Revista Brasileira de Hematologia e Hemoterapia
Brazilian Journal of Hematology and Hemotherapy

www.rbhh.org

Scientific comment

Reticulocyte parameters: why should clinical laboratories evaluate and report them?☆

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Reticulocyte enumeration was described for the first time in the late 1940s. Until the early 1980s, it was performed exclusively by microscopic examination of supravital stained peripheral blood smears. Besides pathophysiologic conditions, technical artifacts and human subjectivity during the microscopic analysis are the primary causes of the compromised accuracy of manual reticulocyte counts. In the early 1950s, Kozenow and Mai[1] were the first to report an enumeration method for reticulocytes, based on fluorescence microscopy with an RNA/DNA-specific fluorochrome (acridine orange). Although fluoresce techniques offer some practical advances over light microscopy, automated reticulocyte enumeration by flow cytometry was proven to be even better. It is faster, technically easier to perform, more objective, easily automated, and less labor-intensive when compared to manual slide-based techniques. Rather than reducing the human interaction, it reduces the sample's turn-around time, and therefore increases cost-efficiency, especially for high throughput analyses. Furthermore, flow cytometry also provides accurate information on the age distribution of the reticulocyte population.

The first decade of the 2000s brought the dawn of a new era in reticulocyte enumeration, when methods using existing hematology analyzers were developed. Currently, blood analyzers offer integrated and user-friendly flow cytometry platforms, which do not require specially trained technical staff in order to perform reticulocyte enumeration, as was necessary in the case of separate flow devices.

Several fluorochromes, such as acridine orange, propidium iodide, ethidium bromide, thioflavin, and auramine were initially used in reticulocyte enumeration by flow cytometry. A great improvement of the counting accuracy was achieved through the development of the thioflavin T-analogue (thiazole orange [TO]), whose excitation occurs in the visible region (λ = 488 nm) and is specific for reticulocyte labeling. This made flow cytometry even more practical; nowadays, Food and Drug Administration-approved TO reagent kits are widely available to clinical laboratories, and automated reticulocyte enumeration tests are a standard routine. Rather than merely counting reticulocytes, flow cytometry also evaluates reticulocyte maturation based on the RNA content. Young or immature reticulocytes are highly fluorescent (high RNA content), while maturing reticulocytes have medium fluorescence intensity, and older reticulocytes have low fluorescence (low RNA content). The mean fluorescence of a TO-stained reticulocyte population, the reticulocyte maturation index (RMI), is a useful clinical indicator of erythropoietic activity and a valuable addition to the conventional reticulocyte count.

In 2013, a survey of the College of American Pathologists (RT, RT-04, 2013) observed that, of 1,055 participating laboratories, 302 used Sysmex XE 2100 (coefficients of variation [CV]: 17.7%), 15 used Sysmex XE 2100C (CV: 13.1%), 413 used Sysmex-5000 (CV: 16.5%), 60 used Sysmex XN-Series (CV: 16.3%); and 267 used Sysmex XT 2000 (CV: 15.3%) hematology analyzers for the index determination of immature reticulocytes. The average CV suggests that clinicians should include approximately 20%

☆See paper by Wollmann M et al. on pages 25-8.

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DOI: 10.5581/1516-8484.20140002

of technical variation into their considerations before making any decision.

In this issue of the Revista Brasileira de Hematologia e Hemoterapia, Wollmann et al. describe a method using fluorescence reticulocyte intensity (low [LFR], medium [MFR], and high [HFR] fluorescence reticulocyte indexes) instead of RMI in order to improve the characterization of iron deficiency anemia (IDA).[2] The authors report that the small number of patients in the sample is sufficient to establish a positive correlation between reticulocyte immaturity and IDA. Similarly, Kojima et al.[3] were able to establish an increase in the reticulocyte count, compared to the control group, by examining only eight patients with IDA. Conversely, Cortellazzi et al.[4] demonstrated that erythroid expansion in sickle cell diseases (Hb SS, Hb SC, and Hb S/ β-thalassemia) leads to an increased release of immature reticulocytes from the bone marrow. No difference with respect to any reticulocyte parameter was observed between the β-thalassemia and IDA groups. In fact, the RMI showed a significant correlation between the total iron-binding capacity (TIBC) and ferritin,[5] reflecting the activity of the bone marrow and erythropoiesis.

In order to better classify IDA anemia, the hemoglobin content of reticulocytes (CHr) is a very important and highly sensitive parameter. When patients with low levels of bone marrow iron stores (considering only those patients with MCV less than 100 fL) were examined, the receiver operator curve analysis of CHr, ferritin, transferrin saturation, and MCV demonstrated that CHr has the highest overall sensitivity and specificity for an accurate prediction of the absence of bone marrow iron stores.[6] The previously mentioned College of American Pathologists[7] survey from 2013 also revealed that 665 laboratories used the same equipment for the RMI, and that the mean CV observed was less than 3% (CHr in pictograms).

In conclusion, extensive evidence has been accumulated suggesting that reticulocyte parameters (flow cytometry counting, RMI, and CHr) are useful for a variety of purposes, e.g. accurately diagnosing anemia patients, assessing the regenerative activity after anemia treatment, monitoring patients receiving recombinant human erythropoietin, or recovering from chemotherapy or bone marrow transplants. For these reasons, clinical laboratories should both record and report these parameters routinely in the future.

Conflicts of interest

The author declares no conflicts of interest.

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