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SHORT COMMUNICATION

Definition of Reference Range for the Immature Granulocytes Parameter Provided by a Hematology Analyzer

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SUMMARY

Background: **Some pathologies or physiological changes may show forms of granulocytes in peripheral blood. Hematology analyzers have brought new parameters such as the detection of immature granulocytes (IG), which may be useful biomarkers. The objective of this study was determined the IG count in a control group to establish the reference range.**
Methods: **A group of healthy donors was used to obtain the reference value of IG in the laboratory of the Hospital de Clínicas de Porto Alegre.**
Results: **The reference range of IG (n = 115) was 0 - 0.06 x 10^9/L and 0 - 0.63%. This reference interval was similar to that of previous studies.**
Conclusions: **The determination of the specific reference interval for each region is an important tool in the direct application of biomarkers in clinical laboratory routines. The use of reference values is essential so that the true positive cases for microscopic review are detected without a large number of false positives, which would impair laboratory efficiency.**
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KEY WORDS

hematology analyzer, immature granulocytes, smear blood review, Sysmex XE

INTRODUCTION

Immature granulocytes (IG) are cells present in the bone marrow that, in normal situations, are rarely found in the peripheral blood. However, in some pathologies or physiological changes, immature forms of granulocytes may occur in circulating blood. The increased need causes the bone marrow to launch the granulocytes into the bloodstream before they can differentiate into segmented granulocytes. In these cases, growth factors and cytokines, produced by tissue macrophages and other cells, induce the release and production of neutrophils by the bone marrow, causing the release of immature forms into the peripheral blood [1].
The classic form of the cytological differential of complete blood count (CBC) is done manually, reading a

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stained blood smear through a microscopic. However, the advent of automation has allowed a great reproducibility of test results and faster turnaround. Currently, automation in hematology allows the determination of more than 32 parameters related to platelets, leukocytes, and erythrocytes, among these the IG, with the proposal of being biomarkers for various clinical conditions [2]. New hematology equipment is available in the market and provides new parameters, the automated CBC has advantages: accuracy, precision, linearity, sensitivity, specificity, quantitative/qualitative alerts, and the possibility of Laboratory Information Systems (LIS) [2].
The validation of these new parameters is essential for the daily routine of the clinical laboratory. It is necessary to have a complete knowledge of the information given by the instrument, so that it is better used in diagnosis, prognosis, and disease monitoring but their clinical utility remains poorly documented. In this study, we determined the IG count in a control group to establish the reference interval.

MATERIALS AND METHODS

For the validation, the protocol 'Standards for Reporting of Diagnostic Accuracy' [3] was followed. The ethics committee of the Research and Graduate Group (GPPG) of the Hospital de Clínicas de Porto Alegre (HCPA) (16-0390) and the National Commission of Ethics in Research (CONEP) approved this work.
In this study, 115 CBC results were used for internal quality control. These samples were obtained from the blood bank donors of Hospital de Clínicas de Porto Alegre (HCPA). The samples were collected from August to October 2016 in tubes with K_2-EDTA, delivered by pneumatic tube transport to the laboratory. The samples were processed within 3 hours after collection in the laboratory of the Clinical Pathology Service of HCPA. The CBC were performed on the Sysmex XE-5000 automated hematology system (Sysmex, Kobe, Japan), which uses, in the DIFF channel, the fluorescence low cytometric technology for the leukocyte differential count [4]. The IMI channel is mainly based on membrane differences between mature and immature cells. Two highly experienced operators, counting 100 leukocytes per slide for each individual in the control group to evaluate false negatives results, performed a manual analysis.
The data were plotted and organized in a Microsoft Office Excel® 2007 worksheet. Statistical analysis was performed using the Statistical Package for Social Sciences 20.0 (SPSS Inc., Chicago, IL, USA). Median and P25 and P75 percentiles were used for descriptive analysis. The P2.5 - P97.5 percentiles were used to determine the normal range.

RESULTS

The median age of the patients was 40 years, ranging from 18 to 67 years, 83 men and 32 women. Table 1 shows the medians and percentile 25 and 75 of CBC and IG count.
The 95% reference range was established for IG# (absolute IG) as 0 - 0.06 x 10^9/L and for IG% (relative IG) as 0 - 0.63%.
The inter-assay precision was calculated by e-Check (XE) control (Sysmex, Kobe, Japan) and processed ten times in the automated apparatus at three different levels. The mean coefficient of variation (CV) was 5.10% (standard deviation = 0.30) for IG# and 5.13% (standard deviation = 0.46) for IG%. These values were below the variation limit defined by the manufacturer, which is 24.1% for IG # and 20.9% for IG%.

DISCUSSION

In the field of hematological analysis, automated analyzers offer several parameters, mainly in the leukocyte differential, such as quantification of IG, which allows us to optimize the process of blood cell release. However, manual review is still often needed. It is essential to set the reference values so that the true positive cases for microscopic review are detected without a large number of false positives, which would impair laboratory efficiency [5].
Each laboratory should determine reference ranges during the validation process of its hematology analyzers [5]. The reference range for IG found in our study was similar values compared to studies of Pekelharing et al., with a sample size of 309 healthy donors, found 0 - 0.06 x 10^9/L and 0 - 0.6%, with a 95% confidence interval [2]. The same values were found by Lima et al. [6] obtained from the analysis of 200 adult outpatients. Bruegel et al. obtained IG reference values lower than 0.03 x 10^9/L and 0.5%, using the 5th and 95th percentiles [7]. However, this was below some other studies [8,9], probably due to the type of sample selected and to the strict criteria used between the hematology laboratory and the HCPA blood bank for accepting the samples that were used for the control group.
Absolute and relative IG measurements showed excellent inter-assay precision using three-level control. However, it was not possible to estimate the reproducibility of IG measurements as performed by other studies [9,10].
The number of IG in relation to the total granulocyte count is a clinical tool with a predictive value with regard to acute bacterial infection. However, studies have shown that the ratio between non-segmented and segmented neutrophils would be a much more accurate index for assessing the severity of bacterial infections. The first stage of bacterial infection, between 12 and 24 hours, occurs with a decrease in total leukocytes, with no left shift in hemogram [1]. Thus, it is evident that

Table 1. Descriptive data of the automated CBC and IG # and % counts for the samples in the Sysmex XE-5000.

	Median	P-25	P-75
Leukocytes (10^9/L)	**6.61**	**5.74**	**7.51**
Erythrocytes (10^{12}/L)	**4.98**	**4.69**	**5.18**
Hemoglobin (g/L)	**148.0**	**142.0**	**155.0**
Hematocrit (%)	**42.90**	**41.00**	**44.70**
Platelets (10^9/L)	**225.0**	**195.0**	**268.0**
IG# (10^9/L)	**0.01**	**0.01**	**0.02**
IG%	**0.20**	**0.10**	**0.30**

IG# - absolute immature granulocyte value, IG% - relative immature granulocyte value (%).

there is a need to obtain early biomarkers that cover this crucial period for the development of infection. More studies are required with other biomarkers available on the market and patient groups with different clinical conditions for a true assessment of the clinical application of IG.

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Declaration of Interest:
The Author(s) declare(s) that there is no conflict of interest.

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