

VARIATION OF RETICULOCYTE COUNT IN BONE MARROW SAMPLES

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Reticulocyte count; Bone marrow aspiration; Immature Reticulocyte Fraction (IRF); Erythropoiesis; Hematological disorders; Aplastic anemia; Hemolytic anemia. Introduction

Article History

Received: 04 May 2026

Accepted: 01 June 2026

Published: 15 June 2026

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Abstract

Reticulocytes are immature erythrocytes that reflect bone marrow erythropoietic activity. Although peripheral blood reticulocyte counts are routinely used, bone marrow reticulocyte assessment provides a more direct and sensitive indicator of erythroid function in hematological disorders.

Objectives To evaluate variations in bone marrow reticulocyte counts across different hematological diseases and compare their relationship with peripheral blood reticulocyte counts and Immature Reticulocyte Fraction (IRF).

Methods This cross-sectional analytical study included 62 patients undergoing diagnostic bone marrow aspiration. Reticulocyte counts were assessed in peripheral blood and bone marrow samples using supravital staining, automated hematology analyzers, and cell counters. IRF and other hematological parameters were also recorded. Data analysis was performed using SPSS software.

Results Significant variations in bone marrow reticulocyte counts were observed among disease groups. Elevated counts were found in hemolytic anemia and post-chemotherapy recovery phases, indicating active erythropoiesis. Reduced counts were observed in aplastic anemia and myelodysplastic syndromes, reflecting ineffective or suppressed marrow activity. A positive correlation was noted between peripheral blood and bone marrow reticulocyte counts; however, bone marrow assessment demonstrated higher sensitivity in detecting erythroid activity.

Conclusion Bone marrow reticulocyte evaluation provides a more precise and sensitive measure of erythropoietic activity compared to peripheral blood assessment. Combined analysis of bone marrow reticulocytes, peripheral counts, and IRF enhances diagnostic accuracy, disease monitoring, and assessment of marrow recovery in hematological disorders.

INTRODUCTION

Reticulocytes are immature erythrocytes that serve as important indicators of bone marrow erythropoietic activity. They represent the final stage of red blood cell maturation before entering the peripheral circulation and provide valuable information regarding the functional status of erythropoiesis. Reticulocyte production is regulated by erythropoietin, iron availability, and the bone marrow microenvironment, making reticulocyte assessment a useful tool disorders (Pan et al., 2022; Diaz-Garzon et al., 2023).

In routine clinical practice, reticulocyte counts are primarily measured in peripheral blood to assess bone marrow response to anemia, hemolysis, and therapeutic interventions. However, peripheral reticulocyte counts reflect only cells that have already been released into circulation and may not accurately represent intramedullary erythropoietic activity. Bone marrow reticulocyte assessment provides a more direct evaluation of erythroid maturation and marrow function, potentially offering greater insight into disorders

characterized by ineffective erythropoiesis, marrow suppression, or regenerative activity (Lim, 2022; Choi et al., 2022).

Despite the clinical significance of reticulocytes, studies examining variations in bone marrow reticulocyte counts across different hematological conditions remain limited. Understanding these variations may improve diagnostic accuracy, facilitate assessment of marrow function, and enhance monitoring of disease progression and treatment response. Therefore, the present study aimed to evaluate the variation of reticulocyte counts in bone marrow samples among patients with different hematological disorders and to determine their relationship with erythropoietic activity.

Marrow stromal changes, fibrosis, infiltration (e.g. malignancy, metastases), or damage to vasculature can impair erythroid maturation or hinder reticulocyte egress (Pan et al., 2022).

The efficiency of maturation is also determined by the functional capacity of erythroblastic islands (support for macrophages, delivery of iron, and cytokine milieu). (Macrophage support is necessary for the stability of erythropoietic nests, as shown by models of erythroblastic islands (Pawsat et al., 2023).

Erythropoietic production must be high under these conditions. The production of reticulocytes in the marrow rises, leading to an increase in the intramedullary reticulocyte-to-precursor ratio and a shift toward younger reticulocytes with higher fluorescence. Typically, peripheral reticulocyte counts rise in tandem (Reiling et al., 2025)

Objectives

1. To determine how different hematological conditions affect the reticulocyte count in bone marrow samples.
2. To determine whether there are any significant differences between reticulocyte counts in peripheral blood and bone marrow.
3. To investigate the relationship between erythropoietic activity and the number of reticulocytes in the bone marrow in various anemic and non-anemic states.

Research Questions

1. How does the number of reticulocytes differ between different samples of bone marrow from patients with various hematological conditions?
 2. Do reticulocyte counts in peripheral blood and bone marrow differ significantly?
 3. How does erythropoietic activity relate to the number of reticulocytes in the bone marrow?
- However, there are issues with bone marrow reticulocyte counting, such as low laboratory standardization, sampling variability, dilution by blood, heterogeneity of marrow regions, and technical staining consistency. In the design of the study, these drawbacks must be carefully addressed (Lim, 2022).

Methodology

2.1 Study design and settings

A cross-sectional analytical study was conducted at the Department of Hematology, Al Razi Institute (affiliated with GCUF), Pakistan, between August and October 2025. Ethical approval was obtained from the Institutional Ethical Review Committee, and written informed consent was secured from all participants.

2.2 Participants

Sixty-two patients (aged 15–70 years) undergoing diagnostic bone marrow aspiration were enrolled via non-probability purposive sampling. Inclusion required adequate marrow cellularity and smear quality, and patient consent. Exclusion criteria included recent blood transfusion (within 2 weeks), active radiotherapy or chemotherapy, and poor-quality smears. Participants were assigned to six diagnostic groups: Normal controls (n=12), IDA (n=12), Hemolytic Anemia (n=12), MDS (n=8), Aplastic Anemia (n=8), and Post-Chemotherapy Recovery (n=10).

2.3 Sample Collection and Laboratory Analysis

Bone marrow aspirates were collected from the posterior superior iliac spine under aseptic conditions and placed in EDTA-containing syringes. Thin marrow smears were prepared, air-dried, and stained with New Methylene Blue (NMB) for supravital reticulocyte identification.

Reticulocyte counts were expressed as a percentage of 1,000 counted erythroid cells. Automated parameters including IRF and Ret-He were obtained using the Sysmex XN-1000 fluorescence-based analyzer. Peripheral blood reticulocyte counts were collected in parallel.

mean $\pm$ standard deviation. One-way ANOVA was used to compare group means, followed by Tukey HSD post-hoc tests for pairwise comparisons. Pearson correlation assessed linear relationships between parameters. ROC curve analysis was performed to evaluate diagnostic accuracy. Statistical significance was set at $p < 0.05$.

2.4 Statistical Analysis: Data were analyzed using SPSS v26.0. Continuous variables are presented as

RESULTS

3.1 Reticulocyte Variation Across Diagnostic Group

Table 1. Mean reticulocyte parameters across six diagnostic groups (Mean $\pm$ SD).

Diagnosis Group	BM Reticulocyte (%) M $\pm$ SD	Peripheral Reticulocyte (%) M $\pm$ SD	IRF M $\pm$ SD	Ret-He (pg) M $\pm$ SD
Normal Controls	1.41 $\pm$ 0.49	1.30 $\pm$ 0.48	0.079 $\pm$ 0.025	29.90 $\pm$ 1.60
IDA	0.74 $\pm$ 0.27	0.63 $\pm$ 0.26	0.063 $\pm$ 0.015	23.64 $\pm$ 2.07
Hemolytic Anemia	5.77 $\pm$ 1.48	6.84 $\pm$ 2.19	0.199 $\pm$ 0.058	28.77 $\pm$ 1.64
MDS	0.75 $\pm$ 0.37	0.61 $\pm$ 0.43	0.061 $\pm$ 0.023	27.33 $\pm$ 1.85
Aplastic Anemia	0.29 $\pm$ 0.17	0.27 $\pm$ 0.15	0.029 $\pm$ 0.010	27.48 $\pm$ 1.03
Post-Chemotherapy Recovery	3.22 $\pm$ 1.29	3.04 $\pm$ 1.17	0.150 $\pm$ 0.039	28.96 $\pm$ 1.57

Note: BM = Bone Marrow; IRF = Immature Reticulocyte Fraction; Ret-He = Reticulocyte Hemoglobin Equivalent; MDS = Myelodysplastic Syndrome.

The highest bone marrow reticulocyte count was observed in patients with Hemolytic Anemia (M = 5.77%, SD = 1.48), reflecting marked erythropoietic stimulation in response to accelerated red cell destruction. Conversely, Aplastic Anemia demonstrated the lowest bone marrow reticulocyte count (M = 0.29%, SD = 0.17), indicating severe bone marrow failure.

A one-way ANOVA revealed statistically significant differences in bone marrow reticulocyte counts among the six diagnostic groups, $F(5, 56) = 62.01$, $p < .001$, $\eta^2 = .847$, indicating a very large effect size.

Table 3.2

One-Way ANOVA Results for Reticulocyte Parameters Across Diagnostic Groups

Variable	F	p	η^2
Hemoglobin	58.97	< .001	.840
BM Reticulocyte (%)	62.01	< .001	.847
Peripheral Reticulocyte (%)	56.60	< .001	.835
IRF	37.60	< .001	.771
Ret-He	20.29	< .001	.644

Post hoc Tukey HSD analyses demonstrated significantly higher bone marrow reticulocyte counts in Hemolytic Anemia compared with Normal Controls, IDA, and Aplastic Anemia ($p < .001$).

Relationship Between Bone Marrow and Peripheral Reticulocyte Counts

Peripheral reticulocyte counts closely mirrored bone marrow reticulocyte counts across all

diagnostic groups. Pearson correlation analysis demonstrated a near-perfect positive relationship between the two measures.

Table 3.3

Pearson Correlation Analysis Among Hemoglobin, Bone Marrow Reticulocyte Count, Peripheral Reticulocyte Count, Immature Reticulocyte Fraction (IRF), and Reticulocyte Hemoglobin Equivalent (Ret-He) (N = 62)

Variable 1	Variable 2	r	r ²	p
Hemoglobin (g/dL)	Bone Marrow Reticulocyte (%)	-.233	.054	.069
Hemoglobin (g/dL)	Peripheral Reticulocyte (%)	-.260	.068	.041*
Hemoglobin (g/dL)	IRF	-.161	.026	.211
Hemoglobin (g/dL)	Ret-He (pg)	.309	.095	.015*
Bone Marrow Reticulocyte (%)	Peripheral Reticulocyte (%)	.984	.968	< .001***
Bone Marrow Reticulocyte (%)	IRF	.809	.655	< .001***
Bone Marrow Reticulocyte (%)	Ret-He (pg)	.307	.094	.015*
Peripheral Reticulocyte (%)	IRF	.792	.627	< .001***
Peripheral Reticulocyte (%)	Ret-He (pg)	.279	.078	.028*
IRF	Ret-He (pg)	.266	.071	.037*

Note. r = Pearson correlation coefficient; r² = coefficient of determination; IRF = Immature Reticulocyte Fraction; Ret-He = Reticulocyte Hemoglobin Equivalent.

Correlations were calculated to assess the relationships among hematological and reticulocyte parameters in patients with various hematological conditions. $p < .05^*$, $p < .01$, $p < .001$. Values of r between .10–.29 indicate weak

correlations, .30–.69 indicate moderate correlations, and $\geq .70$ indicate strong correlations. This analysis addresses the study objective of investigating the relationship between erythropoietic activity and reticulocyte counts in bone marrow and peripheral blood samples.

Table 3.4

Receiver Operating Characteristic (ROC) Curve Analysis of Reticulocyte Parameters for Differentiating Hematological Disorders From Normal Controls (N = 62)

Comparison	Diagnostic Parameter	AUC	95% CI	Sensitivity	Specificity	Diagnostic Accuracy
IDA vs. Normal	Hemoglobin	1.00	[1.00, 1.00]	1.00	1.00	Excellent
IDA vs. Normal	Ret-He (pg)	1.00	[1.00, 1.00]	1.00	1.00	Excellent
IDA vs. Normal	BM Reticulocyte (%)	.889	[.750, 1.00]	1.00	.833	Good
Hemolytic Anemia vs. Normal	BM Reticulocyte (%)	1.00	[1.00, 1.00]	1.00	1.00	Excellent
Hemolytic Anemia vs. Normal	Peripheral Reticulocyte (%)	1.00	[1.00, 1.00]	1.00	1.00	Excellent
Hemolytic Anemia vs. Normal	IRF	.993	[.958, 1.00]	.917	1.00	Excellent
MDS vs. Normal	Hemoglobin	1.00	[1.00, 1.00]	1.00	1.00	Excellent
MDS vs. Normal	Ret-He (pg)	.854	[.668, 1.00]	.625	1.00	Good
Aplastic Anemia vs. Normal	BM Reticulocyte (%)	.958	[.855, 1.00]	1.00	.917	Excellent
Aplastic Anemia vs. Normal	IRF	.984	[.921, 1.00]	.875	1.00	Excellent
Post-Chemotherapy Recovery vs. Normal	IRF	.925	[.801, 1.00]	.900	.917	Excellent
Post-Chemotherapy Recovery vs. Normal	BM Reticulocyte (%)	.875	[.717, 1.00]	.800	1.00	Good

Note: AUC = Area Under the Curve; CI = Confidence Interval; BM = Bone Marrow; IRF = Immature Reticulocyte Fraction; Ret-He = Reticulocyte Hemoglobin Equivalent; IDA = Iron Deficiency Anemia; MDS = Myelodysplastic Syndrome.

ROC analysis demonstrated that reticulocyte-related parameters have strong diagnostic utility in differentiating hematological disorders from normal controls. Hemoglobin, Ret-He, bone marrow reticulocyte count, and peripheral reticulocyte count showed excellent to perfect diagnostic accuracy (AUC = .854–1.00). The highest performance was observed in Hemolytic Anemia, where bone marrow and peripheral reticulocyte counts achieved perfect discrimination (AUC = 1.00). Similarly, IRF

demonstrated excellent accuracy in identifying Aplastic Anemia (AUC = .984) and Post-Chemotherapy Recovery (AUC = .925). These findings indicate that reticulocyte indices are highly sensitive markers of erythropoietic activity and valuable tools for distinguishing hematological conditions and assessing bone marrow function.

Discussion

This study demonstrates that bone marrow reticulocyte counts vary significantly and systematically across hematological conditions, confirming their diagnostic and prognostic utility. The markedly elevated BM reticulocyte counts in hemolytic anemia reflect the expected compensatory erythropoietic surge driven by erythropoietin (EPO) in response to accelerated RBC destruction. These findings align with prior literature demonstrating that stress erythropoiesis promotes premature reticulocyte release so-called "shift reticulocytes" visible both in peripheral blood and bone marrow.

The near-perfect BM-to-peripheral reticulocyte correlation ($r = 0.984$) validates peripheral reticulocyte counting as a useful surrogate of marrow output under many conditions. Nevertheless, BM reticulocyte assessment provides additional information that peripheral measurements cannot: it captures intramedullary inefficiencies, progenitor apoptosis, and early maturation failures not yet reflected in circulating populations. This was especially apparent in aplastic anemia, where the marrow was profoundly hypocellular and BM reticulocyte counts near-absent, despite ongoing anemia a pattern that peripheral counts alone may not unambiguously characterize.

The significantly reduced BM reticulocyte counts in MDS, despite normal or elevated early erythroid precursors, confirm the hallmark ineffective erythropoiesis of this condition where dysplastic progenitors undergo intramedullary apoptosis before completing maturation. This finding underscores the value of measuring reticulocytes specifically, rather than total erythroid precursors, to gauge functional erythropoietic output.

The elevated IRF in post-chemotherapy recovery is consistent with early marrow regeneration: immature reticulocytes are released before full maturation, reflecting a marrow "surge" analogous to what has been described as a leading indicator of hematopoietic engraftment post-transplantation. IRF's excellent ROC performance across multiple disease comparisons (AUC 0.925–0.993) supports its integration into routine marrow assessment protocols.

The markedly depressed Ret-He in IDA (23.64 pg vs. 29.90 pg in normal controls) confirms that iron-restricted erythropoiesis is evident at the reticulocyte level before overt changes in mean corpuscular hemoglobin or serum iron. This has practical implications: Ret-He may enable earlier identification of iron-deficiency states in high-risk populations, prompting timely intervention.

5. CONCLUSION

Bone marrow reticulocyte assessment, complemented by IRF and Ret-He parameters, offers a direct and sensitive window into erythropoietic activity that surpasses what peripheral blood indices can provide alone. BM reticulocyte counts differ significantly and predictably across hematological disorders elevated in active compensatory states (hemolytic anemia, post-chemotherapy recovery) and suppressed in marrow failure and ineffective erythropoiesis (aplastic anemia, MDS, IDA). Incorporating BM reticulocyte parameters into clinical hematology practice has the potential to refine early diagnosis, disease monitoring, and treatment response evaluation. Larger prospective multicenter studies are warranted to validate these parameters as standard tools in hematological assessment.

Suggestions

- Integrate BM reticulocyte assessment into routine hematological workup, justified by the near-perfect BM-to-peripheral correlation
- Standardize staining and reporting protocols across institutions (favoring fluorescence-based automated analysis over manual NMB)
- Adopt IRF as an early clinical biomarker of marrow recovery and treatment response, with AUC data cited
- Use Ret-He for early detection of iron-restricted erythropoiesis before conventional indices change
- Develop disease-specific reference ranges as a foundation for future studies

Limitations

- Small and unequal group sizes ($n = 8-12$), with cautionary interpretation for MDS and aplastic anemia subgroups
- Single-center design limiting generalizability to other populations and healthcare settings
- Cross-sectional methodology preventing assessment of dynamic treatment-response trajectories
- Absence of validated intramedullary normative reference data for this population
- Sampling variability, dilution effects, and residual inter-observer staining inconsistency

Recommendations

- Conduct large-scale multicenter prospective studies (≥ 200 per diagnostic group)
- Establish age-, sex-, altitude-, and ethnicity-stratified reference intervals
- Investigate serial BM reticulocyte monitoring as a prognostic tool in disease management trials
- Expand research to include pediatric populations and underrepresented clinical groups (CKD, HIV, malaria)
- Leverage emerging technologies (scRNA-seq, AI image analysis, digital pathology) for enhanced characterization

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