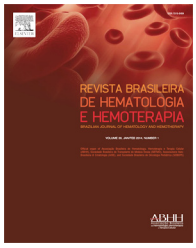
Revista Brasileira de Hematologia e Hemoterapia
Brazilian Journal of Hematology and Hemotherapy

www.rbhh.org



Scientific Comment

Comment on: Evaluation of erythrocyte and reticulocyte parameters as indicative of iron deficiency in patients with anemia of chronic disease☆

*Elisa Piva**

Azienda Ospedaliera-Università di Padova, Padova, Italy

ARTICLE INFO

Article history:
Available online 17 February 2015

The article on the evaluation of the effectiveness of mature red cell and reticulocyte parameters under three conditions: iron deficiency anemia, anemia of chronic disease (ACD), and anemia of chronic disease associated with absolute iron deficiency by Torino et al.[1] is very valuable. Automated reticulocyte counts are widely used in the clinical laboratory due to their greater precision, accuracy and reproducibility compared to those obtained using the microscope. The most important benefit of automated methods is the greater precision of counts. By analyzing a much greater number of reticulocytes (more than ten thousand), the statistical error is minimized.[2] Visual microscopy is still recommended as the comparability method for reticulocytes, despite studies showing that the variation coefficient (VC) ranges from 20% to 40%. Fully automated methods have eliminated inter-observer variability and subjectivity and substantially reduced turnaround time. Automated methods employ a wide variety of reagents for reticulocyte RNA and these show different sensitivity on binding to RNA.[3] Therefore, although automated flow cytometric analysis has led to a significant advance in reticulocyte counting, some limitations still persist in comparability across different laboratories and better methods of standardization and harmonization are needed.[4] Nevertheless, biological and pre-analytical variations can potentially affect test performance and the clinical interpretation of laboratory results.[5] Pre-analytical variations represent a major source of inaccurate laboratory results. Reticulocyte counts are significantly decreased after 24 h of storage at room temperature due to *in vitro* maturation of the reticulocytes. At constant temperatures of 4 °C the counts remain unchanged, with certain limitations for parameters derived or calculated from cellular volumes.[6,7]

Automated reticulocyte counts not only provide enhanced precision and accuracy, but also perform reliable measurements of mRNA content and of cellular indices such as volume, hemoglobin concentration and content. These novel parameters have prompted interest and studies regarding their clinical usefulness, the utility of reporting and their interpretation. Immature reticulocyte fraction (IRF) assesses reticulocyte maturation by the intensity of the staining of reticulocytes, which reflects mRNA content.[8] IRF seems to be useful for the evaluation of engraftment in bone marrow or stem cell transplantation.[9]

☆ See paper by Torino et al. on pages 77–81.

* Corresponding author at: Dipartimento Medicina di Laboratorio, Azienda Ospedaliera-Università di Padova, Via Nicolò Giustiniani 2, 35128 Padova, Italy.
E-mail address: elisa.piva@sanita.padova.it

http://dx.doi.org/10.1016/j.bjhh.2015.02.005

IRF has been proposed as an early marker of engraftment in bone marrow or hematopoietic stem cell transplantation and bone marrow regeneration following chemotherapy.[10] Several studies have demonstrated that increases in the IRF are an indicator of engraftment and precede other parameters, such as absolute neutrophil counts (ANC), reticulated platelet or reticulocyte counts.[11,12] Thus, changes in IRF during erythropoiesis-stimulating agent (ESA) therapy are indicative of the effectiveness of stimulation.[13] Many authors have reported data concerning the clinical utility of IRF in the diagnosis and monitoring of anemia.[14,15] IRF in conjunction with the reticulocyte count, provides essentially the same information as the reticulocyte production index (RPI), making its manual calculation unnecessary. The clinical utility of IRF has been reported in a variety of conditions such as in the monitoring of anemia treatment, neonatal transfusion needs, prognosis in prematurity, in AIDS anemia, renal transplant engraftment due to erythropoietin production, the detection of occult or compensated hemorrhages or hemolysis, aplastic crisis in hemolytic anemias and to verify aplastic anemia.[16] The IRF is a promising parameter that needs consolidation into the clinical practice.

Mean reticulocyte hemoglobin, a measurement of the Hb content of reticulocytes expressed in pg/cell was first measured using Bayer H3 instruments and abbreviated as CHr.[17,18] CHr is the product of the cellular volume and the cellular hemoglobin concentration. Mean reticulocyte hemoglobin has become available in other fully automated hematology analyzers that provide reticulocyte count and maturity. The methodology developed by Sysmex (Sysmex Corporation, Kobe, Japan) for the XE and later for the XN series of automated hematology analyzers provides the reticulocyte hemoglobin or Ret-He parameter, formerly defined as RET-Y. The mean hemoglobin content of reticulocytes (MCHr) and the mean hemoglobin concentration of reticulocytes (CHCr) have become available in the CELL-DYN Sapphire analyzer of Abbott (Abbott Diagnostics, Santa Clara, CA, USA). The reticulocyte hemoglobin expression (RHE) is available in the BC 6800 Mindray analyzer for research use only (Mindray BioMedical Electronics Co, Shenzhen, P.R. China), while the reticulocyte hemoglobin cellular content (RHCc) is provided in the new generation of Pentra blood cell analytical systems (Horiba Medical, Montpellier Cedex, France). RHE and RHCc need to be evaluated and comparison studies should be assessed to verify if the new indices are close to those obtained by other instruments, thus providing reliable results.

Beckman Coulter (Beckman Coulter Inc) provides a new parameter, the red blood cell size factor (RSf), which seems to be in agreement with CHr. The RSf parameter, expressed in fL, joins together the volume of mature red cells (MCV) and the volume of reticulocytes (MRV), according to the following mathematical formula: $RSf = \sqrt{(MCV \times MVR)}$.[19]

Since the life span of the reticulocytes is four days, the measurement of reticulocyte hemoglobin content can directly reflect the functional availability of iron in that time frame.[20] Reticulocyte hemoglobin content is a reliable and early indicator of bone marrow iron status and may detect functional iron deficiency with more sensitivity than biochemical parameters.[21] Reticulocyte hemoglobin content may optimize IV iron therapy and indicate the efficacy of responses to anemia treatment at an early stage. Although its reduction reflects the impairment of hemoglobin production, reticulocyte hemoglobin content is not the appropriate measure to assess iron adequacy in the presence of genetic microcytosis such as thalassemia.[22,23] Iron-sequestration syndromes occur in chronic diseases when iron is not available for erythropoiesis, due to inappropriately high serum hepcidin values, which determine iron sequestration in reticuloendothelial system macrophages.[24] One of the major determinants of the anemia of chronic disease is iron sequestration.[24–27]

Several studies have assessed the value of reticulocyte hemoglobin in conjunction with other parameters to diagnose iron deficiency states. Other studies have assessed the use of both hepcidin and reticulocyte hemoglobin in ACD. Serum hepcidin was shown not to be clinically useful or superior to more standard iron status tests, for managing iron therapy in HD patients on ESA treatment; reticulocyte hemoglobin content and percentage of hypochromic red blood cells were shown to be more useful, either alone or in combination with the transferrin saturation ratio and ferritin levels.[28–30]

The clinical utility of reticulocyte hemoglobin content has been well established as a reliable marker of functional iron deficiency in hemodialysis patients, exhibiting high specificity and sensitivity in the management of IV iron therapy. In patients with chronic kidney diseases and anemia that are undergoing ESA treatment, repletion of iron stores should be ensured before and during therapy. Iron levels must be adequate to optimize hemoglobin production in a balance with erythropoiesis stimulation.[31] The Kidney Disease Outcomes Quality Initiative (NKF KDOQI)™ of the National Kidney Foundation has provided evidence-based clinical practice guidelines where CHr is considered an appropriate test to assess adequacy of iron for erythropoiesis.[32] In the British Guidelines for Laboratory Diagnosis of Functional Iron Deficiency, CHr is one of the recommended tests with a proposed cut-off value of CHr <29 pg.[33]

The reticulocyte hemoglobin content presents some diagnostic limitations. The reticulocyte hemoglobin content is decreased in thalassemia syndromes, where the reduction in CHr seems to be correlated with the degree of impairment in beta chain synthesis, and in other microcytic anemias due to congenital hemoglobin diseases.[34] It can also be elevated in iron-deficient patients with confounding megaloblastic anemia because of the high mean reticulocyte volume associated with megaloblastosis.[35] Therefore, it is important that CHr values are interpreted in the context of the patient's overall erythrocyte physiology, including knowledge of recent blood transfusions, iron therapy, vitamin B12 or folate deficiency, chemotherapy and the results of hemoglobin analysis. Few studies are available on the clinical utility of reticulocyte cell volume however its usefulness seems to be similar to the reticulocyte hemoglobin content in anemia evaluation and monitoring.[35]

With the introduction of automated methods, it has become mandatory to report the absolute count which gives more accurate information on erythropoiesis than the simple reticulocyte percentage.[36] Automated absolute reticulocyte counts have resulted in phasing out the old fashion "hematocrit correction" of reticulocyte percentage. In addition, the obsolete "reticulocyte production index", that corrected the

reticulocyte count both for Hct and maturation time, can be replaced with IRF, which offers the same clinical significance.

Laboratories should report the reticulocyte count as the absolute number of reticulocytes, accompanied by properly determined and method-specific reference ranges. The percentage value may be optional, but it is still important in monitoring bone marrow response when plasma volume is fluctuating as happens in blood boosting in athletes or in kidney diseases. The clinical utility of reticulocyte cellular parameters such as IRF and reticulocyte hemoglobin content has been proven, while MCVr may be optional, even though it could in some instances provide useful information. It may be useful for laboratories to consider providing an interpretation of the reticulocyte analysis. As an example, if the absolute reticulocyte count and IRF are simultaneously increased, an interpretative comment could be added to emphasize the increase of erythropoietic activity. This comment could help physicians assess cases of suspected hemolytic anemia or in monitoring the treatment of anemia. In conclusion, automated reticulocyte counts provide acceptable precision and bias while parameters and indices improve the evaluation of erythropoiesis. Since a qualitative evaluation is performed with reticulocyte maturation parameters and cellular indices, external quality assessment programs should be provided, and interpretative reporting should be offered to clinicians.[37,38] Nevertheless, standardization and harmonization should be encouraged.

Conflicts of interest

The author declares no conflicts of interest.

REFERENCES

1. Torino AB, Gilberti M de F, da Costa E, de Lima GA, Grotto HZ. Evaluation of red cell and reticulocyte parameters as indicative of iron deficiency in patients with anemia of chronic disease. Rev Bras Hematol Hemoter. 2014;36(6):424–9.
2. Tichelli A, Gratwohl A, Driessen A, Mathys S, Pfefferkorn E, Regenass A, et al. Evaluation of the Sysmex R-1000. An automated reticulocyte analyzer. Am J Clin Pathol. 1990;93(1):70–8.
3. d'Onofrio G, Kim YR, Schulze S, Lorentz T, Dörner K, Goossens W, et al. Evaluation of the Abbott Cell Dyn 4000 automated fluorescent reticulocyte measurements: comparison with manual, FACScan and Sysmex R1000 methods. Clin Lab Haematol. 1997;19(4):253–60.
4. d'Onofrio G, Zini G, Rowan M. Reticulocyte counting: methods and clinical application. In: Rowan MR, van Assendelft OW, Preston FE, editors. Advanced laboratory methods in haematology. Arnold Publisher; 2002. p. 78–126.
5. Sandberg S, Rustad P, Johannesen B, Stølsnes B. Within-subject biological variation of reticulocytes and reticulocyte-derived parameters. Eur J Haematol. 1998;61(1):42–8.
6. Costongs GM, Bas BM, Janson PC, Hermans J, Brombacher PJ, van Wersch JW. Short-term and long-term intra-individual variations and critical differences of haematological laboratory parameters. J Clin Chem Clin Biochem. 1985;23(7):405–10.
7. Tarallo P, Humbert JC, Mahassen P, Fournier B, Henny J. Reticulocytes: biological variations and reference limits. Eur J Haematol. 1994;53(1):11–5.
8. Davis BH. Immature reticulocyte fraction (IFR): by any name, a useful clinical parameter of erythropoietic activity. Lab Hematol. 1996;2:2–8.
9. d'Onofrio G, Tichelli A, Foures C, Theodorsen L. Indicators of haematopoietic recovery after bone marrow transplantation: the role of reticulocyte measurements. Clin Lab Haematol. 1996;18(Suppl 1):45–53.
10. Testa U, Rutella S, Martucci R, Scambia G, D'Onofrio G, Pierelli L, et al. Autologous stem cell transplantation: evaluation of erythropoietic reconstitution by highly fluorescent reticulocyte counts, erythropoietin, soluble transferrin receptors, ferritin, TIBC and iron dosages. Br J Haematol. 1997;96(4):762–75.
11. Torres A, Sánchez J, Lakomsky D, Serrano J, Alvarez MA, Martín C, et al. Assessment of hematologic progenitor engraftment by complete reticulocyte maturation parameters after autologous and allogeneic hematopoietic stem cell transplantation. Haematologica. 2001;86(1):24–9.
12. Noronha JF, De Souza CA, Vigorito AC, Aranha FJ, Zulli R, Miranda EC, et al. Immature reticulocytes as an early predictor of engraftment in autologous and allogeneic bone marrow transplantation. Clin Lab Haematol. 2003;25(1):47–54.
13. Dunlop LC, Cohen J, Harvey M, Gallo J, Motum P, Rosenfeld D. The immature reticulocyte fraction: a negative predictor of the harvesting of CD34 cells for autologous peripheral blood stem cell transplantation. Clin Lab Haematol. 2006;28(4):245–7.
14. Torres Gomez A, Casaño J, Sánchez J, Madrigal E, Blanco F, Alvarez MA. Utility of reticulocyte maturation parameters in the differential diagnosis of macrocytic anemias. Clin Lab Haematol. 2003;25(5):283–8.
15. Lesesve JF, Daliphard S, Callat MP, Lenormand B. Increase of immature reticulocyte fraction in myelodysplastic syndromes. Clin Lab Haematol. 2004;26(4):301–2.
16. NCCLS/ICSH methods for reticulocyte counting (flow cytometry and supravital dyes); approved guideline. 2004 NCCLS Document H44-A 2 Wayne, PA, USA.
17. Brugnara C, Laufer MR, Friedman AJ, Bridges K, Platt O. Reticulocyte hemoglobin content (CHr): early indicator of iron deficiency and response to therapy. Blood. 1994;83(10):3100–1.
18. Brugnara C, Hipp MJ, Irving PJ, Lathrop H, Lee PA, Minchello EM, et al. Automated reticulocyte counting and measurement of reticulocyte cellular indices Evaluation of the Miles H*3 blood analyzer. Am J Clin Pathol. 1994;102(5):623–32.
19. Piva E, Brugnara C, Spolaore F, Plebani M. Clinical utility of reticulocyte parameters. Clin Lab Med. 2014, http://dx.doi.org/10.1016/j.cll.2014.10.004.
20. Brugnara C. A hematologic gold standard for iron-deficient states? Clin Chem. 2002;48(7):981–2.
21. Brugnara C. Iron deficiency and erythropoiesis: new diagnostic approaches. Clin Chem. 2003;49(10):1573–8.
22. Mast AE, Blinder MA, Lu Q, Flax S, Dietzen DJ. Clinical utility of the reticulocyte hemoglobin content in the diagnosis of iron deficiency. Blood. 2002;99(4):1489–91.
23. Brugnara C, Adamson J, Auerbach M, Kane R, Macdougall I, Mast A. Iron deficiency: what are the future trends in diagnostics and therapeutics? Clin Chem. 2013;59(5):740–5.
24. Goodnough LT, Nemeth E, Ganz T. Detection, evaluation, and management of iron-restricted erythropoiesis. Blood. 2010;116(23):4754–61.
25. Goodnough LT. Iron deficiency syndromes and iron-restricted erythropoiesis. Transfusion. 2012;52(7):1584–92.
26. Roy CN. Anemia of inflammation. Hematol Am Soc Hematol Educ Prog. 2010;2010(1):276–80.

27. Sun CC, Vaja V, Babitt JL, Lin HY. Targeting the hepcidin–ferroportin axis to develop new treatment strategies for anemia of chronic disease and anemia of inflammation. Am J Hematol. 2012;87(4):392–400.
28. Tessitore N, Girelli D, Campostrini N, Bedogna V, Pietro Solero G, Castagna A, et al. Hepcidin is not useful as a biomarker for iron needs in haemodialysis patients on maintenance erythropoiesis-stimulating agents. Nephrol Dial Transplant. 2010;25(12):3996–4002.
29. Ford BA, Eby CS, Scott MG, Coyne DW. Intra-individual variability in serum hepcidin precludes its use as a marker of iron status in hemodialysis patients. Kidney Int. 2010;78(8):769–73.
30. Thomas C 1, Kobold U, Balan S, Roeddiger R, Thomas L. Serum hepcidin-25 may replace the ferritin index in the Thomas plot in assessing iron status in anemic patients. Int J Lab Hematol. 2011;33(2):187–93.
31. Wish JB. Assessing iron status: beyond serum ferritin and transferrin saturation. Clin J Am Soc Nephrol. 2006; 1(Suppl 1):S4–8.
32. KDIGO clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease. Kidney Int Suppl. 2012;2(4):279–335. Available from: http://www.kidney.org/professionals/kdoqi/guidelines_anemia/cpr12.htm
33. Thomas DW, Hinchliffe RF, Briggs C, Macdougall IC, Littlewood T, Cavill I, et al. British Committee for Standards in Haematology Guideline for the laboratory diagnosis of functional iron deficiency. Br J Haematol. 2013;161(5):639–48.
34. Skarmoutsou C, Papassotiriou I, Traeger-Synodinos J, Stamou H, Ladis V, Metaxotou-Mavrommati A, et al. Erythroid bone marrow activity and red cell hemoglobinization in iron sufficient beta-thalassemia heterozygotes as reflected by soluble transferrin receptor and reticulocyte hemoglobin in content. Correlation with genotypes and Hb A(2) levels. Haematologica. 2003;88(6):631–6.
35. Brugnara C, Mohandas N. Red cell indices in classification and treatment of anemias: from M.M Wintrobes's original 1934 classification to the third millennium. Curr Opin Hematol. 2013;20(3):222–30.
36. Cappelletti P, Biasioli B, Bulian P, Buttarello M, Cenci M, da Rin AG, et al. Linee guida per il referto ematologico; 2002. Available from: http://www.sipmel.it/download/100124-documento.pdf
37. Briggs C. Quality counts: new parameters in blood cell counting. Int J Lab Hematol. 2009;31:277–97.
38. Buttarello M, Plebani M. Automated blood cell counts: state of the art. Am J Clin Pathol. 2008;130(1):104–16.