



## Scientific Comment

# Platelet and reticulocyte new parameters: why and how to use them?☆



Helena Zerlotti Wolf Grotto<sup>a,b,\*</sup>

<sup>a</sup> Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil

<sup>b</sup> Sysmex Latin America & The Caribbean, São Paulo, SP, Brazil

The evolution of automation in hematology has enabled greater reliability and accuracy in the results of the complete blood count (CBC) and the implementation of new technologies has streamlined the laboratory routine, optimizing the time to release tests. Moreover, new laboratory parameters have been recognized as auxiliaries to recognize some clinical conditions.

From the first platelet count performed in a chamber developed by Neubauer in 1924<sup>1</sup> to the present day, there have been increasing improvements in the technology used to identify and quantify platelets. The new generation of hematology analyzers are more accurate to identify cells, providing reliability in the measurement of platelets particularly in cases of severe thrombocytopenia. In addition, the use of fluorescent markers specific for platelets allows the detection of possible interference, for example by decreasing the possibility of cases of false thrombocytosis.<sup>2</sup>

Aspects related to the identification of immature platelets, termed reticulated platelets (RP), date back to 1969. Nucleic acid dyes allowed the detection of younger platelets using an optical microscopy.<sup>3</sup> Thiazole orange dye flow cytometry and the strategy of the 'gate' were introduced later for identifying RP.<sup>4,5</sup> More recently some hematologic analyzers have made it possible to detect newly-released immature platelets. The main clinical applicability of the immature platelet fraction (IPF) is the evaluation of thrombopoietic activity in the bone marrow. An elevated number of immature circulating platelets is observed in thrombocytopenia of peripheral origin caused by excessive consumption of platelets, while

thrombocytopenia caused by inadequate platelet production is characterized by a reduced number of immature platelets entering into the circulation.<sup>6–8</sup>

Another IPF application is the monitoring of the thrombocytopenic phase after chemotherapy and the transplant of precursor cells.<sup>7,8</sup> As younger platelets apparently have greater thrombotic potential and are metabolically and enzymatically more active than adult platelets, some studies propose monitoring of the RP or IPF as indicators of risk for acute coronary syndrome and other inflammatory and thrombotic conditions.<sup>9</sup>

The same technological evolution in terms of accuracy, and availability of new parameters was observed in the erythroid series. The reticulocyte (RTC) count is clinically important both for the pathophysiological classification of anemia, and to monitor marrow response after therapeutic interventions.<sup>10</sup> However, for a long time the RTC count was underused in the clinical and laboratory practice due to three main factors: technical limitations in the detection of the cell, the imprecision of the manual microscopic method, and high coefficient of variations in counts. With the advent of automation, the detection and quantification of these cells are much more accurate and reliable, returning credibility and clinical value to RTC enumeration. Similar to what occurred with platelets, new parameters related to the degree of immaturity of these precursors of red blood cells were introduced by several hematologic analyzer manufacturers. Initially, it was possible to subdivide the reticulocyte population in different degrees of maturity according to the content of RNA inside the cell: more immature reticulocytes have a higher

DOI of original article: <http://dx.doi.org/10.1016/j.bjhh.2016.07.001>.

☆ See paper by Morkis et al. in Rev Bras Hematol Hemoter. 2016;38(4):310–313.

\* Corresponding author at: Sysmex Latin America and The Caribbean, Rua do Paraíso 148, cj 31, CEP 04103-000, São Paulo, SP, Brazil.

E-mails: [lenagrotto@gmail.com](mailto:lenagrotto@gmail.com), [helena.grotto@sysmex.com.br](mailto:helena.grotto@sysmex.com.br)

<http://dx.doi.org/10.1016/j.bjhh.2016.08.001>

1516-8484/© 2016 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

fluorescence intensity than mature cells. The immature reticulocyte fraction (IRF) provides the same information obtained by the reticulocyte count regarding the evaluation of bone marrow response to anemia or erythropoietic activity in general.<sup>10</sup> The advantage is the precocity of the information provided by IRF compared to the RTC count. In cases of regenerative anemia or response to replacement therapy, elevations in the IRF value precede the increase in the absolute number of reticulocytes by several days. For this reason, it is suggested that the IRF should be used as an aid in the evaluation of bone marrow response during mobilization of hematopoietic precursor cells,<sup>11</sup> or as a predictor of recovery from neutropenia in autologous transplantation.<sup>12</sup>

The measurement of the content of hemoglobin of reticulocytes (CHr or Ret-He) reflects the synthesis of hemoglobin in marrow precursors, and allows the detection of early stages of iron deficiency.<sup>13</sup> This parameter has been identified as an auxiliary in the differential diagnosis of anemias.<sup>14</sup> The main advantages of Ret-He are that it is released at the same time as the CBC, and it is more accurate than biochemical markers, such as ferritin and transferrin saturation in detecting iron-deficient erythropoiesis in patients with inflammation or anemia of chronic disease.<sup>10</sup>

In the practice, the determination of Ret-He has been more widely used in patients with chronic kidney disease undergoing a dialysis regime and recombinant human erythropoietin (rHuEPO).<sup>15,16</sup> Under these conditions suitable iron intake is imperative for adequate erythropoiesis. Biochemical dosages have shown limitations in the evaluation of iron status in this group of patients, because they can suffer any effect of inflammatory activity. Reticulocyte hemoglobin content measurement is incorporated into the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) guidelines to monitor rHuEPO therapy.<sup>17</sup>

Limitations in the wider use of these new hematological parameters are related to the lack of standardization and the establishment of reference values for the normal population. Therefore, since the technologies and the nomenclature of new indexes vary according to the manufacturer, it is difficult to compare numeric results obtained from different analyzers.

In the study reported by Morkis et al.,<sup>18</sup> 132 samples of apparently healthy individuals were evaluated in order to establish the reference ranges for IRF, Ret-He and IPF. The observed results are in agreement with published data giving greater reliability in the investigation of the clinical applications of the parameters, and in the analysis of possible alterations found on comparing results from patients with values obtained in the normal population.

The establishment of reference values is a practice that should be adopted whenever a new test is introduced in the laboratory routine. In addition, a harmonization is needed for new parameters and indices between different analyzers.

### Conflicts of interest

The author is a Medical Advisor of Sysmex Latin America and the Caribbean. That company produces the hematologic analyzer used in the study.

### REFERENCES

- Lewis SM. Automation in haematology – present and future trends. *Pure Appl Chem.* 1982;54:2053–8.
- Wada A, Takagi Y, Kano M, Morikawa T. Accuracy of a new platelet count system (PLT-F) depends on the staining property of its reagents. *PLoS One.* 2015;10:e0141311.
- Ingram M, Coopersmith A. Reticulated platelets following acute blood loss. *Br J Haematol.* 1969;17:225–9.
- Lee LG, Chen CH, Chiu LA. Thiazole orange: a new dye for reticulocyte analysis. *Cytometry.* 1986;7:508–17.
- Kienast J, Schmitz G. Flow cytometric analysis of thiazole orange uptake by platelets: a diagnostic aid in the evaluation of thrombocytopenic disorders. *Blood.* 1990;75:116–21.
- Briggs C, Kunka S, Hart D, Oguni S, Machin SJ. Assessment of an immature platelet fraction IPF in peripheral thrombocytopenia. *Br J Haematol.* 2004;126:93–9.
- Briggs C, Hart D, Kunka S, Oguni S, Machin SJ. Immature platelet fraction measurement: a future guide to platelet transfusion requirement after haematopoietic stem cell transplantation. *Transfus Med.* 2006;16:101–9.
- Have LW, Hasle H, Vestergaard EM, Kjaersgaard M. Absolute immature platelet count may predict imminent platelet recovery in thrombocytopenic children following chemotherapy. *Pediatr Blood Cancer.* 2013;60:1198–203.
- Grove EL, Hvas A-M, Kristensen SD. Immature platelets in patients with acute coronary syndromes. *Thromb Haemost.* 2009;101:151–6.
- Buttarelli M. Laboratory diagnosis of anemia: are the old and new red cell parameters useful in classification and treatment, how? *Int J Lab Hematol.* 2016;38 Suppl 1:123–32.
- Noronha JF, Lorand-Metze IG, Grotto HZ. Hematopoietic progenitor cells (HPC) and immature reticulocytes evaluation in mobilization process: new parameters measured by conventional blood cell counts. *J Clin Lab Anal.* 2006;20:149–53.
- Graziuttu ML, Dong L, Miceli MH, Cottler-Fox M, Krishna SG, Fassas A, et al. Recovery from neutropenia can be predicted by the immature reticulocyte fraction several days before neutrophil recovery in autologous stem cell transplant recipients. *Bone Marrow Transplant.* 2006;37:403–9.
- Brugnara C. Reticulocyte cellular indices: a new approach in the diagnosis of anemias and monitoring of erythropoietic function. *Crit Rev Clin Lab Sci.* 2000;37:93–130.
- Canals C, Remancha AF, Sardá MP, Piazuolo JM, Royo MT, Romero MA. Clinical utility of the new Sysmex XE 2100 parameter – reticulocyte hemoglobin equivalent – in the diagnosis of anemia. *Haematologica.* 2005;90:1133–4.
- Garzia M, Di Mario A, Ferraro E, Tazza L, Rossi E, Luciani G, et al. Reticulocyte Hemoglobin Equivalent: an indicator of reduced iron availability in chronic kidney diseases during erythropoietin therapy. *Lab Haematol.* 2007;13:6–11.
- Buttarelli M, Pajola R, Novello E, Rebeschini M, Cantaro S, Oliosi F, et al. Diagnosis of iron deficiency in patients undergoing hemodialysis. *Am J Clin Pathol.* 2010;133:949–54.
- National Kidney Foundation, Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI). NKF-K/DOQI clinical practice guideline and clinical practice recommendations for anemia in chronic kidney disease. *Am J Kidney Dis.* 2006;47 Suppl 3:S11–45.
- Morkis IV, Farias MG, Scotti L. Determination of reference ranges for immature platelet and reticulocyte fractions and reticulocyte hemoglobin equivalent. *Rev Bras Hematol Hemoter.* 2016;38:310–3.