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Validation of the mindray BC6000 hematology analyzer for erythroblast counting in peripheral blood

Validação do analisador hematológico Mindray BC6000 para a contagem de eritroblastos em sangue periférico

Lacy Brito Junior[1]; Adna dos Santos Caldas[1]; Vanessa Ingrid Cardoso Pereira[1]; Roberta Isabella Senna Ferreira[2]; Ana Paula Silveira Paixão[2]

[1] Federal University of Para, Postgraduate Program in Clinical Analysis (PPGAC), Belem, Para, Brazil.
[2] Dr Paulo C Azevedo Clinical Pathology Laboratory, Hematology, Belem, Para, Brazil.

ABSTRACT

Introduction: Differential counting of erythroblasts in blood samples by hematology analyzers still has limitations. Technological advances in blood count equipment have proposed the fully automated counting of these cells, however, not without its validation.

Objective: Validate the automated count of erythroblasts in peripheral blood in the Mindray BC6000 hematology analyzer and verify the existence of correlation of the maturation stages of erythroblasts with the equipment's graphics.

Material and Methods: Prospective study with peripheral blood samples from the Clinical Pathology Laboratory Dr Paulo C. Azevedo, regardless of age and gender, to validate the erythroblast count in the Mindray BC6000 hematology analyzer compared to the manual method (gold standard), in the period of June 2019 to December 2020.

Results: Seventeen peripheral blood samples were analyzed from newborns (09/17 - 52.3%) and from patients older than 2 years (08/17 - 47.7%) who had more than 18% of erythroblasts after morphological analysis of the sample. Statistical analysis of erythroblast counts by the two methodologies showed that the Mindray BC6000 hematological counter has good reproducibility, precision and linearity. There was no correlation between the maturation stages of erythroblasts and the equipment graphics.

Conclusion: The proposed validation showed that the Mindray BC6000 hematological counter has good analytical performance for counting erythroblasts in peripheral blood. However, there is no correlation between the maturation stages of erythroblasts with the graphics generated by the equipment.

Key words: hematology; erythroblasts; laboratory automation.

RESUMO

Introdução: *A contagem diferencial de eritroblastos em amostras de sangue por analisadores de hematologia ainda apresenta limitações. Os avanços tecnológicos nos equipamentos de hemograma têm proposto a contagem totalmente automatizada dessas células, porém, não sem sua validação.*

Objetivo: *Validar a contagem automatizada de eritroblastos no sangue periférico no analisador hematológico Mindray BC6000 e verificar a existência de correlação dos estágios de maturação dos eritroblastos com os gráficos do equipamento.*

Material e Métodos: *Estudo prospectivo com amostras de sangue periférico do Laboratório de Patologia Clínica Dr. Paulo C. Azevedo, independente de idade e sexo, para validação da contagem de eritroblastos no analisador hematológico Mindray BC6000 em comparação ao método manual (padrão ouro), no período de junho de 2019 a dezembro de 2020.*

Resultados: *Foram analisadas 17 amostras de sangue periférico de recém-nascidos (17/09 - 52,3%) e de pacientes maiores*

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de 2 anos (17/08 - 47,7%) que apresentavam mais de 18% de eritroblastos após análise morfológica da amostra. A análise estatística das contagens de eritroblastos pelas duas metodologias mostrou que o contador hematológico Mindray BC6000 tem boa reprodutibilidade, precisão e linearidade. Não houve correlação entre os estágios de maturação dos eritroblastos e os gráficos do equipamento.

Conclusão: *A validação proposta mostrou que o contador hematológico Mindray BC6000 apresenta bom desempenho analítico para contagem de eritroblastos em sangue periférico. Porém, não há correlação entre os estágios de maturação dos eritroblastos com os gráficos gerados pelo equipamento.*

Palavras-chave: *hematologia; eritroblastos; automação de laboratório.*

INTRODUCTION

Technological advances in the automation process of the blood count in recent years have made this test faster in sample analysis, especially with the introduction of conveyors and smear processors and slide staining. But they also brought greater security to the results by improving the identification of normal peripheral blood cells and signaling, through the emission of alerts/alarms (flags), for the presence of immature bone marrow cells in these samples[1-7].

Accuracy in the identification of immature bone marrow cells in peripheral blood has only been possible thanks to the incorporation of impedance, flow cytometry and more recently fluorescence signals of the DNA/RNA content of the cell nucleus for counting and analysis of the leukocyte differential. Thus, allowing the analysis of leukocyte differential dispersion in complexity and cell size graphs, or cell complexity and fluorescence signals of DNA/RNA content[3-12].

Specifically in relation to analyzers that use the principles of electrical impedance, optical dispersion and analysis of cells by DNA/RNA fluorescence signals from the cell nucleus, we can highlight the Mindray BC6000 and Sysmex® hematology analyzers, which have similar methodologies[4,7-9,11,13]. These equipments, in addition to providing the results of counting and differential of leukocytes in five parts, also propose the differential counting of erythroblasts (NRBC) and other body fluids such as pleural and synovial fluids[4,8,12-14].

The incorporation of any new methodology into the routine of a clinical analysis laboratory, however, must first be validated to ensure its analytical quality by evaluating the accuracy of the results. Thus, the International Council for Standardization in Hematology (ICSH), for example, defines as precision the measure of agreement between the estimated value of a given analyte and the observed value obtained using independent techniques[15].

Thus, this study presents the validation of the automated counting of erythroblasts in peripheral blood samples in the Mindray BC6000 hematology device through a comparative method with the manual technique (Gold Standard), and verified the existence of a correlation between the maturation stages of erythroblasts and the graphics generated by the equipment.

METHODOLOGY

Casuistry: Prospective study with routine peripheral blood samples from the Dr Paulo C. Azevedo Clinical Pathology Laboratory, located in the city of Belém, Pará, or bone marrow samples from the same laboratory from an oncology hospital, regardless of age and gender, for characterization of the immunophenotypic profile of the erythroblasts present in these samples and validation of the automated counting of erythroblasts in the Mindray BC6000 hematology counter from June 2019 to December 2020.

For this purpose, 20 bone marrow samples from patients with acute leukemia, disease-free, under therapeutic follow-up were analyzed, that is, in bone marrow recovery after specific treatment with 15 or 30 days post-chemotherapy, as a positive control for the definition of erythroblast populations at different stages of maturity. In addition, 17 samples of peripheral blood from patients undergoing routine blood counts in that laboratory, arising from spontaneous or hospital demand, to verify whether it would be possible to characterize the stage of maturation of peripheral blood erythroblasts in the graphic plotting of the Mindray BC6000 equipment.

Characterization of Samples: Peripheral blood or bone marrow samples from patients of both genders, of any age group, anticoagulated with EDTA (Ethylenediamine Tetraacetic Acid), with a volume of 2ml to 5ml, and with more than 18% of erythroblasts, for each, were included. 100 leukocytes were counted, after morphological analysis by common light microscopy with an immersion objective on a slide stained with May Grunwald by a specialized professional from that laboratory.

Exclusion Criteria: Samples of coagulated peripheral blood or bone marrow, with a volume of less than 2 ml, with less than 18% of erythroblasts after morphological analysis, or from patients with active acute leukemia, were excluded.

Characterization of the presence of erythroblasts by the Mindray BC6000 equipment:

The samples selected by the quantity of erythroblasts after morphological analysis were submitted to acquisition in the Mindray BC6000 equipment, which, according to the manufacturer, has the ability to identify and quantify the presence of erythroblasts in peripheral blood samples.

Immunophenotypic characterization of erythroblasts: For the immunophenotypic characterization of the various stages of erythroblast development in peripheral blood and bone marrow samples, the mononuclear cells were first separated in a conical centrifuge tube with the addition of 2ml of Histopaque-1077® and then the tube was tilted to 45º, gentle addition of 2ml of peripheral blood or bone marrow sample with Pasteur pipette. Afterwards, the samples were centrifuged at 1,500 rpm for 30 minutes and then, using another Pasteur pipette, the layer of mononuclear cells was carefully removed from the interface between the Histopaque gradient and the red blood cells, transferring these cells to another conical centrifuge tube containing 10ml of PBS (0.10%) Euroimun Sodium Perbarate. With another centrifugation at 1,500 rpm for 10 minutes, discarding the supernatant and resuspension of the cell pellet in 5ml of PBS.

Then, 100µL of this solution was transferred to each previously identified conical flow cytometry tube. Subsequently, 5µL of different combinations of commercial monoclonal antibodies, panhematopoietic, CD34 and CD45, or myeloid, CD33, CD117 and CD235a (Glycophorin A), labeled with the fluorochromes FITC, PE, Percyp and APC, were added. Followed by the addition of 200µL red cell lysis solution (Facslysing BD 1:10), incubation in the dark for 10 minutes, and subsequent addition of 1ml (1,000µL) of water to each tube. For further analysis after acquisition of 10,000 events in a BC Coulter DxFlex® flow cytometer, with CytExpert for DxFlex® software (BC, Indianapolis, Indiana, USA), for thirteen colors.

Statistical Analysis: The data obtained were analyzed using the Statistical Package for Social Sciences (SPSS) software, version 26 (SPSS Inc., Chicago, USA) by applying descriptive statistics tests to determine the mean, standard deviation and median referring to the erythroblast counts by the automated and manual methods; of normality by Shapiro-Wilk, to verify if the data had normal distribution, and by Mann-Whitney to compare the medians of the amount of erythroblasts counted by the two methodologies. Being adopted as significance level $p \leq 0.05$.

Ethical Aspects: As this is a prospective study without the researchers' involvement with the research subjects or the use of any personal data of the same, those responsible for the research signed a Data Use Commitment Term (TCUD) with the institutions involved, with a request for a Term Waiver of Free and Informed Consent (TCLE), as provided for in the guidelines and regulatory standards described in Resolution No. 466 of December 12, 2012, complemented by article 17, item VII of Resolution 510/2010.

RESULTS

For the validation process of the automated counting of erythroblasts by the Mindray BC6000 equipment, 17 peripheral blood samples were analyzed, being 09/17 (52.9%) of newborn patients, 05/17 (29.4%) patients they were aged between 2 and 11 years and 03/17 (17.7%) were adults aged between 63 and 75 years. Of these 10/17 (58.8%) patients were female and 07/17 (41.2%) were male (Table 1).

Regarding the erythroblasts counts in the peripheral blood of the analyzed patients, it was observed that, for the newborn samples (9/17), the mean and standard deviation values were 163.0 ± 168.6 for the automated counts and 171.5 ± 185.6 for manual counts. While for samples from patients aged over 2 years (8/17) the mean values and standard deviations observed were 84.1 ± 174.5 for automated counts and 84.2 ± 174.4 for counts erythroblast manuals.

Then, the analysis of the existence of correlation between the erythroblast counts was carried out by manual and automated methods, by performing the normality distribution test, which showed that there was no normal distribution between the two methodologies. In view of this result, the non-parametric Mann-Whitney test was performed, which revealed a value of $p = 0.78$, thus demonstrating that the values of the automated counting of erythroblasts, when compared with the manual counting, are the same, thus being the methodology of Automated erythroblast count on Mindray BC6000 hematology equipment considered statistically validated for routine laboratory use.

Then, immunophenotypic characterizations of the different stages of differentiation of erythroblasts obtained from control samples from bone marrow (Figures 1A, 1B, 1C) and from peripheral blood (Figures 1E, 1F, 1G, 1I, 1J, 1K, 1M, 1N) were performed and 1O). For further analysis the graphical distribution of peripheral blood erythroblasts obtained from the automation equipment in hematology Mindray BC6000 (Figures 1D, 1H, 1L and 1P) in comparison with the respective immunophenotypic characterization of erythroblasts from the same peripheral blood sample.

TABLE 1- Analysis of counts, manual and automated, and immunophenotypic characterization of erythroblasts in peripheral blood samples from patients from the routine of the Dr Paulo C Azevedo Clinical Pathology Laboratory, from June 2019 to December 2020

Sample identification	Sex	Age (years)	Material	Erythroblast Count		Immunophenotypic Characterization of Erythroblasts by Flow Cytometry (%)		
				Automated (%)	Manual (%)	Basophil Erythroblasts CD34 + CD45fracCD117 + CD235a-	Polychromatic erythroblasts CD34-CD45fracCD117 + CD235a+	Erythroblasts Orthochromatic CD235a + CD45-
RN SLA	F	0	SP	22	26	0	2,36	18,93
RN VN	F	0	SP	15	18	0	3,63	63,91
RN GD	F	0	SP	93	93	0,7	1,62	68,7
RN VN	F	0	SP	119	120	2,71	3,05	8
RN FBF	M	0	SP	79	75	1,5	3,24	26,37
RN JV	M	0	SP	474	546	0	3,2	64,29
RN ACL	M	0	SP	363	363	1,46	3,45	68,52
RN TK	F	0	SP	283	284	0	3,11	59,7
RN CG	F	0	SP	19	19	3,71	2,39	50,11
MFL	F	75	SP	27	26	1,71	1,77	9,23
FBA	M	63	SP	18	20	9,73	4,71	53,36
SPK	M	3	SP	17	17	0	21,81	85,26
BNCS	M	11	SP	26	25	4,75	8,66	36,28
MCL	F	63	SP	25	27	0	36,47	11,71
LSGM	F	2	SP	21	20	0	0,92	88,94
PGCG	M	9	SP	23	23	0	3,31	35,85
KVSR	F	2	SP	516	516	1,14	1,39	77,89

Caption: SP: peripheral blood; NB: newborn; CD: cluster of differentiation; F: female; M: male; %: percentage (relative value).

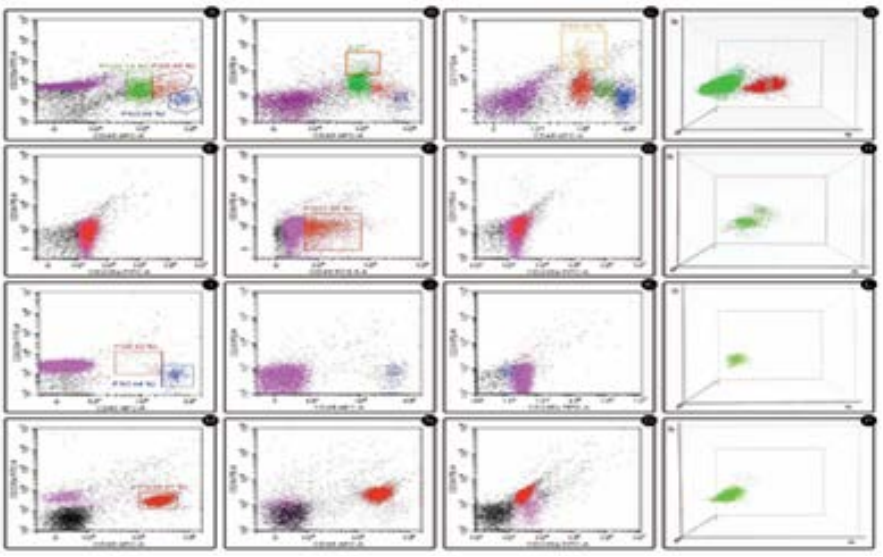

FIGURE 1 – *Graphic representation of cell dispersion of the Dot plots type for the characterization of erythroblast populations in the bone marrow (A, B and C) by flow cytometry, where: in purple are orthochromatic erythroblasts and reticulocytes (Figure 1A to 1N); in green are polychromatic erythroblasts (Figure 1A and 1B); in red basophilic erythroblasts (Fig 1A and 1B); in orange the proerythroblasts (Figure 1C); and lymphocytes in blue (Figure 1A, 1B and 1C) in bone marrow samples. And in Figures 1E, 1F, 1G, 1I, 1J, 1K, 1M, 1N and 1O the populations of erythroblasts in peripheral blood are represented by flow cytometry, where: in purple, they are orthochromatic erythroblasts and reticulocytes; in red are polychromatic erythroblasts; and in black are likely cell debris. The complexity (FS) X nuclear fluorescence (FL) graphs are relative to the peripheral blood samples obtained in the Mindray BC6000 equipment (D, H, L and P), where: in Figure 1D the erythroblasts are represented in green and in red lymphocytes; and in Figures 1H, 1L and 1P, only erythroblasts are shown in green.*

In this sense, it was observed that there is no relationship between the stage of differentiation of erythroblasts, observed in the peripheral blood of a sample, with the respective dispersion of these cells in the graphs obtained from the BC6000 automation equipment in hematology - Mindray®. As we can see, for example, in Figures E, F and G, where the presence of 78.11% of orthochromatic erythroblasts (pink) and 21.89% of polychromatic erythroblasts (red) is observed, these may even suggest the existence of correlation with the respective graphical dispersion by the Mindray BC6000 equipment. However, when the analysis is performed in relation to Figures M, N and O, where there is a predominance of polychromatic erythroblasts (36.47%), it is observed that, even so, the graphic dispersion of the Mindray BC6000 equipment does not change in regarding its special distribution.

DISCUSSION

As already presented, this research consisted of evaluating the performance of the Mindray BC6000 hematology analyzer for the automated counting of erythroblasts when compared to the manual counting on a slide (gold standard). In this sense, the results obtained with the analyzed automated methodology proved to be capable of accurately counting erythroblasts and were thus validated for use in the routine of the clinical analysis laboratory that supported the research. Thus ensuring precision in the results and more safety and speed in the release of blood count reports.

These data corroborate other studies in the literature that compare the count of erythroblasts in peripheral blood using non-automated and automated methodologies, and which also showed no statistical difference between these methodologies[3,5,9,10-12,14,16].

It is known, however, that there is today on the market a large number of automated hematology analyzers available for routine use in clinical analysis laboratories, however, each using a variety of technologies for the analysis of peripheral blood samples. This fact, however, requires that each laboratory, before placing one of these devices in its diagnostic routine, has to perform their previous validation[1,3-7,14,16-18].

TAN, NAVA and GEORGE16 in their studies on the capacity of the Cell-Dyn Sapphire (Abbott®) and UniCel DxH 800 (Beckman Coulter) hematological equipment to count and differentiate erythroblasts from other cell types, for example, showed that the Cell- Dyn Sapphire (Abbott®) showed 93.5% sensitivity for counting erythroblasts, while the UniCel DxH 800 equipment (Beckman Coulter) had 73.0% specificity for the same count, when compared to the gold standard methodology (manual). Thus suggesting that erythroblast counting using equipment that uses methodologies similar to the Cell-Dyn Sapphire (Abbott®), such as BC6000 (Mindray®), have greater sensitivity for counting these cells.

SHEN et al[12] in their studies, in turn, compared automated and manual erythroblast counts in peripheral blood samples from several patients. In this validation, the equipment used for this counting of erythroblasts was the Mindray BC6000 and, similar to what was observed in our study, there was no significant difference in the results obtained when compared to the manual counting ($p>0.05$). These authors then suggest that this equipment has 80.0% sensitivity and 96.9% specificity for counting erythroblasts.

In our studies, however, we also sought to identify whether the graphical plot of the erythroblast count generated by the equipment would be able to differentiate the maturation stages of erythroblasts present in the peripheral blood sample. For this purpose, as already presented, immunophenotyping of erythroblasts from peripheral blood samples was performed by flow cytometry, and subsequent analysis of the plotting graphics of the Mindray BC6000 equipment, where it was observed that, although the equipment reproduces the erythroblast count with precision, the generated graphic projection does not allow to differentiate in which stage of maturation the erythroblasts are. This result, however, has no consequences for the patient, since the maturation stage of erythroblasts in peripheral blood, so far, does not seem to have any implications for its treatment or prognosis.

A similar study carried out by GRIMALD and SCOPACASA[19] with samples from patients who had a hematological disease with the presence of immature cells of the myeloid lineage and erythroblasts acquired in the hematological analyzer CELL-DYN 4000 (Abbott®), which uses similar technology to the equipment Mindray BC6000 also showed similar results in terms of precision for counting erythroblasts and the same difficulty in graphically identifying the stages of maturation of these cells.

Thus, the validation proposed in our study showed that the Mindray BC6000 hematological counter has good analytical performance for counting erythroblasts in peripheral blood. However, the graphics generated by the equipment do not show correlation with the maturation stages of erythroblasts.

Conflicts of Interests: The authors declare they have no competing interests.

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CORRESPONDING AUTHOR

Lacy Brito Junior 0000-0001-9102-5817
lcdbrito2@gmail.com