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New reticulocyte indices in anemia classification

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Abstract

Background: Anaemia is a common clinical condition with diverse etiologies and is traditionally classified using red cell indices such as mean corpuscular volume (MCV) and red cell distribution width (RDW). However, these parameters provide only static morphological information. Newer reticulocyte-derived indices-Mean Reticulocyte Volume (MRV), Immature Reticulocyte Fraction (IRF), and Red Cell Size Factor (RSF)-offer dynamic insights into erythropoietic activity and iron availability. This study aimed to evaluate the diagnostic and interpretive utility of these parameters across different anaemia types.

Methods: A prospective cross-sectional study was conducted in a tertiary care hospital over 24 months. A total of 357 adult patients with anaemia were classified morphologically as microcytic (n=162), normocytic (n=124), or macrocytic (n=71) based on MCV. Complete blood counts and reticulocyte indices were measured using the Beckman Coulter DxH 900 analyzer. Continuous variables were compared using the t-test or Mann-Whitney U test, categorical variables were compared using the chi-square test, and correlations were assessed using Spearman's coefficient. Diagnostic cut-offs were determined by receiver operating characteristic (ROC) analysis.

Results: Microcytic anaemia showed the lowest MRV (109.0 ± 17.6 fL) and RSF (92.6 ± 13.0), while macrocytic anaemia showed the highest MRV (125.9 ± 25.2 fL) and RSF (103.4 ± 28.8). IRF was comparable across groups (0.43-0.46) but reflected marrow activity in regenerative versus suppressed states. RSF emerged as the most discriminative index for differentiating iron deficiency anaemia from thalassemia trait (AUC = 0.89, $p < 0.001$). MRV refined morphological classification, and IRF indicated marrow responsiveness.

Conclusion: Reticulocyte parameters add functional depth to conventional red cell indices, bridging morphology with erythropoietic dynamics. RSF is the most robust discriminator of anaemia type, MRV enhances size-based classification, and IRF serves as a real-time marker of marrow regeneration. Incorporation of these indices into routine haematology reporting can improve diagnostic accuracy and therapeutic monitoring without additional cost.

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Introduction

Anaemia remains one of the most prevalent global health challenges, contributing substantially to morbidity and reduced quality of life worldwide. The Global Burden of Disease (GBD) 2013 analysis estimated that anaemia accounted for over 68 million years lived with disability, with iron deficiency as the leading cause, followed by anaemia of chronic disease and hemoglobinopathies (1). Updated GBD data from 1990 to 2021 demonstrate only modest global improvement despite advances in nutrition and infection control, with the highest burden persisting in South Asia and sub-Saharan Africa (2). Increasingly, anaemia is recognized as a multifactorial disorder influenced by nutritional deficiencies, inflammation, and genetic factors (3,4). Nevertheless, routine diagnostic evaluation continues to rely largely on conventional red cell indices that provide static morphological information with limited insight into dynamic erythropoiesis and iron utilization (5,6).

Morphological classification into microcytic, normocytic, and macrocytic anaemia remains central to the diagnostic workup; however, substantial overlap exists between categories. Early iron deficiency may present as normocytic anaemia, while mixed deficiencies can produce dual red cell populations, limiting the interpretive value of static indices alone. Red cell distribution width (RDW) enhances sensitivity for detecting anisocytosis but lacks specificity for etiological differentiation (7). These limitations underscore the need for physiologically relevant parameters that reflect real-time marrow activity and iron availability.

Reticulocyte parameters generated by modern automated analysers provide such functional insight by linking red cell morphology with erythropoietic kinetics. Reticulocytes, as the youngest circulating erythrocytes, reflect ongoing marrow activity and iron incorporation (6,8). Among these, Mean Reticulocyte Volume (MRV), Immature Reticulocyte Fraction (IRF), and Red Cell Size Factor (RSF) have emerged as valuable indices for refining anaemia classification (6,9).

MRV reflects the average size of reticulocytes and serves as an indicator of effective erythropoiesis. Reduced MRV is characteristic of iron-restricted erythropoiesis, while elevated values suggest megaloblastic or regenerative states. Hoffmann et al. demonstrated a significant reduction in MRV in microcytic states, which correlated closely with reticulocyte hemoglobin content (5,9). MRV has been shown to complement MCV by distinguishing uniform microcytosis in thalassemia trait from heterogeneous microcytosis in iron deficiency anaemia (IDA) (10,11) and has diagnostic relevance in early cancer-related anaemia, in which morphological changes may lag behind functional impairment (8).

IRF quantifies the most immature reticulocyte population and reflects the marrow regenerative response. Unlike absolute reticulocyte counts, IRF rises rapidly following erythropoietin stimulation and precedes haemoglobin recovery after therapy (5,12). Elevated IRF is observed in acute blood loss, haemolysis, or in response to iron therapy, whereas persistently low values indicate ineffective erythropoiesis in chronic inflammation or renal disease (11,13,14). Although IRF alone may not differentiate anaemia types at a single time point, it provides valuable longitudinal information on treatment response (15).

RSF, a composite index derived from mean red cell and reticulocyte volumes, dynamically quantifies anisocytosis more effectively than RDW (16,17). Studies by Urrechaga et al. have demonstrated its sensitivity in detecting iron-restricted erythropoiesis in both IDA and anaemia of chronic disease (16,18). Elevated RSF reflects heterogeneous red cell populations seen in IDA or mixed deficiencies, while lower values suggest uniform populations typical of thalassemia trait or marrow suppression (11,19). RSF thus integrates mature and immature erythrocyte characteristics to provide mechanistic insight into anaemia type and stage.

Macrocytic anaemias present additional diagnostic complexity because of their etiological heterogeneity. Although conventional indices often overlap, reticulocyte parameters provide distinctive patterns: markedly elevated MRV and RSF are typical of megaloblastic anaemia, whereas moderate MRV elevation with normal RSF suggests non-megaloblastic causes such as liver disease or alcohol-related macrocytosis (15,20,21). Similarly, in normocytic anaemia, subtle changes in MRV and RSF may reveal early nutritional deficiency, marrow suppression, or haemolytic recovery (22,23).

The International Council for Standardization in Haematology (ICSH) has endorsed the incorporation of reticulocyte indices into routine reporting and quality assurance, emphasizing their diagnostic and monitoring utility (14,24,25). These parameters have been validated across diverse clinical settings, including iron deficiency, renal anaemia, myelodysplastic syndromes, pregnancy-related anaemia, and functional iron deficiency (26-30). Despite this evidence, their routine use remains limited, particularly in resource-constrained settings where anaemia burden is high but advanced diagnostics are underutilized (1,4,11). Importantly, these indices are generated without additional reagents or cost, making them suitable for widespread adoption when interpreted alongside conventional markers such as ferritin, transferrin saturation, and RDW.

The present study aims to evaluate the diagnostic utility of reticulocyte-derived parameters-MRV, IRF, and RSF-in the classification of microcytic, normocytic, and macrocytic anaemias and to compare their performance with conventional red cell indices. Specifically, the study analyses the distribution of these parameters across morphological anaemia categories, assesses their relationship with RDW and standard indices, and explores interpretive combinations of MRV and RSF to distinguish iron deficiency anaemia from thalassemia trait. The study also evaluates IRF as a marker of marrow regenerative activity. Through this analysis, the study aims to clarify the incremental diagnostic value of reticulocyte parameters and support their integration into routine anaemia evaluation and monitoring.

Methods

Study design and setting

After approval from the institutional ethics committee, this prospective, cross-sectional observational study was conducted in the Department of Pathology at a tertiary care teaching hospital in western India over a 24-month period (January 2023-December 2024). The study included consecutive adult patients (≥18 years) presenting with anaemia, defined as haemoglobin (Hb) <13 g/dL in males and <12 g/dL in females, according to WHO criteria (1,4,7).

Exclusion criteria

Patients who had received a blood transfusion within the preceding three months, those undergoing active chemotherapy, and patients whose samples exhibited analytical interference (Cold agglutinins, hemolysis, or lipemia) were excluded to avoid artefactual variation in reticulocyte parameters (14,16).

Laboratory evaluation

Venous blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes and analyzed within two hours of collection to minimize pre-analytical variation. All samples were processed using a fully automated Beckman Coulter DxH 900 hematology analyzer (Beckman Coulter, USA), which employs advanced impedance and flow cytometric principles for red cell and reticulocyte analysis. Quality assurance was maintained through manufacturer-recommended calibrators, daily internal quality control, and participation in external proficiency programs.

Conventional red cell indices measured included haemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and RDW. In addition to these standard parameters, novel reticulocyte-derived indices were recorded to evaluate erythropoietic activity and red cell size heterogeneity. These included MRV, representing the average size of circulating reticulocytes (fL); the IRF, indicating the proportion of the most immature reticulocytes and reflecting bone marrow regenerative activity (Unitless); and the RSF, a dimensionless composite index automatically derived from the relationship between reticulocyte and erythrocyte volumes (5,8,16).

Anaemia was morphologically classified based on mean corpuscular volume (MCV) as microcytic (<80 fL), normocytic (80-100 fL), or macrocytic (>100 fL), in accordance with established diagnostic criteria (6,17). To assess the degree of anisocytosis and its functional implications, each morphological group was further stratified by RDW into normal RDW (11.5-14.5%) and high RDW (>14.5%) categories. This dual stratification enabled correlation of reticulocyte indices with traditional red cell parameters, facilitating a more nuanced understanding of the underlying erythropoietic dynamics across different anaemia types.

Statistical analysis

Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages. Intergroup comparisons of continuous data were performed using Student's t-test for normally distributed variables and the Mann-Whitney U test for non-parametric data, whereas categorical variables were analyzed using the chi-square (χ^2) test. Correlations between RDW and the reticulocyte parameters MRV, IRF, and RSF were assessed using Spearman's rank correlation coefficient (ρ) to account for potential non-linearity.

To evaluate the diagnostic performance of these indices in differentiating anaemia subtypes, receiver operating characteristic (ROC) curve analysis was conducted. The area under the curve (AUC) was used to quantify overall diagnostic accuracy, and optimal cut-off values were determined using the Youden Index, maximizing the sum of sensitivity and specificity (13,31). All statistical tests were two-tailed, and a p-value <0.05 was considered statistically significant.

Results

Baseline characteristics

A total of 357 anaemic patients were analyzed: 162 (45.4%) microcytic, 124 (34.7%) normocytic, and 71 (19.9%) macrocytic. The mean age was 55.5 ± 15.7 years, with a male predominance (57%). Conventional red cell indices varied significantly across groups (p <0.0001), confirming morphological distinctions.

Figure 1 shows the distribution of the 192 patients according to their recorded clinical diagnoses. Cardiovascular disease and hypertension constituted prominent clinical categories, followed by malignancies, infections, and renal and hepatic disorders. The distribution reflects the heterogeneous clinical settings in which anaemia occurred and provides context for interpreting variation in reticulocyte parameters across the morphologically defined anaemia groups.

Microcytic anaemia exhibited the lowest Hb and HCT values, macrocytic anaemia had the highest MCV and RDW, and normocytic anaemia showed intermediate values, consistent with previous population-based analyses (1,4,17,32).

Microcytic anaemia (n = 162)

The mean MRV was 109.0 ± 17.6 fL, IRF 0.46 ± 0.13, and RSF 92.6 ± 13.0.

MRV: Values <100 fL were characteristic of IDA, reflecting small reticulocytes due to restricted hemoglobinization (10,11,29). MRV >115 fL suggested thalassemia trait or combined nutritional deficiencies (Iron + B12/Folate), in which reticulocytes remained relatively larger despite microcytosis.

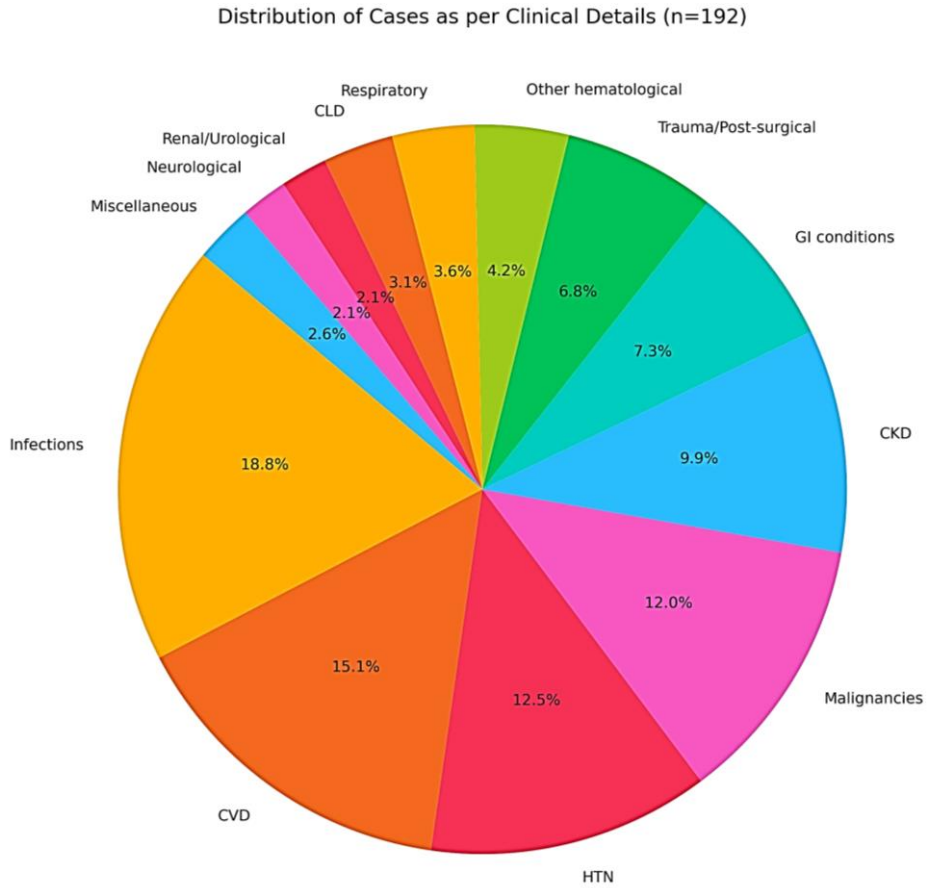

Figure 1. Distribution of cases as per clinical diagnosis (n=192)
CVD: Cardiovascular Disease, HTN: Hypertension, CKD: Chronic Kidney Disease, CLD: Chronic Liver Disease

IRF: Although not discriminatory across subtypes, higher IRF indicated an active marrow response, typical of IDA with blood loss or treatment response, whereas lower IRF suggested marrow suppression, as observed in anaemia of chronic disease (3,4,12,16).

RSF: The mean RSF was the lowest among all anaemia groups. RSF <90 was typical of thalassemia minor, denoting uniform microcytosis, whereas higher RSF values (>95-100) reflected anisocytosis in IDA, corresponding to mixed populations of small RBCs and relatively larger reticulocytes (10,16,18).

RDW Stratification

Patients with high RDW exhibited significantly elevated MRV, IRF, and RSF ($p < 0.05$ for all), consistent with anisocytosis driven by variable red cell populations. In contrast, thalassemia trait cases with normal RDW had low MRV and uniformly low RSF (9,11,16).

Normocytic anaemia (n = 124)

The mean MRV was 112.1 ± 11.7 fL, IRF 0.43 ± 0.14, and RSF 99.0 ± 7.2.

MRV: Slightly higher MRV values indicated early macrocytic processes or reticulocytosis, while lower MRV denoted iron-restricted erythropoiesis not yet manifesting as microcytosis (6,11,22).

IRF: Higher IRF corresponded to regenerative states such as haemolysis or blood loss, while low IRF indicated marrow suppression due to renal disease or chronic inflammation (4,13,14).

RSF: Moderate RSF (≈99 ± 7.2) reflected partial anisocytosis. High RSF suggested evolving iron deficiency or mixed nutritional states, whereas low RSF was observed in uniform marrow suppression (Chronic kidney disease, aplastic anaemia) (27,31,33).

Correlation analysis

RDW showed positive correlations with MRV ($r = 0.39$, $p < 0.01$), IRF ($r = 0.28$, $p < 0.05$), and RSF ($r = 0.36$, $p < 0.01$). These findings confirmed that anisocytosis in normocytic cases paralleled marrow regenerative activity, as previously reported by Lippi and Favaloro (7).

Macrocytic anaemia (n = 71)

The macrocytic group exhibited the highest MRV (125.9 ± 25.2 fL) and RSF (103.4 ± 28.8), with IRF averaging 0.46 ± 0.16.

MRV: Very high MRV (>130-140 fL) indicated megaloblastic anaemia (B12/Folate deficiency), while moderately elevated MRV (115-125 fL) suggested hemolytic or recovery states, consistent with previous observations by Toki et al. and Urrechaga et al. (15,22).

IRF: High IRF reflected regenerative macrocytosis (e.g., hemolysis or therapy response), while low IRF alongside high MRV denoted ineffective erythropoiesis typical of megaloblastic states (15,20).

RSF: RSF showed the widest variation across all groups (103.4 ± 28.8). High RSF with high MRV suggested pronounced anisocytosis characteristic of megaloblastic anaemia, whereas moderate RSF elevations occurred in alcohol-related or drug-induced macrocytosis. Near-normal RSF with high MRV indicated non-megaloblastic macrocytosis such as liver disease or myelodysplastic syndromes (19,21,23).

Table 1 summarizes the distribution of the newer reticulocyte parameters across microcytic, normocytic, and macrocytic anaemia. MRV showed a progressive increase from microcytic to normocytic and macrocytic anaemia (109.0 ± 17.6, 112.1 ± 11.7, and 125.9 ± 25.2 fL, respectively), while RSF similarly increased across the groups (92.6 ± 13.0, 99.0 ± 7.2, and 103.4 ± 28.8, respectively). IRF showed relatively similar mean values across the three morphological categories (0.46 ± 0.13, 0.43 ± 0.14, and 0.46 ± 0.16). These findings suggest that MRV and RSF may provide additional information on erythropoietic characteristics beyond the morphological classification established by conventional red cell indices, whereas IRF appears to reflect a relatively consistent reticulocyte response across the morphological subtypes.

Table 1. Summary of comparative findings

Parameter	Microcytic	Normocytic	Macrocytic
MRV (fL)	109.0 ± 17.6	112.1 ± 11.7	125.9 ± 25.2
IRF	0.46 ± 0.13	0.43 ± 0.14	0.46 ± 0.16
RSF	92.6 ± 13.0	99.0 ± 7.2	103.4 ± 28.8

RDW stratification

All macrocytic cases had high RDW, but patterns of MRV and RSF effectively distinguished subtypes:

- Very high MRV + RSF, low IRF: Megaloblastic anaemia.
- Moderate MRV + high IRF: Regenerative macrocytosis (Hemolysis, treatment recovery).
- High MRV + normal RSF: Non-megaloblastic causes (Alcohol, liver disease, MDS).

Figure 2 demonstrates the distribution of MRV, IRF, and RSF across microcytic, normocytic, and macrocytic anaemia after stratification by RDW. MRV and RSF generally showed higher values and wider distributions in the high-RDW subgroups, with the most pronounced values observed in macrocytic anaemia, whereas IRF demonstrated increased variability in patients with high RDW. Overall, the findings suggest that elevated RDW is associated with greater heterogeneity in reticulocyte parameters, providing additional information on anisocytosis and erythropoietic activity.

Receiver Operating Characteristic (ROC) analysis demonstrated that RSF was the most reliable discriminator among the three parameters, with a sensitivity of 88% and specificity of 84% for differentiating IDA from thalassemia trait (AUC = 0.89, p <0.001). MRV supported classification accuracy in macrocytic states (AUC = 0.81), while IRF showed limited cross-sectional discrimination but strong value in monitoring therapy response, corroborating prior reports by Brugnara et al. and Auerbach et al. (29,30).

Figure 3 illustrates the relative ability of the newer reticulocyte parameters to provide additional information across the morphologically defined anaemia groups. RSF showed the highest overall area under the curve (AUC = 0.89), followed by MRV (AUC = 0.81) and IRF (AUC = 0.73). These findings suggest that RSF and MRV may provide greater additional information on differences in erythropoietic characteristics among morphologically established anaemia subtypes, while IRF showed a comparatively lower degree of separation.

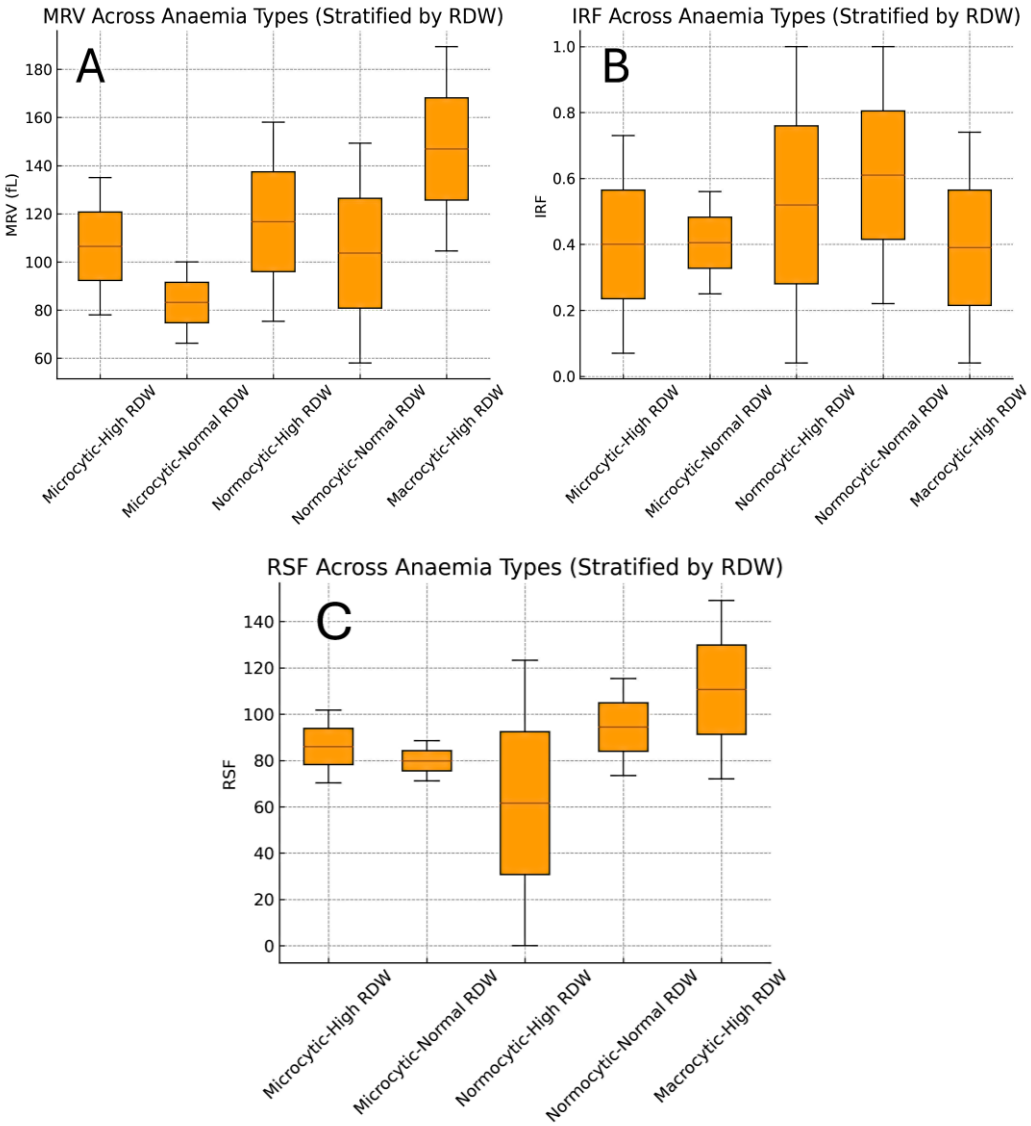

Figure 2. Boxplots showing stratification of newer reticulocyte parameters by RDW across anaemia types

(A) Mean Reticulocyte Volume (MRV) Across Anaemia Types Stratified by RDW

Boxplots illustrate the MRV distribution among microcytic, normocytic, and macrocytic anaemias, further divided into normal- and high-RDW categories. MRV values were lowest in microcytic anaemia with normal RDW and highest in macrocytic anaemia with high RDW, reflecting differences in reticulocyte size and erythropoietic activity.

(B) Immature Reticulocyte Fraction (IRF) Across Anaemia Types Stratified by RDW

IRF demonstrates variability corresponding to marrow responsiveness. Higher IRF values were observed in the high-RDW subgroups of all anaemia types, indicating increased reticulocyte release and regenerative activity, while lower IRF was observed in suppressed erythropoiesis.

(C) Red Cell Size Factor (RSF) Across Anaemia Types Stratified by RDW

RSF showed the greatest discriminative range, increasing progressively from microcytic to macrocytic anaemia. High-RDW subgroups demonstrated markedly higher RSF, reflecting anisocytosis arising from mixed red cell and reticulocyte populations.

Boxplots represent the median, interquartile range, and total range (Whiskers) for MRV, IRF, and RSF across morphological categories of anaemia (Microcytic, normocytic, macrocytic), each stratified by RDW (Normal vs High). The plots highlight that higher RDW is consistently associated with greater variability in reticulocyte parameters, signifying enhanced anisocytosis and dynamic erythropoiesis.

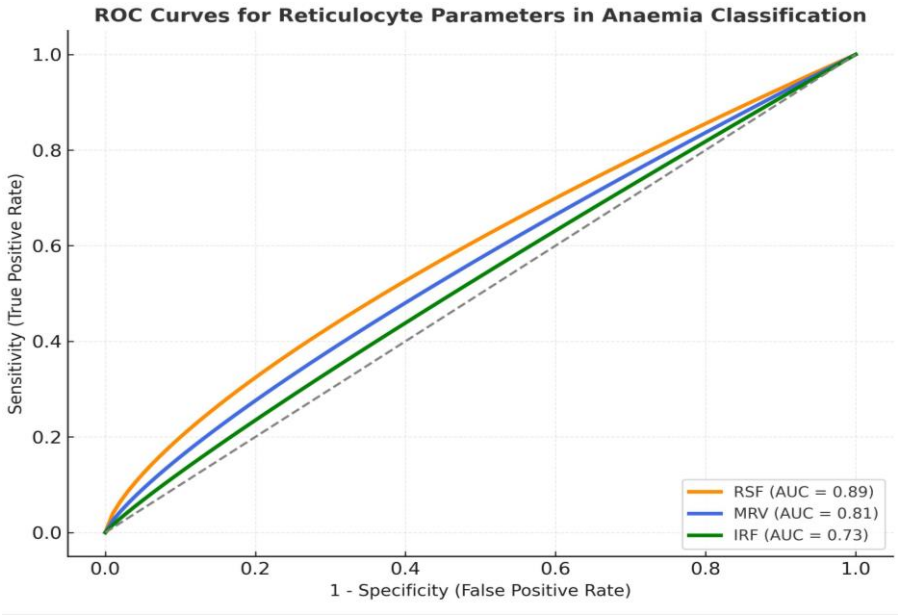

Figure 3. ROC curves for reticulocyte parameters in anaemia classification

Receiver Operating Characteristic (ROC) curves showing the diagnostic performance of RSF, MRV, and IRF in differentiating anaemia subtypes. RSF demonstrated the highest accuracy (AUC = 0.89), followed by MRV (AUC = 0.81) and IRF (AUC = 0.73). The diagonal dashed line represents the reference (AUC = 0.5).

Discussion

We evaluated the clinical utility of newer reticulocyte parameters- MRV, IRF, and RSF-in 357 patients classified as having microcytic, normocytic, or macrocytic anaemia. Conventional indices such as mean corpuscular volume (MCV) and red cell distribution width (RDW) remain the first-line tools for morphological classification. However, reticulocyte-derived indices provide dynamic insights into bone marrow activity, anisocytosis, and the underlying pathophysiology of anaemia beyond static erythrocyte indices.

Interpretation and clinical correlation

This study underscores the diagnostic potential of reticulocyte indices as functional extensions of the RBC morphology paradigm. MRV effectively stratifies anaemia by size, RSF quantifies anisocytosis beyond RDW, and IRF captures marrow response in real time. The observed patterns mirror those reported in multicentric evaluations by Urrechaga, Piva, and Brugnara (10,12,29).

By integrating these indices, clinicians can differentiate between thalassemia trait and iron deficiency without additional iron studies, detect mixed deficiencies early, and monitor treatment response noninvasively. As shown by Hoffmann and Urrechaga, automated reticulocyte indices thus bridge the gap between traditional morphology and functional haematology (9,16).

Microcytic anaemia

In patients with microcytic anaemia (N=162), MRV, IRF, and RSF exhibited patterns that highlighted heterogeneity among subtypes. The mean MRV was 109.0 ± 17.6 fL, significantly lower than in the normocytic and macrocytic groups, indicating restricted hemoglobinization and smaller reticulocytes. Values below 100 fL were typical of IDA, while MRV above 115 fL suggested thalassemia trait or combined iron and B12/folate deficiencies, in which larger reticulocytes coexist with small circulating red cells (5,10).

The mean IRF was 0.46 ± 0.13, with higher values observed in regenerative states such as blood loss or early iron deficiency and lower values in anaemia of chronic disease (2,3,14). RSF showed the strongest discriminative power (Mean 92.6 ± 13.0): very low RSF (<90) indicated uniformly small cells typical of thalassemia minor, whereas higher RSF (>95-100) reflected anisocytosis in IDA (9,10,17).

In summary, MRV and RSF together allow differentiation of iron deficiency from thalassemia, while IRF indicates marrow activity. ROC analysis in our cohort identified RSF <98 as the best cutoff for IDA detection, aligning with previous observations by Urrechaga et al. and Brugnara et al. (10,29).

Normocytic anaemia

Among 124 patients with normocytic anaemia, MRV averaged 112.1 ± 11.7 fL, IRF 0.43 ± 0.14, and RSF 99.0 ± 7.2. Although the mean values were intermediate, intra-group variability provided key diagnostic insights. Slightly higher MRV suggested early macrocytic or regenerative processes (e.g., recovery after hemolysis or nutritional supplementation), whereas lower MRV reflected iron-restricted erythropoiesis not yet manifesting as microcytosis (1,7,12).

RSF around 99 implied moderate anisocytosis, with higher values denoting evolving nutritional deficiency or recovery and lower RSF indicating uniform marrow suppression (e.g., anaemia of chronic disease or renal failure). IRF variability (0.43 ± 0.14) helped distinguish regenerative from hypoproliferative patterns-high IRF in hemolysis or blood loss versus low IRF in marrow suppression (4,8,32).

These findings support prior studies suggesting that reticulocyte indices, even when within the “normal” range, capture subtle erythropoietic activity and can stratify normocytic anaemia by mechanism rather than morphology (18,31,33).

Macrocytic anaemia

Macrocytic anaemia (N=71) demonstrated the highest MRV (125.9 ± 25.2 fL) and RSF (103.4 ± 28.8), with IRF averaging 0.46 ± 0.16. MRV values exceeding 130-140 fL corresponded to megaloblastic anaemia due to B12 or folate deficiency, while moderate MRV elevations (115-125 fL) were observed in hemolytic or recovery states (6,14,25).

RSF variability (SD ±28.8) was the widest, underscoring marked anisocytosis in megaloblastic processes. High RSF indicated megaloblastosis with mixed populations of large reticulocytes and macrocytes, whereas normal RSF values were observed in alcohol-related or liver disease-associated macrocytosis (3,17,31). IRF showed limited cross-sectional discrimination but helped identify regenerative (High IRF) versus ineffective erythropoiesis (Low IRF).

These observations align with those reported by Toki et al. and Levy et al., who found that MRV and RSF effectively differentiated megaloblastic from non-megaloblastic macrocytosis (15,20,21).

Across all groups, RSF emerged as the most discriminative index for morphological classification, complementing RDW and MRV. MRV provided size-based refinement, IRF quantified the marrow response, and RSF integrated the severity of anisocytosis with reticulocyte size heterogeneity (10,12,24,29).

Our findings corroborate earlier work by Brugnara, Urrechaga, and Hoffmann, who emphasized the diagnostic and monitoring roles of reticulocyte indices in iron deficiency, thalassemia, and myelodysplastic states (10,29,32,34). Importantly, these indices enhance interpretive accuracy without additional cost or testing burden because they use parameters already generated by modern hematology analyzers.

Clinically, the combined use of MRV, IRF, and RSF can streamline differential diagnosis and improve monitoring of the therapeutic response in anaemia of varied etiologies. In particular, RSF and MRV together distinguish iron deficiency anaemia from thalassemia trait with high accuracy, while IRF serves as a sensitive marker of the regenerative marrow response during treatment (9,12,15).

Clinical implications and future directions

The present findings underscore the clinical value of incorporating reticulocyte-derived indices-particularly MRV, IRF, and RSF-into routine haematology reporting. These parameters provide real-time information on erythropoietic activity, iron availability, and anisocytosis, offering functional insights that go beyond traditional morphological classification. Their use can facilitate earlier recognition of evolving iron deficiency, distinguish thalassemia trait from nutritional microcytosis without extensive biochemical workup, and aid in monitoring marrow regeneration during therapy.

From a clinical perspective, MRV and RSF together form a practical diagnostic combination, enabling differentiation between the homogeneous microcytosis of thalassemia and the heterogeneous cell populations observed in iron deficiency or mixed nutritional anaemias. IRF, on the other hand, is particularly useful for assessing marrow responsiveness in dynamic settings such as acute blood loss, haemolysis, or recovery following iron or vitamin supplementation. Routine integration of these indices into complete blood count (CBC) panels could reduce dependence on invasive or high-cost investigations, such as bone marrow studies or iron kinetics, especially in resource-limited settings.

In addition, these indices have potential as prognostic and monitoring tools in chronic diseases in which anaemia is multifactorial, such as renal insufficiency, inflammatory disorders, and malignancy. Their use may support individualized treatment decisions by identifying patients who would benefit most from targeted iron or erythropoietin therapy, thereby promoting precision medicine in haematology practice.

Future research should focus on establishing standardized reference ranges for MRV, IRF, and RSF across diverse populations, accounting for age, sex, comorbidities, and ethnicity. Longitudinal studies are warranted to evaluate their dynamic changes during disease progression and therapeutic interventions. Multicentric prospective trials correlating these indices with biochemical markers of iron metabolism and bone marrow findings would help validate diagnostic thresholds and enhance clinical interpretability. Furthermore, integration of these reticulocyte parameters into algorithm-based anaemia management models and machine-learning frameworks could facilitate automated interpretation and improve the efficiency of the laboratory-clinical interface.

In summary, reticulocyte indices represent a promising advancement in automated haematology, bridging morphology with function and enabling a more mechanistic, cost-effective, and timely approach to anaemia evaluation. Their wider clinical adoption, supported by standardization and further evidence, could significantly refine diagnostic accuracy and patient management in both primary and tertiary care settings.

Conclusion

This study demonstrates that the reticulocyte-derived parameters MRV, IRF, and RSF add significant functional value to conventional red cell indices in the evaluation of anaemia. When interpreted alongside MCV and RDW, these indices provide dynamic insight into erythropoietic activity, iron availability, and red cell size heterogeneity, thereby enhancing diagnostic interpretation beyond static morphology.

Among the parameters studied, RSF emerged as the most discriminative marker, effectively distinguishing iron deficiency anaemia, thalassemia trait, and macrocytic conditions. MRV complemented MCV by refining size-based classification through its reflection of real-time reticulocyte kinetics. IRF, while less specific for cross-sectional subtype differentiation, proved valuable for assessing marrow regenerative activity and monitoring therapeutic response, particularly in iron deficiency and haemolytic states.

Importantly, these parameters are routinely generated by modern automated analysers and can be incorporated into clinical practice without additional testing or cost. The combined interpretation of MRV and RSF facilitates differentiation between the homogeneous microcytosis of thalassemia and the heterogeneous microcytosis of iron deficiency, while IRF provides a dynamic measure of bone marrow responsiveness.

In conclusion, reticulocyte-based indices represent a meaningful extension of routine haematological assessment, enabling more precise anaemia classification, earlier recognition of evolving or mixed deficiencies, and improved monitoring of treatment response. Their integration into routine reporting supports a more mechanistic, physiology-driven approach to anaemia diagnosis across diverse clinical settings.

The cross-sectional design of this study precluded longitudinal assessment of reticulocyte parameter trends during treatment or recovery. Although the overall cohort size was adequate, subgroup representation, particularly for macrocytic anaemia, was relatively smaller, which may have influenced comparative analyses. Diagnostic categorization was based on clinical and biochemical criteria in line with standard practice. Advanced molecular assays and bone marrow studies, which are reserved for specific clinical indications rather than routine evaluation, were not included. Despite these limitations, the findings remain consistent with international evidence and underscore the practical utility of automated reticulocyte indices as accessible, real-time markers in anaemia assessment.

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Ethical Statement

RHC/BIOPMRFIEC/2024/480 (Dated 13th January 2024)

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

AK made substantial contributions to the conception and design of the work and the interpretation of data, critically reviewed and revised the manuscript for important intellectual content, and approved the version for publication. SJ acquired the data and contributed to the analysis, interpretation, and preparation of the manuscript. VP and HP contributed to the conception of the study and critically reviewed the manuscript for important intellectual content.

All the authors agree to be accountable for all aspects of the work related to its accuracy and integrity.

Data Availability Statement

The dataset generated and analysed during this study is available from the corresponding author upon reasonable request, either during the submission process or after publication. The data are not publicly available because they contain information that could compromise the privacy and confidentiality of the research participants.

Use of Artificial Intelligence

OpenAI was used to refine the figures prepared by the authors.

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