International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Use of Immature Reticulocyte Fraction in Clinical Settings

Vineetha .V[1], Kavitha Thilak[2]

[1]Research Scholar, Srinivas University, Mangalore, Karnataka, India
Orcid-ID: 0009-0006-3288-2409
Email: *vineethavmelel[at]gmail.com*

[2]Research Scholar, Srinivas University, Mangalore, Karnataka, India
Orcid-ID: 0000-0003-0566-4765
Email: *kavithathilak[at]gmail.com*

Abstract: ***IRF is an indicator of erythropoiesis used by automated analyzers. The manual reticulocyte count includes immature and mature reticulocytes while counting all RNA stained cells, takes a lot of time and effort. Reticulocyte analysis by flow cytometry is more accurate and sensitive. The measurement of fluorescence intensity enables the measurement of reticulocyte maturity. IRF provides an understanding of erythropoietic activity in the bone marrow and how it responds to therapy. It is a straight forward, efficient, affordable, repeatable, and trustworthy parameter. It is critical for the assessment of aplastic anaemia in pancytopenia, acute leukaemia patients', post-chemotherapy bone marrow recovery, and for directing stem cell harvest in autologous peripheral blood stem cell transplant. To compare the results from various analyzers, it is essential to get an international agreement on the definition and reference range of IRF. The data was gathered from Google Scholar that was published in various scientific journals. The clinical utility of IRF was evaluated systematically in the research publications. The benefits, drawbacks, and applications of IRF are determined based on review. Numerous studies with promising findings have been evaluated. There is an urgent need for the normal value standardization. Further research in the area will aid to overcoming current difficulties.***

Keywords: Immature reticulocyte fraction (IRF), High-fluorescence reticulocytes (HRF), Medium-fluorescence reticulocytes (MRF), Low-fluorescence reticulocytes (LRF), Reticulocyte haemoglobincontent (CHr)

1. Introduction

The reticulocyte count is helpful for both detecting anaemias and tracking the bone marrow's response to treatment and it indicates the erythropoietic activity of the bone marrow. By simultaneously supplying additional parameters and indicators such the immature reticulocyte fraction, the reticulocyte volume, and the haemoglobin content and concentration, automated flow-cytometric analysis has significantly advanced reticulocyte counting. IRF has been suggested as a potential early engraftment marker. Reticulocyte volume is a helpful indicator when tracking the therapeutic response of anaemias, and reticulocyte haemoglobin content is useful in determining the functional iron available for erythropoiesis [1]. One of the most common adverse effects of anticancer medication is anaemia. After chemo-and radiation, reliable laboratory tests confirming haematological recovery are required. Reticulocyte counts can be used to quantitatively track the efficiency of erythropoiesis. Using flow cytometry, these cells' RNA content may be measured and classified as low, middle, and high-fluorescence reticulocytes (HFR). Their maturation is tied to this dispersion. The HFR fraction is made up of the youngest reticulocytes. The sum of the HFR and MFR fractions is known as the immature reticulocyte fraction (IRF) [2, 3]. Depending on the type of equipment, different fluorochromes including acridine orange, and thiazole orange, as well as dyes like oxazine 750 and new methylene blue, are utilised. The improved reproducibility of flow cytometric examination of reticulocytes makes it a desirable alternative option to microscopic enumeration [4, 5]. Reticulocyte volume and content changes occur significantly more quickly than those of RBC [6]. The immature reticulocyte fraction (IRF), has been the subject of numerous research in recent years, particularly for the differential diagnosis of anaemias, monitoring of it's therapy, and for follow-up or bone marrow recovery in various clinical circumstances [7, 8].

The more immature reticulocytes with elevated RNA concentrations were referred to as IRF. The amount of RNA traces in the cell, which may be stained with a fluorescent dye specifically made for nucleic acids, determines the maturity of the reticulocytes. Reticulocytes are categorized as having low (LFR), medium (MFR), or high fluorescence (HFR) depending on the strength of the fluorescence they release. The IRF can offer real-time data on the quality of erythropoiesis and is equal to the sum of the medium and high fluorescence fractions [(HFR + MFR) x100]. [9, 10].

In order to better understand how well this metric can reflect erythropoietic activity, investigations have looked into the relationship between IRF and the diagnosis of anaemia. Despite the relative ease of getting the IRF, results have been contradictory, and its application in clinical practice is currently restricted because there is no established reference value, no method of standardization for haematological analyzers, and little understanding of major clinical effects [11, 12, 13]. Therefore, the goal of the current study was to give a thorough and critical analysis of the clinical use of IRF in the differential diagnosis of anaemias, as well as its application possibilities and constraints. A brief history on the potential clinical value of IRF for assessing haematological recovery in numerous clinical situations was also added as a relevant complement in this study.

Licensed Under Creative Commons Attribution CC BY

2. Literature Survey

Sl No	Research Area	Outcome	References
1.	IRF in iron deficiency anaemia and thalassemia's differential diagnosis	Assessed 383 adult individuals, including 126 patients with severe iron deficiency anaemia, 121 patients with mild-thalassemia, and 136 patients with minor-thalassemia. With a mean IRF estimate of 8.7%, 12.9%, and 16.7%, respectively, the researchers found a significant difference between the three groups.	Urrechaga *et al. [14]*
2.	IRF in iron deficiency anaemia and thalassemia's differential diagnosis	When compared to healthy people (8.8%), patients with severe iron deficiency anaemia had a higher mean IRF (22.1%), followed by patients with mild iron deficiency anaemia (17.1%), and patients with minor-thalassemia (13.2%). These results suggest that when compared to patients with moderate-thalassemia, IRF is elevated in the more severe forms of iron deficient anaemia.	Noronha et al. [15]
3.	IRF in iron deficiency anaemia and thalassemia's differential diagnosis	Compared to patients with-thalassemia trait (18.0%), patients with iron deficiency anaemia had a considerably greater percentage of IRF (22.3%); however, there was no change when compared to patients with β-thalassemia (18.3%).	Velasco-Rodriguez et al. [16]
4.	IRF in iron deficiency anaemia and thalassemia's differential diagnosis	Patients with heterozygous-thalassemia (7.3%), patients with iron deficiency anaemia (6.9%), and control subjects (6.2%) all had a similar percentage of high fluorescence reticulocytes.	LimaandGrottoet al. [17]
5.	IRF in iron deficiency anaemia and thalassemia's differential diagnosis	99 pregnant women between the ages of 18 and 40 who were getting prenatal care and had no medical background or prescriptions for extra iron were evaluated. The authors looked at this to assess the specificity and sensitivity of IRF for the early identification of iron deficiency during pregnancy. The authors discovered that the IRF value changed between pregnant women with and without iron insufficiency, with 76.1% sensitivity and 53.1% specificity, using a cut-off value of 0.35.	Canalejo et al. [18]
6.	IRF in iron deficiency anaemia and thalassemia's differential diagnosis	149 subjects experienced IRF, with healthy subjects' IRF averaging 1.13% and patients with iron deficiency anemia's IRF averaging 2.10%. Several investigations have demonstrated that IRF increased as serum iron levels dropped and peaked in patients with a pronounced iron deficiency. This finding suggested a higher level of erythropoietic activity in patients as compared to healthy individuals. During typical erythropoiesis, reticulocytes gradually mature in the peripheral circulation and lose their RNA as they do so. Reduced haemoglobin synthesis and enhanced transferrin receptor development are the effects of iron deficiency. The amount of intracellular RNA is inversely associated with reticulocyte fluorescence.	Choi and Son et al. [19]
7.	IRFin thalassemia and macrocytic anaemias diagnosis	141 people—including patients with thalassemia, intermediate thalassemia, and healthy individuals—were tested for reticulocyte maturity. In comparison to healthy persons, the IRF value revealed a considerable rise in both and-thalassemia, but there was no difference between the thalassemia groups. These findings support that thalassemia patients active and compensatory erythropoiesis causes a larger discharge of immature reticulocytes into the bloodstream as compared to healthy people, and that this finding might be used to raise a diagnostic suspicion.	Butthep et al. [20]
8.	IRFin thalassemia and macrocytic anaemias diagnosis	Individuals with myelodysplastic syndromes, megaloblasticanaemia, and non-megaloblastic macrocytic anaemias are included in the differential diagnosis of macrocytic anaemias. Non-megaloblastic macrocytic anaemia would likely not be diagnosed at very high IRF values (>16).	Torres Gomwz A et al. [21]
9.	IRF in the diagnosis of heridatory spherocytosis and other haemolytic anemias	The scientists found that among people with HS, the ratio of reticulocytes to IRF was greater than 7.7. The authors came to the conclusion that using this limit as a precondition would allow for the use of HS screening. Also, in all mild HS cases (Hb> 12g/dL), the Ret/IRF ratio was higher than 19, suggesting that this metric may be used for mild HS screening. Sensitivity, specificity, positive and predictive negative values of the diagnostic test used to differentiate HS from other hemolytic and iron deficiency anaemias were 100%, 99.3%, 75.0%, and 100%, respectively, when compared to other criteria used in the diagnosis of anaemias. Moreover, the Ret/IRF ratio seems to be more effective in the differential than the existing values.	Mullier et al. [22]
10.	IRF in the diagnosis of heridatory spherocytosis and other haemolytic anemias	Autoimmune hemolytic anaemia-AIHA (45.4%), deficiency of G6PD (42.2%), HS (33.3%), thalassemia (30.6%), and, finally, the group of healthy individuals (28.1%), were shown to have significantly different average IRF levels. The physiopathology of each disease can be used to account for these variances. Intense hemolysis, which is defined by the early destruction of erythrocytes as a result of the immunological humoral response or brought on by infections, can develop from both AIHA and anaemia caused by a G6P enzyme shortage. In both situations, the bone marrow must exhibit enough compensatory hyperplasia in an effort to keep the bloodstream's blood cell count at a healthy level.	Xu et al. [23]
11.	IRF used as a sign for the hematologic recovery	Discovered that IRF is a very robust indication of postchemotherapy aplasia in addition to being the first evidence of hematologic recovery.	Luczyński et al. [24]
12.	IRF used as a sign for the hematologic recovery	Children with acute lymphatic leukaemia had a haematological recovery of the bone marrow earlier with the IRF than with the ANC.	Rauf et al. [25]

Licensed Under Creative Commons Attribution CC BY

13.	IRF used as a sign for the hematologic recovery	After hematopoietic stem cell transplantation, IRF can be thought of as a novel tool for hematopoietic evaluation because this parameter rises before the absolute neutrophil count (ANC) and stays elevated.	Morkis IV et al. [26]
14.	IRF used as a sign for the hematologic recovery	IRF was shown to be 4 days sooner than neutrophil count and 2 days before the immature platelet fraction in detecting the hematologic recovery of individuals after allogeneic hematopoietic progenitor cell transplantation.	Gonçalo et al. (2011) [27]

3. Methodology

The most recent information acquired from a range of secondary sources, including published literature from numerous scholarly publications, was used to produce the literature evaluation. Relevant articles were selected by an automated search on Google Scholar. To better comprehend the clinical utility of immature reticulocyte fraction, only recent secondary sources were looked at for literature evaluation.

The following section reviews

1) IRF in iron deficiency anaemia and thalassemias differential diagnosis
2) IRF in thalassemia and macrocytic anaemias diagnosis
3) IRF in the diagnosis of heriditory spherocytosis and other haemolyticanaemias
4) Other clinical applications and limitations of immature reticulocyte fraction

4. Results and Discussion

Clinical utilities of immature reticulocyte fraction from recent literatures were reviewed in this literature review. Based our study, we have summarized that:-

Reticulocyte count in manual method is the assay traditionally used to assess the status of erythropoiesis in haematological illnesses with changes in erythropoietic activity. Compared to the microscopic method, the automated reticulocyte count based on flow cytometry has generated a far more precise and objective measurement of the proportion and total quantity of reticulocytes. IRF evaluation is not only a quick procedure, but it also has an advantage over other parameters for differentiating anaemias due to the speed with which the information is obtained, which shows the bone marrow activity in real time.

Research included in this time of this study that the assessment of the IRF aids in the clinical presentation of anaemias, with better efficacy in hemolytic anaemias of high intensities, like as anaemia caused by a G6PD deficiency and severe hereditary spherocytosis. Also, because it progressively increases as the iron supplies decline, it serves as an early indicator of iron deficiency anaemia. IRF is a metric that relates to bone marrow activity, which means that it is directly related to that level of activity. The intensity of erythropoiesis varies depending on the pathological processes of each type of anaemia.

In addition to the parameters that reflect reticulocyte maturation, a number of indices obtained from reticulocytes, including MCVr, CHr (Reticulocyte haemoglobin content), and MCHCr, have been described and may have applications in a range of clinical diseases. They enable the evaluation of the functional status of erythropoiesis, which is essential for the diagnosis and treatment of iron deficiency, in addition to rhEPO (Recombinant Human Erythropoietin) therapy.

Although studies on the topic have been published since 1989, there are still certain limitations. IRF application in clinical practise is challenging because to the lack of reference values for both healthy people and people with various anaemias. The type of haematological analyzer employed by each researcher affects reference values, hence diagnostic cutoff is method-dependent [26, 27, 28].

In individuals with severe iron deficiency anaemia compared to mild forms, IRF values tended to be greater, according to the findings reported in the current review. Intriguingly, patients with iron deficient anaemia had an increased tendency for IRF levels compared to those with thalassemias. Unexpectedly, the IRF values reported by Cell-Dyn 3500 for heterozygous beta-thalassemia, iron deficiency anaemia, and healthy people were comparable, and no difference was found among these groups.

It should be highlighted that consistency on how to express the maturity of reticulocytes among the many haematological analyzers is essential based on the diversity of results reported in the literature, i.e., some expressions as IRF, some as RMI (Reticulocyte maturity index), and others merely the percentage of IRF. Although these measures are capable of indicating bone marrow activity, their diverse methods of acquisition may make it difficult to compare the outcomes. Lack of a certified external quality assessment for these indices as well as internal quality control is another drawback of these indices, which makes it difficult to comprehend and make therapeutic decisions.

New reticulocyte measures, however, are probably going to help with many haematological illnesses' monitoring and diagnosis. IRF values from different haematological analyzers vary, and this is an important distinction when considering potential clinical uses. IRF and other reticulocyte indices will unquestionably play a crucial part in the monitoring of the treatment for different kinds of anaemia once this limitation is solved.

Clinical application can only take place if the reference intervals have been established using the pre-analytical variables (time, temperature, anticoagulants), which, while being suggested as necessary nearly two decades ago, has not yet been realised [29, 30, 31].

5. Conclusion

In conclusion, although IRF is important in many clinical settings, care must be taken when interpreting the findings

Licensed Under Creative Commons Attribution CC BY

due to the inconsistent data supplied by laboratories employing various haematological analyzers. Because of this, it is impossible to compare the results, and the proper interpretation is hampered by the high variability of the IRF values as a function of the approach

6. Future Scope

The recent application of IRF as a prognostic tool for various diseases is one of the recent innovation, most of its scientific parts are still untouched. Some clinical applications of IRF remain unclear. Even though IRF provides fresh insight into the diagnosis and prognosis of a wide variety of diseases at very fast and at a low cost. In order to provide better treatment strategies for the patients it's very much suggested to implement the usage of IRF as a modern prognostic tool.

References

[1] Elisa Piva, Carlo Brugnara, Federica Spolaore & Mario Plebani. (2014). Clinical Utility of Reticulocyte Parameters. Clinics in laboratory medicine, 35 (3), 133-163.
[2] Choi JW, Pai SH. Reticulocyte subpopulations and reticulocyte maturity index (RMI) rise as body iron status falls. Am J Hematol, 2001; 67: 130-5.
[3] Turowski D, Wysocka J, Butkiewicz AM. Peripheral blood reticulocytes and their reference range values for percentage, absolute count, and immature fraction in children, measured with flow cytometry. Folia HistochemCytobiol, 2000; 38: 31-6.
[4] Hoffmann JJML, Urrechaga E, Aguirre U. Microcytaireanemie: thalassemie of ijzergebrek? Eenmeta-analyse van diagnostische indices. Ned TijdschrKlinChemLabgeneesk. 2015; 40: 213-4.
[5] Lozoff B. Iron deficiency and child development. Food Nutri Bull. 2007; 28 (4 Suppl): S560-71. Epub 2008/02/27.
[6] Oliveira RAG. Blood count. How to do and interpret. 2nd ed: LMP; 2016. 643 p.
[7] Oliveira RAG. Blood count. How to do and interpret. 2nd ed: LMP; 2016. 643 p.
[8] 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.
[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] 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 05/25/2010.
[11] 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.
[12] Piva E, Brugnara C, Chiandetti L, Plebani M. Automated reticulocyte counting: state of the art and clinical applications in the evaluation of erythropoiesis. ClinChem Lab Med. 2010; 48 (10): 1369-80. Epub 2010/07/30.
[13] 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 anemia. J ClinPathol. 2016; 69 (2): 149-54. Epub 2015/08/13.
[14] 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.
[15] Noronha JF, Grotto HZ. Measurement of reticulocyte and red blood cell indices in patients with iron deficiency anemia and beta-thalassemia minor. ClinChem Lab Med. 2005; 43 (2): 195-7. Epub 2005/04/22.
[16] 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 anemia. J ClinPathol. 2016; 69 (2): 149-54. Epub 2015/08/13.
[17] 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.
[18] Canalejo K, Aixalá M, Casella A, Capanera P, Medina J, Jelen A. Evaluation of the fraction of inmature reticulocytes as a parameter of iron deficiency in embarrassment. Acta biochemistry latinamerican clinic. 2011; 45: 81-5.
[19] Choi JW, Son BK. Soluble transferrin receptor concentration is not superior to log ferritin for evaluating erythropoiesis in adolescents with iron deficiency anemia. ClinChimActa. 2005; 355 (1-2): 83-9.
[20] 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.
[21] Torres Gomez A, Casaño J, Sanchez 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.
[22] 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.
[23] 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.
[24] 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.

Licensed Under Creative Commons Attribution CC BY

[25] 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.
[26] 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.
[27] 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.
[28] Tsuda I, Tatsumi N. Maturity of reticulocytes in various hematological disorders. Eur J Haematol. 1989; 43 (3): 252-4.
[29] Morkis I, Farias M, Scotti L. Determination of reference ranges for immature platelet and reticulocyte fractions and reticulocyte hemoglobin equivalent. Brazilian Journal of Hematology and Hemotherapy. 2016; 38.
[30] Brugnara C. Iron deficiency and erythropoiesis: new diagnostic approaches. Clin Chem. 2003; 49 (10): 1573-8. Epub 2003/09/23.
[31] 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 ClinPathol. 1994; 47 (8): 736-9. Epub 1994/08/01.

Author Profile

Vineetha V, Assiatant Professor, Muthoot College of Allied health sciences, Kozhencherry, Pathanamthitta, Orcid-ID: 0009-0006-3288-2409: E-mail: vineethavmelel[at]gmail.com

Kavitha Thilak, Professor, Head of the Department - Medical Laboratory Technology, Al - Ameen College of Medical Sciences, Orcid-ID: 0000-0003-0566-4765: E-mail: kavithathilak[at]gmail.com

Licensed Under Creative Commons Attribution CC BY