzed the interobserver variation in the cytological diagnoses of squamous intraepithelial lesions. They found that there was agreement among the five observers in only 10% of the cases reviewed and among three or more observers in 72% of the cases, emphasizing the importance of the evaluation of agreement for the diagnoses as a method of quality control in microscopy[11].

The left shift was also the object of comparative analysis by the study and was evaluated from the percentage of blasts and IG in 100 leukocytes counted under the microscope. The presence of deviation should be reported mainly due to its importance in the diagnosis and evaluation of acute infections, as it results from increased marrow activity, with the release of immature forms of the bone marrow into the peripheral blood[1]. However, there is no consensus in the literature regarding the morphological limits for what should be considered as a blast, thus making counting variable among microscopy expert.

The criterion used for this study combines recommendations from several scientific societies and defines the blast as a cell that presents 10-14 µm in diameter, with a non-segmented nucleus or showing rudimentary lobes connected by a thick filament, abundant and clear cytoplasm containing fine and well distributed granulation[5].

Regarding the presence or absence of deviation, in this study the absolute value of blasts was considered, taking as reference the values defined by age group[7]. For this parameter, a substantial agreement was observed between the analysts-control (*kappa*: 0.72). The reading of each analysts-control was compared with that of the analysts on duty and it was found, respectively, *kappa* of 0.41 and 0.57 (moderate agreement).

A low agreement in this case can have negative impacts on the clinical evaluation of patients, since the percentage of blasts is widely used as a laboratory parameter in the diagnosis and follow-up of inflammatory and infectious processes treatment.

Nunes *et al.* (2007)[12] studied interobserver agreement in histological sections of mammary carcinomas for immunohistochemistry. Interobserver agreement was found between moderate and very good among the five study observers. Observers' experience was pointed out as the main influence factor for agreement rates, thus highlighting the need for continuous training of the microscopy expert, which should cover theory and practice.

According to the standardized criteria used in the study, the abnormalities considered for reporting lymphocytic atypia include increased cell size, nucleus immaturity with presence of nucleolus and not condensed chromatin, irregular nuclear outline or abnormal lobulation, basophilia or vacuolization of the cytoplasm and irregular cell contour[5].

The clinical importance of quantifying lymphocytic atypia is due to its association with infections of viral etiology, and they are rare in the smears of healthy adults and more frequent in children. The percentages found in the study patients were relatively low (up to 3%).

The agreement observed between analysts-control was moderate (*kappa*: 0.47) and slight when compared with the analysts on duty (*kappa*: 0.09 and 0.20). A lower agreement in the analysis of atypia in relation to the other parameters was expected and, to a certain extent, it can be attributed to the subjectivity of the observer analysis, since it depends on the number of fields observed by the microscopy expert and their location.

CONCLUSION

Among the CBC analysis steps, microscopy is the one that presents the greatest difficulty of standardization and training of professionals. In this regard, both the ability to detect the structures

present in the slide (accuracy) and the reproducibility between the observers (precision) must be considered[13].

The minor agreements were observed for the poikilocytosis and lymphocytic atypia variables, in which reporting and classification cannot be performed by the equipment and, therefore, depend only on the microscopy expert reading.

The need for standardization of the criteria for classification of changes in blood cells' morphology in the laboratory studied was evidenced. We also recommended carrying out training and professional qualification, specifying the criteria to be followed to standardize the results.

It is also necessary to establish a methodology to evaluate intra and interobserver reproducibility, to investigate the causes of variability and to plan corrective actions to promote a better degree of accuracy and precision among analysts, thus guaranteeing the quality of the results released.

RESUMO

Introdução: *Recomendações em literatura e publicações de sociedades científicas têm proposto sistemas e níveis de classificação para comunicar alterações hematoscópicas no hemograma. Visando a uniformização dos resultados liberados no laboratório clínico, a padronização em hematoscopia apresenta-se como uma ação importante dentro da garantia da qualidade, contribuindo de modo coerente para diagnóstico acurado e adequado acompanhamento de pacientes.* ***Objetivo:*** *Verificar a adequação dos critérios de classificação das alterações hematocópicas adotados pelos profissionais do laboratório do Hospital Geral de Vitória da Conquista, Bahia, Brasil.* ***Material e método:*** *No período de 12 semanas, foram selecionadas 159 lâminas de hemogramas provenientes de 53 pacientes de quatro unidades de terapia intensiva (UTIs) do hospital, sendo três lâminas por paciente. Foram realizadas leituras das lâminas pelo plantonista do laboratório e por duas analistas-controle que utilizaram critérios padronizados por Palmer (2015), sendo todas as análises cegas e independentes. Os resultados foram comparados por meio do teste de concordância* kappa *de Cohen entre as analistas-controle e os plantonistas.* ***Resultados:*** *Para os parâmetros analisados, observou-se entre as analistas-controle concordância moderada a excelente e, quando comparadas com os plantonistas, verificou-se concordância ligeira a substancial.* ***Discussão:*** *A menor concordância ocorreu na expressão de poiquilocitose e atipia linfocitária, que não são detectadas pelos hemocitômetros, sendo necessária a capacidade técnica do analista.* ***Conclusão:*** *O estudo evidenciou uma necessidade de padronização dos critérios para hematoscopia usados pelos analistas para obtenção de resultados reprodutíveis que espelhem qualidade e confiabilidade por parte dos prescritores. Para tanto, é necessário investimento em treinamentos dos profissionais envolvidos na revisão de lâminas hematológicas.*

Unitermos: *hematologia; microscopia; controle de qualidade.*

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