Value of immature reticulocyte fraction in anemias and other hematologic conditions: a brief narrative review

Valor da fração de reticulócitos imaturos em anemias e outras condições hematológicas: uma breve revisão narrativa

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Abstract
New hematological parameters, such as immature reticulocyte fraction (IRF), tend to become important tools in clinical practice. IRF identifies the most immature reticulocytes that contain a large amount of ribonucleic acid, being an important parameter to evaluate bone marrow activity in real time for differential diagnosis of anemias, monitoring of its treatment, and for follow-up or bone marrow recovery in various clinical conditions. However, there is still a long way to go before IRF can be used in clinical practice. Thus, it is urgent to establish reference values and to standardize of the methodologies used by different hematological analyzers and how to express the results. This narrative review provides a critical perspective on IRF, its potential of clinical use and limitations.

Keywords
Reticulocytes; Iron-Deficiency Anemia; Hemolytic Anemia; Bone Marrow; Thalassemia

Anemias are a serious public health problem in the world due to their high prevalence and close relationship with the development of children, mainly in the cognitive, motor, social, emotional and neurophysiological areas.[1-3] Although, anemias present varied etiopathogenesis can share common clinical manifestations. Thus, identification of laboratory abnormalities is essential for the differential diagnosis, treatment and follow-up of this disease.[1]

The blood count corresponds to the first laboratory test for the identification and initial direction of the anemia investigation.[4] According to the World Health Organization (WHO), anemia is a condition in which hemoglobin concentration in the blood is below the reference values, being 13 g/dL and 12 g/dL for men and women, respectively.[3] After the identification of anemia, erythrocyte parameters such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) aid a search of the possible causal mechanisms. In addition, they are the basis for the morphological classification including normochromic and normocytic, microcytic and hypochromic, and macrocytic anemias.[5]

Considering that microcytic anemia is the most commonly encountered anemia in general medical practice, mainly in children and adolescents, expanding the possibilities of laboratory tests for their differential diagnosis is of great importance.[6] The types of anemia with these morphological characteristics include iron deficiency anemia, thalassemias, anemia of chronic disease and sideroblastic anemia.[7]

Erythrocyte parameters, and both reticulocyte count and indices are also considered very useful tools in the differential diagnosis of anemia and its treatment.[8] Reticulocytes, described by the first time by Wilhelm Henrich in

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1865 as “Granulated” erythrocytes are immature red blood cells (RBC) with varying amounts of RNA[9] and may indicate whether anemia has aregenerative pattern and whether the bone marrow is responding to treatment.[9-11]

Automated reticulocyte counting was made possible by methods based on detection of fluorescence or change in absorbance. Various fluorochromes such as polimetidine, acridine orange and orange thiazole, and dyes such as oxazine 750 and new methylene blue are used according to the type of equipment. As reported previously, flow cytometric analysis of reticulocytes is an attractive alternative procedure because of its higher reproducibility when compared to microscopic enumeration method.[12]

By means of automation and the intensity of emitted fluorescence or light absorption, reticulocytes can be identified and classified into three fractions based on their degree of maturation.[13] Thus, the types I (high fluorescence or light absorption), II (medium fluorescence or light absorption) and III (low fluorescence or light absorption) have been introduced. Also, from the automation features, new reticulocyte indices were introduced for volume (MCVr), hemoglobin content (MCHr) and hemoglobin concentration (MCHCr), as well as the volume dispersion measures (RDWr) and hemoglobin content (CHr) of reticulocytes.[13]

Reticulocytes are known to circulate a maximum of 4 days in the blood until they become fully mature erythrocytes, while the erythrocytes live approximately 120 days in the circulation. In light of this knowledge, changes in the volume and content of reticulocytes are much faster compared to RBC.[13] This characteristic of reticulocytes enables them both for differential diagnosis and for investigation of response to anemia treatment by means of calculating the new indexes previously mentioned.

The reticulocyte count is also clinically important for the pathophysiological classification of anemia. If a decreased number of reticulocytes is found, this indicates an inadequate production of erythrocytes by the bone marrow. On the other hand, an increase in reticulocyte count indicates destruction of erythrocytes or an excessive blood loss.[5]

Several studies have been published in recent years[8,9,14-16] about the immature reticulocyte fraction (IRF), the younger fraction of reticulocytes, especially for the differential diagnosis of anemias, monitoring of its treatment, and for follow-up or bone marrow recovery in various clinical conditions.

IRF: DEFINITION AND MEASUREMENT

The availability of fluorochromes and dyes that bind to RNA, together with the advancement of technology, allowed the automated reticulocyte counting and new parameters derived from it, such as RMI (reticulocyte maturity index), HFR (high fluorescence reticulocytes) and IRF.[13, 17]

The term IRF was introduced to indicate the more immature reticulocytes containing high amounts of RNA.[18] The maturity of the reticulocytes is based on the amount of RNA traces present in the cell, which can be stained with a fluorescent dye specific for nucleic acids as polymethin or others. According to the intensity of fluorescence emitted, reticulocytes are classified as low (LFR), medium (MFR) or high (HFR) fluorescence. The IRF corresponds to the sum of the medium and high fluorescence fractions [(HFR + MFR) x100] and is able to provide real-time information on the quality of erythropoiesis. On the other hand, the RMI corresponds to [(HFR + MFR) x100]/LFR.[9,13,19]

According to Buttarello,[8] IRF and reticulocyte count can be considered as an index of acceleration and a quantitative measure of the effectiveness of erythropoiesis, respectively. Moreover, these parameters are useful for discriminating those anemias characterized by an increase in erythropoiesis (hemolytic anemias) or loss of blood resulting in an increase in both total reticulocyte and in IRF; anemias consequent to the reduced bone marrow production (chronic renal disease) with both parameters decreased and, finally, those anemias provoked by acute infections or myelodysplasic syndromes in which occur a dissociation between total reticulocyte count (reduced or normal) and IRF (can be increased). Still, accordinxg to the same author,[8] the increase in IRF precedes in several days the increase in reticulocyte count during replacement of cobalamin, folates and iron for the treatment of nutritional anemias.

In this sense, studies have investigated the association between IRF and the diagnosis of anemia, in an attempt to better understand the ability of this parameter to reflect erythropoietic activity.[17,20,21] However, results have been conflicting and despite the relative ease of obtaining the IRF, its use in clinical practice is still limited due to the lack of establishment of reference value, standardization of the methodologies used by hematological analyzers and knowledge of significant clinical outcomes.[8]

Thus, the objective of the present study was to provide a comprehensive and critical review about the clinical usefulness of IRF in the differential diagnosis of anemias, in addition to its potentialities of use and limitations. As a timely complementation, some background was also included on the possible clinical utility of IRF for evaluating hematological recovery in many clinical conditions.

SEARCH STRATEGY AND SELECTION CRITERIA

An electronic search for original or review articles published in English and Portuguese was conducted without date limit, through Medline Ovid, Embase, Web of Science and LILACS/Scielo databases. The search was performed using a combination of relevant keywords, such as reticulocyte

maturation, immature reticulocyte fraction, IRF, immature reticulocytes, reticulocyte maturation profile, iron deficiency anemia, microcytic anemia, macrocytic anemia, anemia of chronic disease, anemia of inflammation, thalassemia, beta-thalassemia, alpha-thalassemia, monitoring of erythroid regeneration, bone marrow transplant, hemolytic anemia, medullary aplasia. "OR" and "AND" tools were utilized to combine the words. A total of 47 articles were raised and after careful reading of their titles and abstracts, 9 articles were excluded. The remaining 38 articles in strict association with the objectives of this review were included. In addition to articles, some books were used that included an overview about IRF. The criterion of inclusion of articles was based on the reading of their titles and abstracts, through which the subjects of greatest interest were identified, highlighting the applicability and limitations of the IRF. Articles that did not meet these requirements were excluded.

IRF IN THE DIFFERENTIAL DIAGNOSIS OF IRON DEFICIENCY ANEMIA AND THALASSEMIAS

The differential diagnosis between iron deficiency anemia (IDA), mainly on its mild and moderate forms, and thalassemia on its heterozygous form is very complex as they share laboratory and clinical characteristics. However, differential diagnosis is important to provide a prompt and adequate indication for iron supplementation to the patients with IDA, to avoid unnecessary iron therapy in patients with thalassemia. Also, a correct IDA diagnosis may prevent severe and lethal forms of thalassemic syndromes in the context of pre-nuptial counseling in areas with high prevalence of the disease. In this case, however, blood counts are insufficient and other tests are required, such as hemoglobin electrophoresis, HbA2 and Fetal Hb dosages, serum levels of ferritin and iron and DNA analysis.[1] However, in areas where thalassemia is endemic usually have limited or unavailable health care resources. Thus, introduction of low-cost and easy-to-perform biomarkers are highly desirable in the differential diagnosis of microcytic and hypochromic anemias.[22] The IRF has been shown to be an alternative parameter in the differentiation of these anemias.[19,21,23] In this context, some studies have been addressed as follows.

Urrechaga *et al.*[24] evaluated 383 adult patients including 136 patients with minor β-thalassemia, 121 with mild iron deficiency anemia and 126 patients with severe iron deficiency anemia. The investigators observed a significant difference among the three groups, with a mean IRF estimate of 8.7%, 12.9% and 16.7%, respectively. A significant difference was observed when all groups were compared to the healthy individuals, who had an IRF of 4.4%. Noronha and Grotto[23] also observed a higher mean IRF in patients with severe iron deficiency anemia (22.1%), followed by patients with mild iron deficiency anemia (17.1%) and patients with minor β-thalassemia (13.2%), when compared to the healthy individuals (8.8%). These findings indicate that IRF is increased in the more severe forms of iron deficiency anemias when compared to patients with minor β-thalassemia.

Velasco-Rodriguez *et al.*[21] have found a significantly higher percentage of IRF in patients with iron deficiency anemia (22.3%) when compared to patients with β-thalassemia trait (18.0%), but there was no difference when compared with patients with dβ-thalassemia (18.3%). On the other hand, in a study by Limaand Grotto,[25] the percentage of high fluorescence reticulocytes was not significantly different between patients with heterozygous β-thalassemia (7.3%), patients with iron deficiency anemia (6.9%) and control subjects (6.2%). However, it is important to highlight that these authors evaluated only the percentage of high fluorescence reticulocytes, not the sum of high and medium fluorescence reticulocytes as in the other three studies mentioned previously, which may explain the difference in the results.

In iron deficiency anemia, as well as in thalassemia, inefficient erythropoiesis can be observed. Interestingly, individuals with iron deficiency anemia, despite having a suppressed erythropoiesis, presented a higher IRF, suggesting a more intense erythropoietic activity. However, in the three previous studies,[21,23,24] the absolute number of reticulocytes was lower in patients with iron-deficiency anemia compared to those with thalassemia, although the latter had more prone to higher IRF. Therefore, one can speculate that patients with iron deficiency anemia probably behave as in myelodysplastic syndromes in which there is a dissociation between total reticulocyte count and IRF may be observed, that is, a lower absolute number of reticulocytes and a higher IRF. On the other hand, this unexpected event could be explained by the supplementation of iron by the patients resulting in an increase in IRF that precedes in several days the increase of reticulocyte count.

Although iron replacement has not been mentioned by the previously cited investigators, it cannot be ruled out. Another hypothesis is that the higher proportion of immature reticulocytes in iron deficiency anemia, when compared to patients with thalassemia, could be a consequence of a higher mRNA expression of the transferrin soluble receptor (sTfR). In response to the insufficient supply of iron, sTfR synthesis is proportionally increased and may result in an increase of erythroid precursor's levels.[25] The table 1 shows the main characteristics of four studies comparing maturity of reticulocytes between patients with iron deficiency anemia and thalassemia, and Table 2 between iron deficiency anemia and healthy individuals.

Table 1 - Summary of cross-sectional studies comparing the maturity of reticulocytes between iron deficiency anemia and thalassemia.

Design of the study and sample size	Participants' characteristics	Hematologic analyzer and parameter	Main results
Velasco-Rodríguez et al., 2016[21] N = 428 . Spain.	dβ-thalassemia (n = 43) β-thalassemia (n = 179) Iron deficiency anemia (n = 206)	ADVIA 2120 (Siemens/ USA) IRF	dβ–thalassemia: 18.3% β-thalassemia: 18.0% Iron deficiency anemia: 22.3% Significant difference between β-thalassemia and iron deficiency anemia
Urrechaga et al., 2011[24] N = 473. Spain.	β-thalassemia, minor (n = 136) Iron deficiency anemia, mild (n = 121) Iron deficiency anemia, severe (n = 126) Healthy individuals (n = 90)	Sysmex XE-5000 (Sysmex/ Japan) IRF	β-thalassemia: 8.7% Iron deficiency anemia, mild: 12.9% Iron deficiency anemia, severe: 16.7% Healthy individuals = 4.4%. Significant difference between healthy individuals and both types of anemia
Noronha and Grotto, 2005[23] N = 108. Brazil.	Iron deficiency anemia, mild (n = 14) Iron deficiency anemia, severe (n = 19) β-thalassemia, minor (n = 25) Healthy individuals (n = 50)	Sysmex XE-2100 (Sysmex/ Japan) IRF	Iron deficiency anemia, severe: 22.1% Iron deficiency anemia, mild: 17.1% β-thalassemia, minor: 13.2% Healthy individuals: 8.8% Significant difference between healthy individuals and both types of anemia and β-thalassemia
Lima and Grotto, 2003[25] N = 149. Brazil.	β-thalassemia, heterozygous (n = 43) Iron deficiency anemia (n = 49) Healthy individuals (n = 57).	Cell-Dyn 3500 (Abbott/ USA) HFR	β-thalassemia, heterozygous: 7.3% Iron deficiency anemia: 6.9% Healthy individuals: 6.2% No significant difference among the groups

HFR: high fluorescence reticulocytes; IRF: immature reticulocytes fraction.

Table 2 - Summary of cross-sectional studies comparing the maturity of reticulocytes between iron deficiency anemia and healthy individuals.

Design of the study and sample size	Participants' characteristics	Hematologic analyzer and parameter	Main results
Canalejo et al., 2011[26] N = 99. Argentina.	Pregnant women ageing between 18 and 40 years without history of disease or prescription of iron.	Cell-Dyn 3700 (Abbott USA) IRF	IRF values showed significant differences: > 0.35 in pregnant women with iron deficiency; < 0.35 in healthy women. Sensibility: 76.1%. Specificity: 53.1%.
Choi and Son, 2005[27] N = 149. South Korea.	Iron deficiency anemia (n = 76), average age: 15.9 ± 1.6; Healthy individuals (n = 73), average age: 16.3 ± 1.4.	Sysmex R-3000 (Sysmex/Japan) IRF	Iron deficiency anemia: 2.10%; Healthy individuals: 1.13%. There was significant difference between the groups.

IRF: immature reticulocytes fraction.

Canalejo *et al.*[26] evaluated 99 pregnant women, aged between 18 and 40 years, during prenatal care, with no history of disease or prescription of supplementary iron. The authors aimed to assess the specificity and sensibility of IRF for the early diagnosis of iron deficiency during pregnancy. The authors observed that the IRF value was different between pregnant women with and without iron deficiency, with 76.1% of sensitivity and 53.1% of specificity, using as cut-off a value of 0.35.

Choi and Son[27] evaluated the IRF of 149 patients and the means of IRF found were 1.13% for healthy individuals and 2.10% for patients with iron deficiency anemia. These studies have demonstrated that IRF started to increase as serum iron levels decreased reaching its peak when the patients had an evident iron deficiency. This finding suggests an increased erythropoietic activity when patients were compared with healthy individuals. In normal erythropoiesis, reticulocytes mature gradually in the peripheral blood losing their RNA. By other side, iron deficiency leads to a restricted synthesis of hemoglobin and to an increase in the production rate of transferrin receptor. The fluorescence intensity of reticulocytes is directly proportional to the amount of intracellular RNA. Therefore, higher emission of MFR and HFR in iron deficiency anemia could be influenced by the amount of RNA of the intracellular transferrin receptors.[25]

IRF IN THE DIAGNOSIS OF THALASSEMIA AND MACROCYTIC ANEMIAS

The diagnosis of thalassemia requires, in addition to the blood count, electrophoresis of hemoglobin, and other complimentary tests, such as molecular, to confirm and differentiate the types of thalassemia. IRF may be useful as a screening parameter since it presents significant differences between healthy individuals and patients with thalassemia; however, it was not effective in differentiating the types of thalassemia.

Butthep *et al.*[28] evaluated reticulocyte maturity in 141 individuals, among them patients with α and β-thalassemia, intermediary thalassemia and healthy individuals. IRF value showed significant increase both in α and β-thalassemia in relation to healthy individuals, but there was no difference between the groups of thalassemia. These results reinforce that active and compensatory erythropoiesis in the bone marrow of patients with thalassemia lead to a greater release of immature reticulocytes in the bloodstream when compared to healthy individuals and is useful to raise a diagnostic suspicion.

Another possible application of IRF would be in the differential diagnosis of macrocytic anaemias including patients with myelodysplastic syndromes, megaloblastic anemia and non-megaloblastic macrocytic anemias. Very high values of IRF (>16) would most likely exclude the diagnosis of non-megaloblastic macrocytic anemia.[29]

IRF IN THE DIFFERENTIAL DIAGNOSIS BETWEEN HEREDITARY SPHEROCYTOSIS AND OTHER HEMOLYTIC ANEMIAS

Differental diagnosis of hereditary hemolytic anemias is complex in mild, moderate and severe cases, and requires more specific complimentary tests, in addition to the blood count. Three studies comparing the maturity of reticulocytes in patients with hereditary spherocytosis (HS) and in patients with other hemolytic anemias were found, and the IRF was shown to be an important parameter in the screening of patients with HS. Table 3 shows the main characteristics of the three studies that compared the maturity of reticulocytes between patients with HS and other hemolytic anemias.

Table 3 - Summary of cross-sectional studies comparing the maturity of reticulocytes between hereditary spherocytosis and other hemolytic anemias.

Design of the study and sample size	Participants' characteristics	Hematologic analyzer and parameter	Main results
Xu et al., 2015[30] n = 370. China.	HS (n = 53); α-thalassemia (n = 55); β-thalassemia (n = 54); Anemia by deficiency of G6PD (n = 56); AIHA (n = 52); Healthy individuals (n = 100).	Beckman-Coulter LH780 (Beckman Coulter /USA) IRF	AIHA: 45.4%; Anemia by deficiency of G6PD: 42.2%; HS: 33.3%; α and β-thalassemias: 30.6%; Healthy individuals: 28.0%. Significant difference among groups..
Lazarova et al., 2014 [31] n = 410. Belgium.	Patients with at least one of the screening tests positive for HS.	Beckman Coulter DxH800 (Beckman Coulter/USA) Ret/ IRF	Ret/IRF ratio showed 92% of sensitivity and 89% of specificity for HS diagnosis based on the reference intervals for healthy individuals determined by authors (0.71 to 2.34 for women, 0.8 to 2.69 for men).
Mullier et al., 2011[32] n = 400. Belgium.	HS (n = 45); Other hemolytic diseases (n = 108); Microcytic anemias (n = 93): iron deficiency (n = 64) and functional iron deficiency (n = 29); Healthy individuals (n = 61).	Sysmex XE-5000 (Sysmex/Japan) Ret/IRF	All 45 confirmed cases of HS had a Ret/IRF ratio higher than 7.7. Hence, this value may be used as reference to track all spherocytosis cases. Among all 45 cases, all considered as mild spherocytosis (Hb > 12 g/dL, n = 12) had a Ret/IRF ratio higher than 19, showing to be a reference value that could be used to track mild HS.
Bobée et al., 2018[15] n = 700. France.	Severe HS (n = 9); Moderate HS (n = 25); HS without anemia (n = 13); β-thalassemia minor (n = 30); Anemia by deficiency of G6PD (n = 23); Anemia by deficiency of PK (n = 17); Sickle cell trait (n = 28); Sickle cell disease (n = 30); Iron deficiency anemia (n = 33); Membranopathies (n = 3); Healthy individuals (n = 489).	Sysmex XE-5000 (Sysmex/Japan) Ret/IRF	Severe HS: 21.6%; Ret/IRF: 13.6 Moderate HS:13.0%; Ret/IRF: 22.8 HS without anemia: 6.5%; Ret/IRF: 37.1 β-thalassemia minor: 10.2%; Ret/IRF: 7.5 Anemia by deficiency of G6PD: 11.6%; Ret/IRF: 9.2 Anemia by deficiency of PK: 16.5%; Ret/IRF: 36.2 Sickle cell trait: 9.6%; Ret/IRF: 7.6 Sickle cell disease: 24.8% Ret/IRF: 9.6 Iron deficiency anemia:19.9% Ret/IRF: 3.3 Membranopathies: 11.7% Ret/IRF: 10.7 Healthy individuals: 13.2% Ret/IRF:7.9 Ret/IRF ratio showed 94.1% of sensitivity and 79.6% of specificity for HS screening.

HS: hereditary spherocytosis; AIHA: autoimmune hemolytic anemia; G6PD: glucose 6 phosphate dehydrogenase; PK: pyruvate kinase; IRF: immature reticulocytes fraction, Ret: reticulocytes absolute count.

Mullier *et al.*[32] evaluated the efficiency of IRF in differentiating HS from other hemolytic anemias and compared this diagnostic tool with the current existent methods. The group with HS was compared to 108 patients with other hemolytic diseases, 93 patients with iron deficiency and 61 healthy individuals. Authors noted that patients with HS had a reticulocytes/IRF ratio higher than 7.7. The authors concluded that this limit could be used as a pre-condition for HS screening. Moreover, all cases of mild HS (Hb > 12g/dL) had a Ret/IRF ratio higher than 19 and, consequently, this value could be used for mild HS screening. The efficacy of the diagnostic tool used to differentiate HS from other hemolytic and iron deficiency anemias was compared to other parameters used in the diagnosis of anemias, obtaining sensibility, specificity, positive and predictive negative values of 100%, 99.3%, 75.0% and 100%, respectively. Thus, Ret/IRF ratio seems to be more efficient in the differential diagnosis among these different anemias when compared to the existing parameters. In addition, Lazarova *et al.*[31] obtained similar results, with the Ret/IRF ratio showing 92% of sensibility and 89% of specificity in the differential diagnosis of HS. Bobée *et al.,*[15] after a series of analyzes, elaborated HS-optimized screening tools, and one of its parameters is reticulocytes/IRF ratio higher than 9.1.

Still concerning to the efficiency of IRF in differentiating HS from other hemolytic anemias, Xu *et al.*[30] observed that the average of IRF was significantly different among the following groups: autoimmune hemolytic anemia – AIHA (45.4%), deficiency of G6PD (42.2%), HS (33.3%), thalassemia (30.6%) and, finally, the group of healthy individuals (28.1%). These differences can be explained by the physiopathology of each disease. AIHA and anemia by deficiency of the G6P enzyme can lead to intense hemolysis, characterized by the premature destruction of erythrocytes as a result of the immunological humoral response or triggered by infections, respectively. In both cases, the bone marrow needs to show sufficient compensatory hyperplasia to try to maintain sufficient blood cells number in the bloodstream.

HS is caused by qualitative and/or quantitative changes in the proteins of erythrocyte membranes, thus it is possible to observe morphological alterations that lead to the reduction of the red blood cells lifespan. The severity of anemia is profoundly variable as it is related to the type of mutation affecting the individual. Authors did not highlight the classification of spherocytosis (mild, moderate or severe) or thalassemia (minor and major for beta; and mild, trace or disease of the hemoglobin H for alpha) of the patients included in the study. Thus, the possibility of existing moderate or mild anemia among the patients may have resulted in a lower IRF, since individuals with mild to moderate hemolysis would not have bone marrow hyperplasia in the same degree of AIHA and anemia due to G6PD deficiency.

IRF FOR EVALUATING HEMATOLOGICAL RECOVERY

Laboratory tests indicating hematological recovery are very useful in certain clinical conditions. Effective erythropoiesis can be assessed and sequentially monitored by quantitative measurement of IRF. In 2006, Luczyński *et al.,*[33] conducted a study to find the most sensitive indicator of anemia among reticulocyte subpopulations assessed by flow cytometry in children with different types of cancer. These authors have found that IRF is not only the first sign of hematologic recovery but also a very strong indicator of postchemotherapy aplasia. In addition, autologous stem cell transplant recipients at risk for infection due to neutropenia (absolute neutrophil count < or = 100/microl) can be identified by IRF several days before neutrophil recovery which reflects erythroid engraftment and hence a recovering marrow.[34] Within this context, another study,[35] reported that IRF was an early predictor of bone marrow recovery post chemotherapy in patients with acute leukemia, compared with absolute neutrophil count (ANC). As advantages, the authors emphasize that IRF is not affected by infection, is easily measured, inexpensive and reliable parameter to evaluate bone marrow regeneration. Another study developed by Rauf *et al.,*[36] reported that the IRF revealed a hematopoietic recovery of the bone marrow of children with acute lymphatic leukemia after chemotherapy earlier than the ANC. Moreover, IRF can be considered as a new tool for hematopoietic evaluation after hematopoietic stem cell transplantation, since this parameter increases before absolute neutrophil count (ANC) and persists increased.[37]

In another study, Gonçalo and col. (2011) evaluated the hematologic recovery of patients after allogeneic hematopoietic progenitor cell transplantation and found that this recovery was detected 4 days earlier by IRF than by neutrophil count (NEU) (11 vs 15 d) and 2 days before the immature platelet fraction (10 vs 12 d). For patients undergoing a nonmyeloablative regimen (NMA), the prediction was even higher for IRF 5 days compared to NEUT (10 vs 15 d).[38]

Since parvo B19 infection may provoke aplastic anemia and that after its treatment high and medium fluorescence reticulocytes appear in the peripheral blood three to five days prior to the peak in absolute reticulocytes, IRF can be an useful tool for assessment of earlier erythroid regeneration.[39] IRF has also been used to evaluate recovery of erythropoiesis after the frequent side effects of antitumor treatment as well as by the disease itself[33] and may also reflect success of the erythroid engraftment and, therefore, a recovered marrow.[34]

Finally, from the clinical point of view, hematological recovery after treatment of hematological and oncological diseases, such as bone marrow transplantation and chemo and radiotherapy may also be evaluated early by IRF, which allows more specific treatment or individualized approach.

IRF: DISCUSSION OF CLINICAL APPLICATIONS AND TECHNICAL LIMITATIONS

Reticulocyte count in manual method has been the assay traditionally used to evaluate the status of erythropoiesis in haematological disorders with disturbances in erythropoietic activity. However, automated reticulocyte count based on flow cytometry has provided much objective and exactly measure of percentage and absolute number of reticulocytes than microscopic method. In addition to being a fast method, evaluation of IRF presents advantage in comparison to other parameters for discriminating anemias due to the precocity with which the information is obtained which represents the bone marrow activity in real time.

In addition to the parameters that describe reticulocyte maturation and potential use in various clinical conditions, other indexes derived from reticulocytes such as MCVr, CHr and MCHCr, have been described. They allow the evaluation of the functional status of erythropoiesis, a useful parameter in the diagnosis and treatment of iron deficiency, in addition to rhEPO therapy.

Studies addressed in this review showed that the evaluation of the IRF assists in the differential diagnosis of anemias, showing more efficiency in iron deficiency anemia on its severe and moderate forms and in hemolytic anemias of greater intensity, such as anemia by deficiency of G6PD and severe hereditary spherocytosis. Moreover, it is an early marker for iron deficiency anemia, since it increases gradually as the iron supplies decrease. IRF is a parameter related to bone marrow activity, that is, it is directly related to the intensity of the erythropoiesis which varies according to the physiopathology of each type of anemia.

However, although there are studies being published on the subject since 1989,[40] limitations still exist. Lack of reference values both for healthy individuals and for individuals with different anemias makes IRF use difficult in clinical practice. Reference values vary among researchers depending on the type of hematological analyzer used and, therefore, diagnostic cutoff are method dependent.[8] For example,an analysis of the data presented in Tables 1, 2 and 3 revealed great variability of the IRF according to the hematological analyzer. For example, for the control group the IRF ranged from <0.35%, using Cell-Dyn 3700 (Abbott Diagnostics, Santa Clara, CA) for healthy women, to 28.0% in healthy subjects, using the LH780 (Beckman Coulter, Miami, FL). Even in Sysmex hematological analyzers (Toa Medical Electronics Co, Kobe, Japan) IRF values ranging from 0.73% (R-3000) to 8.8% (XE 2100) were observed. Likewise, discrepancies among hematological analyzers were also observed for iron deficiency anemia, thalassemias and other anemias. Several hematological analyzers showed very high IRF values for anemia, such as iron deficiency anemia and thalassemias: ADVIA 120 (Bayer, Tarrytown, USA), Sysmex XE 5000 (Toa Medical Electronics Co, Kobe, Japan), Sysmex 2100 (Toa Medical Electronics Co, Kobe, Japan); while others such as Sysmex 3000 (Toa Medical Electronics Co, Kobe, Japan) showed much lower IRF values, which did not differed significantly from healthy controls.

In agreement with Butarello,[8] an analysis of the data presented in Tables 1, 2 and 3 have confirmed that the results are still very discrepant when supplied by different analyzers.This fact implies the urgency of the standardization of method-dependent parameters, since IRF and other more recent RBCs indices appear to be much more sensitive indicators of sudden erythropoietic changes than the conventional parameters.

According to the data presented in the current review, IRF values tended to be higher in patients with severe iron deficiency anemia compared to mild forms. Interestingly, IRF values showed a tendency to increase in patients with iron deficiency anemia in relation to those with thalassemias. Surprisingly results of IRF provided by Cell-Dyn 3500 for beta-thalassemia heterozygous, iron-deficiency anemia and healthy individuals were similar and no significant difference was observed among these groups.

On the basis of the diversity of results found in literature, it should also be noted that standardization on how to express the maturity of reticulocytes among the various hematological analyzers is imperative, i.e. some expressions as IRF, others as RMI and some only the percentage of IRF. Although all parameters are capable of reflecting bone marrow activity, they are obtained differently which may compromise comparability of the results. Another limitation of these indices is the lack of an accredited external quality assessment for such indices as well as internal quality control, which hinders the interpretation and clinical decision making.

However, new reticulocyte parameters are likely to improve the diagnosis and monitoring of many hematological diseases. Different hematological analyzers produce different results for IRF and this is a critical point related to the possible clinical applications. Once this limitation is overcome IRF and other reticulocyte indices certainly will assume an essential role in differential diagnosis of different types of anemia and in the monitoring of their treatment.

As mentioned by Morkis *et al.* 2016,[41] and many others[8,20,42,43] clinical application can only occur when the reference intervals were determined according to the pre-analytical variables (time, temperature, anticoagulants) and standardization of the methodology, that although it has already been pointed out as required nearly two decades ago,[44] it has not yet been realized.

In summary, although IRF is of recognized importance in many clinical situations, caution is required in interpreting the results considering the lack of consistency between data provided by laboratories using different hematological analysers. Thus, the great variability of the IRF values as a

function of the method prevents any comparison of results and the correct interpretation is impaired.

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Resumo

Novos parâmetros hematológicos, como a fração de reticulócitos imaturos (IRF), tendem a se tornar ferramentas importantes na prática clínica. O IRF identifica os reticulócitos mais imaturos, que contêm grande quantidade de ácido ribonucleico, sendo um importante parâmetro para avaliar a atividade da medula óssea, em tempo real, para o diagnóstico diferencial das anemias, acompanhamento do seu tratamento, e para o acompanhamento ou recuperação da medula óssea em diversas condições clínicas. No entanto, ainda há um longo caminho a percorrer antes que a IRF possa ser usada na prática clínica. Assim sendo, é urgente estabelecer os valores de referência e padronizar as metodologias utilizadas pelos diferentes analisadores hematológicos e como expressar seus resultados. Esta revisão narrativa fornece uma perspectiva crítica sobre o IRF e seu potencial para o uso clínico, bem como suas limitações.

Palavras-chave

Reticulócitos; Anemia Ferropriva; Anemia Hemolítica; Medula Óssea; Talassemia

REFERENCES

1. Hoffmann JJML, Urrechaga E, Aguirre U. Microcytaire anemie: thalassemie of ijzergebrek? Een meta-analyse van diagnostische indices. Ned Tijdschr Klin Chem Labgeneesk. 2015;40:213-4.
2. Lozoff B. Iron deficiency and child development. Food Nutr Bull. 2007;28(4 Suppl):S560-71. Epub 2008/02/27.
3. WHO. Worldwide prevalence of anaemia 1993–2005: WHO global database on anaemia. Benoist B, McLean E, Egli I, Cogswell M, editors. Geneva2008.
4. Portaria nº 1247, de 10 de novembro de 2014. Aprova o Protocolo Clínico e Diretrizes Terapêuticas da Anemia por Deficiência de Ferro., (2014).
5. Cascio MJ, DeLoughery TG. Anemia: Evaluation and Diagnostic Tests. Med Clin North Am. 2017;101(2):263-84. Epub 2016/12/08.
6. Iolascon A, De Falco L, Beaumont C. Molecular basis of inherited microcytic anemia due to defects in iron acquisition or heme synthesis. Haematologica. 2009;94(3):395-408. Epub 2009/02/03.
7. Massey AC. Microcytic anemia. Differential diagnosis and management of iron deficiency anemia. Med Clin North Am. 1992;76(3):549-66. Epub 1992/05/01.
8. Buttarello 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. Epub 2016/05/16.
9. Piva E, Brugnara C, Spolaore F, Plebani M. Clinical utility of reticulocyte parameters. Clin Lab Med. 2015;35(1):133-63. Epub 2015/02/14.
10. Wollmann M, Gerzson BM, Schwert V, Figuera RW, Ritzel Gde O. Reticulocyte maturity indices in iron deficiency anemia. Rev Bras Hematol Hemoter. 2014;36(1):25-8. Epub 2014/03/14.
11. Joao AR, Pinto S, E C. Subpopulações dos reticulócitos e fração de reticulócitos imaturos como indicadores de aumento da eritropoese em doentes com anemia por deficiência de ferro. Rev Bras Hematol Hemoter. 2008;30(3):188-92.
12. van Houte AJ, Bartels PC, Schoorl M, Mulder C. Methodology-dependent variations in reticulocyte counts using a manual and two different flow cytometric procedures. Eur J Clin Chem Clin Biochem. 1994;32(11):859-63. Epub 1994/11/01.
13. Oliveira RAG. Hemograma. Como fazer e interpretar. 2ª ed: LMP; 2016. 643 p.
14. Grotto HZ. Platelet and reticulocyte new parameters: why and how to use them? Rev Bras Hematol Hemoter. 2016;38(4):283-4. Epub 2016/11/20.
15. Bobee V, Daliphard S, Schrapp A, Lahary A. Screening of hereditary spherocytosis and pyruvate kinase deficiency by automated blood count using erythrocytic and reticulocytic parameters. Int J Lab Hematol. 2018;40(6):697-703. Epub 2018/09/06.
16. Liao L, Xu Y, Wei H, Qiu Y, Chen W, Huang J, *et al.* Blood cell parameters for screening and diagnosis of hereditary spherocytosis. J Clin Lab Anal. 2019;33(4):e22844. Epub 2019/04/05.
17. 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(2):93-130. Epub 2000/05/16.
18. Chang CC, Kass L. Clinical significance of immature reticulocyte fraction determined by automated reticulocyte counting. Am J Clin Pathol. 1997;108(1):69-73. Epub 1997/07/01.
19. Urrechaga E, Borque L, Escanero JF. Analysis of reticulocyte parameters on the Sysmex XE 5000 and LH 750 analyzers in the diagnosis of inefficient erythropoiesis. Int J Lab Hematol. 2011;33(1):37-44. Epub 2010/05/25.
20. Piva E, Brugnara C, Chiandetti L, Plebani M. Automated reticulocyte counting: state of the art and clinical applications in the evaluation of erythropoiesis. Clin Chem Lab Med. 2010;48(10):1369-80. Epub 2010/07/30.
21. Velasco-Rodriguez D, Alonso-Dominguez JM, Gonzalez-Fernandez FA, Villarrubia J, Sopena M, Abalo L, *et al.* Reticulocyte parameters of delta beta thalassaemia trait, beta thalassaemia trait and iron deficiency anaemia. J Clin Pathol. 2016;69(2):149-54. Epub 2015/08/13.
22. Hoffmann JJ, Urrechaga E, Aguirre U. Discriminant indices for distinguishing thalassemia and iron deficiency in patients with microcytic anemia: a meta-analysis. Clin Chem Lab Med. 2015;53(12):1883-94. Epub 2015/11/05.
23. Noronha JF, Grotto HZ. Measurement of reticulocyte and red blood cell indices in patients with iron deficiency anemia and beta-thalassemia minor. Clin Chem Lab Med. 2005;43(2):195-7. Epub 2005/04/22.
24. Urrechaga E, Borque L, Escanero JF. Erythrocyte and reticulocyte parameters in iron deficiency and thalassemia. J Clin Lab Anal. 2011;25(3):223-8. Epub 2011/05/14.
25. de Lima GA, Grotto HZ. Soluble transferrin receptor and immature reticulocytes are not useful for distinguishing iron-deficiency anemia from heterozygous beta-thalassemia. Sao Paulo Med J. 2003;121(2):90-1. Epub 2003/07/19.
26. Canalejo K, Aixalá M, Casella A, Capanera P, Medina J, Jelen A. Evaluación de la fracción de reticulocitos inmaduros como parámetro de ferropenia en el embarazo. Acta bioquímica clínica latinoamericana. 2011;45:81-5.
27. Choi JW, Son BK. Soluble transferrin receptor concentration is not superior to log ferritin for evaluating erythropoiesis in adolescents with iron deficiency anemia. Clin Chim Acta. 2005;355(1-2):83-9.
28. Butthep P, Wisedpanichkij R, Jindadamrongwech S, Fucharoen S. Elevated erythropoietin and cytokines levels are related to impaired reticulocyte maturation in thalassemic patients. Blood Cells Mol Dis. 2015;54(2):170-6. Epub 2014/12/06.
29. 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.

30. Xu Y, Yang W, Liao L, Deng Z, Qiu Y, Chen W, *et al.* Mean reticulocyte volume: a specific parameter to screen for hereditary spherocytosis. Eur J Haematol. 2016;96(2):170-4. Epub 2015/05/06.
31. Lazarova E, Pradier O, Cotton F, Gulbis B. Automated reticulocyte parameters for hereditary spherocytosis screening. Ann Hematol. 2014;93(11):1809-18. Epub 2014/06/10.
32. Mullier F, Lainey E, Fenneteau O, Da Costa L, Schillinger F, Bailly N, *et al.* Additional erythrocytic and reticulocytic parameters helpful for diagnosis of hereditary spherocytosis: results of a multicentre study. Ann Hematol. 2011;90(7):759-68. Epub 2010/12/22.
33. Luczynski W, Ratomski K, Wysocka J, Krawczuk-Rybak M, Jankiewicz P. Immature reticulocyte fraction (IRF)--an universal marker of hemopoiesis in children with cancer? Adv Med Sci. 2006;51:188-90. Epub 2007/03/16.
34. Grazziutti 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(4):403-9. Epub 2006/01/10.
35. Raja-Sabudin RZ, Othman A, Ahmed-Mohamed KA, Ithnin A, Alauddin H, Alias H, *et al.* Immature reticulocyte fraction is an early predictor of bone marrow recovery post chemotherapy in patients with acute leukemia. Saudi Med J. 2014;35(4):346-9. Epub 2014/04/22.
36. Rauf SE, Khan SA, Ali N, Afridi NK, Haroon M, Arslan A. Immature Reticulocyte Fraction and Absolute Neutrophil Count as Predictor of Hemopoietic Recovery in Patients with Acute Lymphoblastic Leukemia on Remission Induction Chemotherapy. Turk J Haematol. 2016;33(2):131-4. Epub 2015/08/06.
37. Morkis IV, Farias MG, Rigoni LD, Scotti L, Gregianin LJ, Daudt LE, *et al.* Assessment of immature platelet fraction and immature reticulocyte fraction as predictors of engraftment after hematopoietic stem cell transplantation. Int J Lab Hematol. 2015;37(2):259-64. Epub 2014/07/30.
38. Goncalo AP, Barbosa IL, Campilho F, Campos A, Mendes C. Predictive value of immature reticulocyte and platelet fractions in hematopoietic recovery of allograft patients. Transplant Proc. 2011;43(1):241-3. Epub 2011/02/22.
39. Wyrick-Glatzel J, Conway-Klaassen J. Clinical utility of the IRF: assessment of erythroid regeneration following parvo B19 infection. Clin Lab Sci. 2002;15(4):208-12. Epub 2003/06/05.
40. Tsuda I, Tatsumi N. Maturity of reticulocytes in various hematological disorders. Eur J Haematol. 1989;43(3):252-4.
41. Morkis I, Farias M, Scotti L. Determination of reference ranges for immature platelet and reticulocyte fractions and reticulocyte hemoglobin equivalent. Revista Brasileira de Hematologia e Hemoterapia. 2016;38.
42. Brugnara C. Iron deficiency and erythropoiesis: new diagnostic approaches. Clin Chem. 2003;49(10):1573-8. Epub 2003/09/23.
43. Watanabe K, Kawai Y, Takeuchi K, Shimizu N, Iri H, Ikeda Y, *et al*. Reticulocyte maturity as an indicator for estimating qualitative abnormality of erythropoiesis. J Clin Pathol. 1994;47(8):736-9. Epub 1994/08/01.
44. Buttarello M, Bulian P, Farina G, Petris MG, Temporin V, Toffolo L. Five fully automated methods for performing immature reticulocyte fraction: comparison in diagnosis of bone marrow aplasia. Am J Clin Pathol. 2002;117(6):871-9. Epub 2002/06/06.

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