Scholars Journal of Medical Case Reports
Abbreviated Key Title: Sch J Med Case Rep
ISSN 2347-9507 (Print) | ISSN 2347-6559 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Hematology

Interest of the Parameter 'Immature Reticulocyte Fraction (IRF)' in the Diagnostic Approach and the Follow-Up of the Anemies: Experience of the Laboratory of the Haematology Service of the Military Hospital Avicenne in Marrakech

Dr. Aziki Mehdi[1*], Dr. Zakaria Faraji[1], Dr. Sara El Malihi[1], Pr. Mohamed Chakour[1], A. Raissi[2]

[1,2]Hematology Department; Avicenne Military Hospital, Marrakech, Morocco

DOI: https://doi.org/10.36347/sjmcr.2025.v13i08.020 | **Received:** 29.05.2025 | **Accepted:** 23.07.2025 | **Published:** 12.08.2025

***Corresponding author:** Dr. Aziki Mehdi
Hematology Department; Avicenne Military Hospital, Marrakech, Morocco

Abstract | **Original Research Article**

Anemia is a frequent pathology requiring precise evaluation to guide management. The aim of this retrospective study, carried out on 94 patients in the haematology laboratory of the Avicenne Military Hospital in Marrakech (over 12 months), was to assess the diagnostic value of IRF (Immature Reticulocyte Fraction) in distinguishing between regenerative and aregenerative anaemias, by comparing it with the reticulocyte count. IRF, measured by the Sysmex XN-1500, reflects the rate of release of RNA-rich immature reticulocytes, marking the erythropoietic activity of the bone marrow. The results showed a significant positive correlation between RFID and reticulocytes ($r = 0.515$; $p < 0.001$), as well as excellent sensitivity (94.4%) and specificity of 61.8% at a cut-off of 15%.ROC curve analysis confirmed the performance of IRF (AUC = 0.840), and the Kappa concordance coefficient (0.778) between IRF at T0 and reticulocytes at T48 validated its ability to detect a bone marrow response early.Of the 94 cases, 75.5% presented with anaregenerative anaemia and 24.5% with regenerative anaemia. Patients with regenerative anaemia had a mean RFI of 25%, compared with 12.94% in patients with aregenative anaemia ($p<0.05$). ***Conclusion:*** RFID appears to be a rapid, reliable and non-invasive automated haematological tool for the differential diagnosis of anaemia. Its ability to reflect the erythropoietic activity of the anaemia patients is a major advantage.

Keywords: Anemia - Diagnosis - RFID - Immature reticulocytes.

INTRODUCTION

Assessment of erythropoietic activity is an essential step in the diagnosis and monitoring of anaemia. The reticulocyte count, a direct reflection of the bone marrow's capacity to produce new red blood cells, is central to this assessment. In this context, the Immature Reticulocyte Fraction (IRF) has emerged as an innovative parameter, made accessible thanks to technological advances in automated haematology analysers [1]. Initially referred to as the reticulocyte maturity index, IRF is now the internationally accepted term for quantifying the proportion of young reticulocytes.

Based on RNA content measured by fluorescence intensity or light scatter, RFI distinguishes three subpopulations: LFR (Low Fluorescence Reticulocytes), MFR (Medium Fluorescence Reticulocytes) and HFR (High Fluorescence Reticulocytes) [2]. IRF corresponds to the sum of maturing and immature reticulocytes (HFR + MFR), and is expressed as a fraction from 0.00 to 1.00 [1,3].

It reflects the proportion of young cells with a high RNA content, larger cell size and higher light scatter [4]. Clinically, RFI is a sensitive and reliable measure of erythropoiesis [5]. Combined with the reticulocyte count, it allows effective monitoring of bone marrow activity [6], which is useful for differentiating between regenerative anaemias and anaemia.

The objectives of our study were to:

- Propose a new 'IRF' parameter for the diagnosis and monitoring of anaemia;
- Study its performance;
- Compare it with the reticulocyte parameter (correlation study).

MATERIALS AND METHODS

1. Type, location and duration of the study

This was a prospective study carried out in the haematology laboratory of the Avicenne Military Hospital in Marrakech, over a period of 6 months, from January to June 2024. The laboratory is equipped with three automated cyto-haematology machines, three automated haemostasis machines, a centrifuge and a GeneXpert molecular biology system. Technicians receive samples and carry out internal quality control prior to any analysis.

2. Data collection

Clinical, sociodemographic and biological data were collected from the medical records of patients referred to the laboratory, focusing specifically on haematological parameters relevant to the study.

3. Data entry and statistical analysis

Data were entered using Microsoft Word and Excel. Statistical analysis, including the construction of an ROC curve, was carried out using SPSS software. The Student's T-test was used to compare the means, with a significance threshold set at $p < 0.05$.

4. Diagnostic procedures

The analyses were carried out in three phases

- Pre-analytical phase: fasting peripheral venous blood sample taken on an EDTA tube, identified and transported rapidly to the laboratory at room temperature.
- Analytical phase: analyses carried out using the Sysmex XN-1500 automated system, allowing automated measurement of 29 haematological parameters.
- Post-analytical phase: validation and clinical interpretation of the results obtained.

4.1. Blood count

The blood count performed by the Sysmex XN-1500 automated system includes the complete blood count (CBC) and the blood smear. Erythrocyte parameters analysed included haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin content (MCHC), as well as reticulocyte count, mean fraction (MFR), high fraction (HFR) and IRF. The reference ranges are those provided by the manufacturer (Sysmex Corporation).

Figure 1: Sysmex XN-1500 machine used to perform CBC in the laboratory

4.2 Measurement of IRF :

The immature reticulocyte fraction (IRF) was assessed by flow fluorocytometry using the Sysmex XN-1500. After permeabilisation of the reticulocyte membranes, fluorescent dyes (polymethine and ozazine) penetrated the cells to label the intracellular RNA. Cell size (FSC) and fluorescence intensity (SFL) are measured using a semiconductor diode laser. Two-dimensional scattergrams allow reticulocytes to be classified into LFR, MFR and HFR subpopulations. The IRF corresponds to the sum of the MFR and HFR fractions, representing the most immature reticulocytes. This automated method offers a standardised, rapid result in less than a minute.

4.3. Diagnostic performance of IRF :

The diagnostic performance evaluated is as follows:

- Sensitivity: 94.4% [95% CI: 0.898-0.990].
- Specificity: 61.8% [95% CI: 0.521-0.715].
- Positive predictive value (PPV): 49.1% [95% CI: 0.390-0.590].

- Negative predictive value (NPV): 96.5% [95% CI: 0.930-1.000].

RESULTS

1. Demographic profile

During the study period from 22 December 2022 to 22 December 2023, we included 94 patients with anaemia. Of these, 55.3% were men and 44.7% were women, with a male/female sex ratio of 1.24. The mean age was 55.59 years (range 5-87 years), with a predominance of the 61-80 age group (45.75%).

2. Clinical profile

The majority of patients came from outpatient clinics (61.70%) and the clinical haematology department (19.15%). The other patients came from various medical departments.

In terms of anaemia type, normocytic normochromic anaemia predominated (52.13%), followed by microcytic hypochromic anaemia (38.30%) and macrocytic anaemia (9.57%).

3. Biological profile

Biological analysis revealed an overall mean RFI of 16.35 (standard deviation: 8.55; extremes: 3.4 to 44.5) and a mean reticulocyte count of 8.66% (standard deviation: 5.41).

The mean FRIs according to the type of anaemia were:

- Microcytic hypochromic anaemia: 19.06 ± 7.95
- Normocytic normochromic anaemia: 14.87 ± 8.84
- Macrocytic anaemia: 12.24 ± 7.67

The comparative study between regenerative and aregenative anaemias showed a significant difference ($p<0.05$), with a mean RFI of 25.23 ± 8.73 for regenerative anaemias, compared with 14.59 ± 7.60 for aregenative anaemias.

4. **Correlation between RFI and reticulocyte count**

The correlation between RFI and reticulocyte count was positive, moderate to strong, and statistically highly significant, with a Spearman correlation coefficient of 0.659 ($p<0.001$).

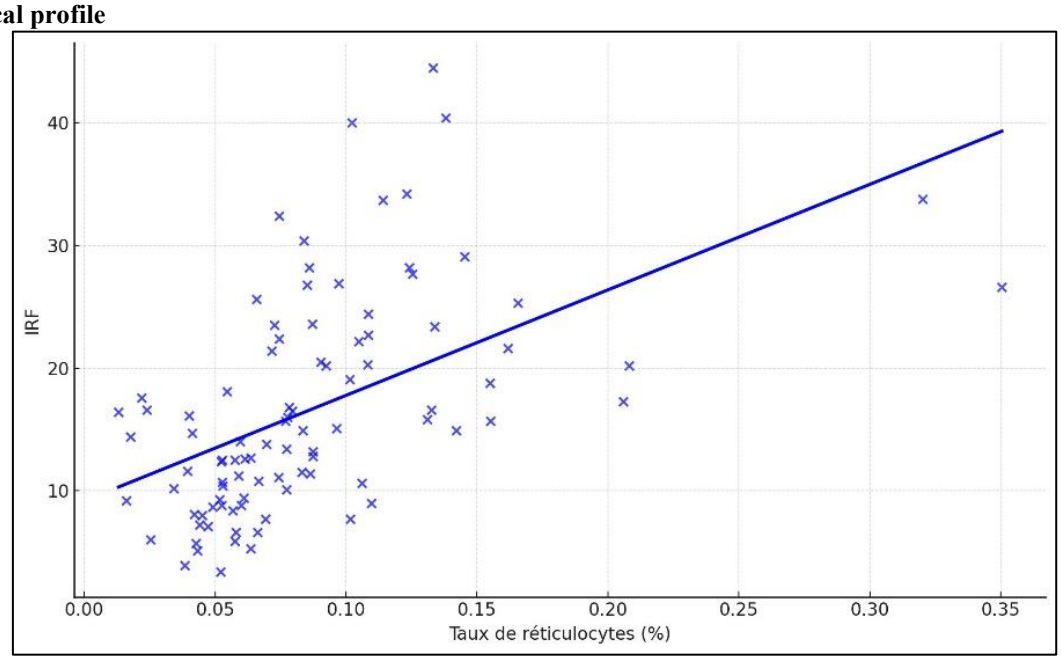

Figure: Correlation between IRF and reticulocyte count

5. IRF cut-off

The ROC curve established an optimal IRF cut-off of 15% to distinguish regenerative from aregenerative anaemias, with a sensitivity of 94.4% and a specificity of 61.8%. The area under the curve (AUC) was 0.840, indicating good diagnostic ability.

6. Predictive power of IRF

A concordance study between initial IRF and reticulocyte count after 48 hours in 36 patients showed substantial agreement (Kappa coefficient: 0.778; $p<0.001$), confirming the strong predictive power of IRF in assessing bone marrow regeneration in anaemias.

DISCUSSION

Our prospective study, which included 94 patients with anaemia over a period of one year, analysed the clinical relevance of IRF and reticulocyte count in the differential diagnosis of anaemia.

Immature reticulocytes, characterised by a high RNA content, are released into the peripheral blood during intense stimulation of erythropoiesis, particularly in cases of haemorrhage, certain anaemias or treatments that stimulate bone marrow activity [5]. IRF, the ratio of

immature reticulocytes to total reticulocytes, is a sensitive parameter of erythropoiesis, measured by automated haematology analysers [5].

The overall mean IRF was 16.35% (± 8.55), in line with the literature [3,4]. For microcytic hypochromic anaemias, the mean RFI was 19.06% (± 7.95), similar to that reported by Sedick *et al.,* (19.58%) [7]. However, other studies, such as those by Tanweer *et al.,* (14 ± 4.3%) (42) and Bansari *et al.,* (9.6 ± 5.8%) [8], show lower means, possibly explained by methodological, ethnic or sample size differences. The high mean in our study probably reflects efficient bone marrow regeneration in response to easy and well-known treatments for iron deficiency anaemia [5,9].

The mean reticulocyte count in this group (0.1011) was lower than in other studies (Sedick: 1.76; Tanweer: 1.1; Bansari: 0.95) [7,8,10]. This could be explained by reduced or inefficient erythropoiesis typical of certain iron deficiency anaemias or chronic diseases, with a dissociation between a high IRF and a low total reticulocyte count [5,11].

In macrocytic anaemias, our mean IRF (12.24 ± 7.67) is significantly lower than that found by Jonali *et al.,* (52 ± 12) [12]. These differences could be due to various causes of macrocytic anaemia in our population (vitamin deficiencies or bone marrow aplasias), conditions known to inhibit bone marrow activity, giving rise to a low immature fraction (<10%) (25). The mean reticulocyte count was also lower in our series (0.11) compared with Jonali *et al.,* (0.81), reinforcing the idea of interpopulation and aetiological variability [12].

The significant positive correlation observed between RFI and reticulocyte count ($r=0.659$; $p<0.001$) confirms the clinical utility of RFI as a reliable indicator of bone marrow regeneration, particularly in differentiating between regenerative and aregenerative anaemias.

Our ROC analysis determined an optimal RFI threshold of 15% (sensitivity: 94.4%, specificity: 61.8%), which is in line with thresholds reported in the literature, in particular those in the series by Sedick (15.15%), Chakma (15.85%) and Chang (23%) [7,13,14]. This threshold value for IRF is an effective way of distinguishing between regenerative and aregenerative forms.

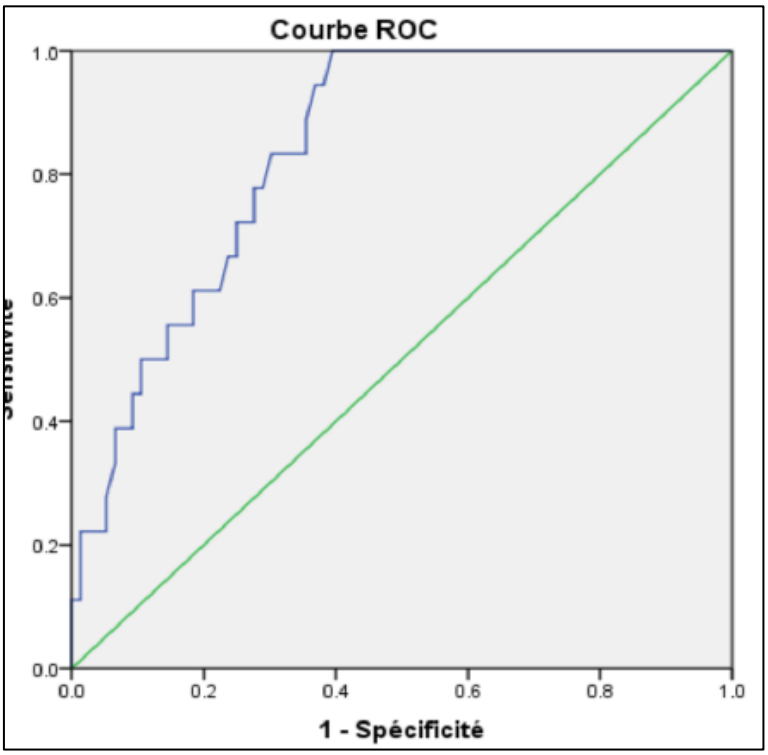

Figure 10: ROC curve showing the sensitivity and specificity of RFID in differentiating between anaemias

Finally, a concordance study between initial IRF and reticulocyte count at 48h in 36 patients revealed a high Kappa coefficient (0.778; $p<0.001$), demonstrating that IRF is a reliable early indicator of bone marrow response [3,15]. This observation corroborates those of Kaplan *et al.,* showing a significant early increase in IRF after iron treatment in iron deficiency anaemia, thus clarifying the predictive role of IRF compared with traditional reticulocyte count [2].

CONCLUSION

Anemias are common and may be of central or peripheral origin. The haemogram and reticulocyte

count, although essential, do not detect bone marrow variations at an early stage. IRF offers a rapid, cost-effective and non-invasive alternative for diagnosing and monitoring anaemia. Our study identified a discriminatory threshold of 15%, with a sensitivity of 94.4% and a specificity of 61.8%, confirming that IRF is an early and reliable indicator of erythropoiesis.

REFERENCES

1. Piva E, Brugnara C, Spolaore F, *et al.,* (2015) Clinical utility of reticulocyte parameters. *Clin Lab Med* 35: 133–163.
2. Sysmex Educational Enhancement and Development. SEED Hématologie. Importance du comptage des réticulocytes.
3. Sah AK, Rao DS, Sah AK, *et al.,* (2024) Clinical Significance of Reticulocytes, *Red Blood Cells - Functions and Significance*, IntechOpen.
4. Briggs C (2009) Quality counts: new parameters in blood cell counting. *Int J Lab Hematol* 31: 277–297.
5. Adane T, Asrie F, Getaneh Z (2021) CLINICAL UTILITY OF IMMATURE RETICULOCYTE FRACTION. *Journal of Clinical Chemistry and Laboratory Medicine* 4: 1–8.
6. d'Onofrio G, Tichelli A, Foures C, *et al.,* (1996) Indicators of haematopoietic recovery after bone marrow transplantation: the role of reticulocyte measurements. *Clin Lab Haematol* 18 Suppl 1: 45–53.
7. Sedick Q, Elyamany G, Hawsawi H, *et al.,* (2021) Diagnostic accuracy of reticulocyte parameters on the sysmex XN 1000 for discriminating iron deficiency anaemia and thalassaemia in Saudi Arabia. *Am J Blood Res* 11: 172–179.
8. Anovadia DB, Trivedi DDP, Savjiyani DD, *et al.,* (2023) Clinical significance of immature reticulocyte fraction (IRF) and reticulocyte maturity indices in differential diagnosis of anemia. *Int J Clin Diagn Pathol* 6: 16–19.
9. Ch. Jyothi , K. Lakshmi Chinmayee et al (2024) Évaluer la pertinence clinique de la fraction réticulocytaire immature (FRI) et indices de maturité des réticulocytes pour distinguer les diverses causes de anémie chez les patients. *Journal international des sciences de la vie, de la biotechnologie et de la recherche pharmaceutique*.
10. Ahmed T, Mahmood A, Uddin N, *et al.,* (2021) DIAGNOSTIC SIGNIFICANCE OF MEASURING RETICULOCYTE MATURITY INDICES IN IRON DEFICIENCY ANAEMIA. *Pakistan Armed Forces Medical Journal* 71: 2109–2113.
11. Mariane Cristina Souza Value of immature reticulocyte fraction in anemias and other hematologic conditions: a brief narrative review - Revista RBAC. Available from: https://www.rbac.org.br/artigos/value-of-immature-reticulocyte-fraction-in-anemias-and-other-hematologic-conditions-brief-narrative-review/.
12. Das J, Khonglah Y, Tiewsoh I, *et al.,* (2022) Utility of reticulocyte indices in the diagnosis of pancytopenia. *J Family Med Prim Care* 11: 1335–1340.
13. Chang CC, Kass L (1997) Clinical significance of immature reticulocyte fraction determined by automated reticulocyte counting. *Am J Clin Pathol* 108: 69–73.
14. L'anémie de la personne âgée | AMUB Available from: https://www.amub-ulb.be/node/1248.
15. Kaplan M, Yılmaz N, Özet G (2018) Yeni tanı demir eksikliği anemisi olan hastalarda bazal immatür retikulosit fraksiyonu (IRF) düzeyi ve demir tedavisine IRF yanıtının değerlendirilmesi. *otd* 10: 431–435.