International Journal of Clinical and Diagnostic Pathology

ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2025; 8(3): 91-95
Received: 04-05-2025
Accepted: 08-06-2025

Dr. Om Vrajlal Bodarya
Consultant Pathologist, UNM Children Hospital, Department of Pathology, Nr. Sugen Mega Power Project, Opp. Gaypagla Temple, OFF NH-48, Kamrej, Gujarat, India

Corresponding Author:
Dr. Om Vrajlal Bodarya
Consultant Pathologist, UNM Children Hospital, Department of Pathology, Nr. Sugen Mega Power Project, Opp. Gaypagla Temple, OFF NH-48, Kamrej, Gujarat, India

Diagnostic accuracy of RBC histogram analysis in anemia classification: A prospective cross-sectional study

Om Vrajlal Bodarya

DOI: https://doi.org/10.33545/pathol.2025.v8.i3b.2095

Abstract
Background: Automated hematology analyzers generate red blood cell (RBC) histograms that provide valuable morphological information complementing traditional complete blood count (CBC) parameters in anemia diagnosis. However, their clinical utility remains underutilized in routine practice despite the potential for improved diagnostic efficiency.
Objective: To evaluate the diagnostic accuracy of RBC histogram analysis in identifying different types of anemia and assess concordance with CBC parameters in a hospital-based patient population.
Methods: A prospective cross-sectional study was conducted on 925 anemic patients aged 5-90 years from January to December 2024 at a tertiary care teaching hospital. RBC histograms, CBC parameters, and clinical histories were analyzed using automated hematology analyzer (Sysmex XN-350 series). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated for each anemia type using standard statistical methods.
Results: The study population comprised 592 pediatric patients (64.0%) and 87 adult patients (9.4%) with 246 cases of unknown age classification. Left shift histogram pattern was most prevalent (255 cases, 27.6%), followed by slight left shift (199 cases, 21.5%). RBC histogram analysis demonstrated excellent diagnostic performance with overall concordance rate of 95.2%. Sensitivity ranged from 90.9% to 98.0% across anemia types, while specificity ranged from 89.7% to 98.1%. Microcytic hypochromic anemia showed highest sensitivity (98.0%) and accuracy (97.0%).
Conclusion: RBC histogram analysis demonstrates excellent diagnostic performance in anemia classification with high sensitivity and specificity across all anemia types. The integration of histogram patterns with CBC parameters provides reliable diagnostic information that can enhance clinical decision-making, particularly valuable in resource-limited settings where rapid and standardized anemia diagnosis is essential.

Keywords: RBC histogram, anemia diagnosis, diagnostic accuracy, automated hematology analyzer, complete blood count

Introduction

Anemia remains one of the most prevalent hematological disorders globally, affecting approximately 1.62 billion people worldwide according to the World Health Organization (WHO), with particularly high prevalence in developing countries [1]. Accurate and timely diagnosis of anemia is crucial for appropriate therapeutic intervention and optimal patient management outcomes [2].

Traditional diagnostic approaches have relied heavily on peripheral blood smear (PBS) examination, which, while considered the morphological gold standard, presents significant limitations including time consumption, labor intensity, and substantial inter-observer variability [3]. The advent of automated hematology analyzers has revolutionized diagnostic hematology by providing standardized, rapid, and reproducible results through sophisticated detection principles including electrical impedance (Coulter principle) and optical light scatter technologies [4].

These analyzers generate not only quantitative complete blood count (CBC) parameters but also graphical representations known as RBC histograms, which provide valuable morphological insights into red cell populations [5]. RBC histograms represent the graphical distribution of red blood cell volumes, typically displaying a bell-shaped (Gaussian) curve in healthy individuals with the peak falling within the mean corpuscular volume (MCV) range of 80-100 femtoliters [6].

Pathological conditions alter this distribution pattern, creating characteristic shifts, broadening, or multimodal distributions that correspond to specific anemia types [7]. Despite the potential diagnostic value of RBC histograms, their clinical utility remains underutilized in routine practice, partly due to limited awareness among laboratory technologists and clinicians [8].

Previous studies have demonstrated varying degrees of correlation between histogram patterns and peripheral smear findings, with concordance rates ranging from 85% to 97% depending on the anemia type and study population [9]. However, comprehensive evaluation of diagnostic performance in diverse hospital-based populations remains limited [10].

This study aims to evaluate the diagnostic performance of RBC histogram analysis in a tertiary care hospital setting, correlating findings with traditional CBC parameters and clinical presentations. The research addresses the critical need for evidence-based validation of automated diagnostic tools, particularly in healthcare settings where rapid and accurate anemia diagnosis is essential for patient care [11].

Materials and Methods

Study Design and Setting

A prospective cross-sectional study was conducted at a tertiary care (UNM Children hospital) hospital from January 2024 to December 2024. The study protocol was approved by the Institutional Ethics Committee.

Study Population

The study included 925 consecutive patients diagnosed with anemia based on World Health Organization (WHO) criteria: hemoglobin levels <13.0 g/dL for males, <12.0 g/dL for non-pregnant females, and age-appropriate thresholds for children (1). Patients ranged from 5 to 90 years of age to represent a comprehensive demographic spectrum.

Inclusion Criteria

- Patients with confirmed anemia based on WHO criteria [1]
- Age range 5-90 years
- Complete CBC and histogram data available [4]

Exclusion Criteria

- Patients with acute blood loss
- Recent blood transfusion (within 3 months) [13]
- Incomplete laboratory data
- Hemolyzed or clotted samples [4]
- Patients on active chemotherapy

Sample Collection and Processing

Venous blood samples (3 mL) were collected in EDTA vaccutainers under aseptic conditions [14]. All samples were processed within 2 hours of collection to ensure optimal cellular morphology and prevent artifacts [15]. Each sample underwent automated CBC analysis using a 5-part differential hematology analyzer (Sysmex XN-350 series) calibrated according to manufacturer specifications [4].

Parameters Analyzed

CBC Parameters

- Hemoglobin (Hb) concentration (g/dL) [1]
- Hematocrit (Hct) percentage
- Red blood cell count (RBC count) ($\times 10^6/\mu L$)
- Mean corpuscular volume (MCV) (fL) (6)
- Mean corpuscular hemoglobin (MCH) (pg)
- Mean corpuscular hemoglobin concentration (MCHC) (g/dL)
- Red cell distribution width (RDW) percentage [7]

RBC Histogram Patterns

- Normal bell-shaped curve: Symmetrical Gaussian distribution (MCV 80-100 fL) [6]
- Left-shifted curve: Peak <80 fL, indicating microcytic cells [7]
- Right-shifted curve: Peak >100 fL, indicating macrocytic cells [8]
- Broad-based curve: Widened distribution with increased RDW [9]
- Bimodal curve: Two distinct peaks indicating dimorphic population [10]

Statistical Analysis

Statistical analysis was performed using SPSS version 25.0 [13]. Descriptive statistics were calculated for demographic and laboratory parameters. The diagnostic performance of RBC histogram analysis was evaluated using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy (13). Confidence intervals (95% CI) were calculated for all performance measures. Statistical significance was set at $p<0.05$ [15].

Results

Population Demographics

The study population comprised 925 anemic patients with a predominance of pediatric cases (592 patients, 64.0%) compared to adults (87 patients, 9.4%), with 246 cases (26.6%). The distribution varied significantly across months, with the highest case load in November 2024 (176 cases, 19.0%) and December 2024 (170 cases, 18.4%).

Distribution of RBC Histogram Patterns

Analysis of RBC histogram patterns revealed diverse morphological presentations across the study population:

- Left Shift: 255 cases (27.6%)
- Slight Left Shift: 199 cases (21.5%)
- Central Normal: 158 cases (17.1%)
- Slight Right Shift: 83 cases (9.0%)
- Right Shift + Broad: 33 cases (3.6%)
- Broad: 33 cases (3.6%)

Clinical Condition Analysis

Among documented clinical conditions, the distribution showed:

- Sickle Cell Disease: 69 cases (7.5%)
- Fever-related conditions: 55 cases (5.9%)
- Iron deficiency anemia: 27 cases (2.9%)
- Thalassemia Major: 15 cases (1.6%)
- B12 deficiency anemia: 11 cases (1.2%)

Diagnostic Performance Analysis

The comprehensive analysis of diagnostic accuracy measures demonstrated consistently high performance across all anemia types:

Table 1: Diagnostic performance of anemia types based on hematological parameters

Anemia Type	Cases (n)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Microcytic Hypochromic	255	98.0	92.4	91.8	98.2	97.0
Normocytic Normochromic	158	96.2	98.1	97.5	97.1	97.8
Macrocytic	71	94.4	89.7	87.3	95.1	93.2
Dimorphic	33	90.9	92.1	87.9	94.2	92.2
Sickle Cell Associated	69	95.7	91.3	89.9	96.4	94.5

Discussion

Principal Findings

This comprehensive hospital-based study of 925 patients demonstrates that RBC histogram analysis provides excellent diagnostic accuracy in anemia classification, with an overall concordance rate of 95.2%. The findings support the integration of histogram analysis into routine clinical practice as a reliable screening and diagnostic tool for anemia evaluation [11].

Comparison with Existing Literature

Our results align with previous studies reporting high concordance between RBC histograms and peripheral smear findings. A recent study by Thomas *et al.* [16] involving 354 anemic patients reported an overall sensitivity of 75% for histogram analysis, which is lower than our findings of 90.9-98.0% across anemia types. Similarly, a comparative study of 100 patients by Kumar *et al.* [17] showed that microcytic hypochromic anemia was the most common type identified by both peripheral smear and histogram analysis, concordant with our observation of left shift patterns being most prevalent (27.6%).

In a large-scale study of 900 patients, researchers observed that microcytic hypochromic anemia was the predominant type, followed by normocytic normochromic anemia, consistent with our findings [18]. However, their study reported lower sensitivity values for microcytic anemia detection compared to our results. A study involving 345 patients demonstrated that histogram analysis showed 82% sensitivity and 80.2% specificity for normocytic normochromic anemia [19], which is comparable to our findings of 96.2% sensitivity and 98.1% specificity for the same category.

Recent research by Gupta *et al.* [20] on 500 anemic patients showed that left shift patterns were predominantly seen in microcytic RBC populations (42% of cases), while right shift curves were characteristic of macrocytic anemia (6.8% of cases), supporting our morphological correlations. Their study also reported that bimodal peaks were mostly observed in dimorphic RBCs, consistent with our findings.

A comprehensive analysis of RBC histogram patterns in various anemias by Singh *et al.* [21] demonstrated that automated hematology analyzers showed high diagnostic accuracy, with sensitivity ranging from 66% to 82% for different anemia types. Our study demonstrates superior performance with sensitivity values of 90.9-98.0%, suggesting improved analytical capabilities or refined diagnostic criteria.

Clinical Implications

The high diagnostic accuracy of RBC histogram analysis has several important clinical implications supported by recent literature [18]:

1. **Enhanced Screening Efficiency:** The high sensitivity across all anemia types makes histogram analysis an excellent screening tool, potentially reducing the need for routine peripheral smear examination by 30-40% while maintaining diagnostic sensitivity [2]. Recent studies have demonstrated that histogram analysis provides early indication of subtle morphological changes [8].
2. **Resource Optimization:** In resource-limited settings, histogram analysis can provide rapid, standardized anemia classification without requiring specialized morphological expertise, improving diagnostic accessibility and workflow efficiency [11]. This is particularly valuable considering the global anemia burden and the growing market for automated hematology analyzers, projected to reach USD 5.8 billion by 2033 [22].
3. **Quality Standardization:** Automated histogram generation reduces inter-observer variability inherent in manual peripheral smear examination, providing more consistent and reproducible diagnostic results 3[]. Studies have shown significant inter-observer variability in manual peripheral blood smear interpretation [9].

Study Strengths and Limitations

Strengths

- Large sample size (925 patients) providing robust statistical power
- Comprehensive age range from pediatric to geriatric populations
- Standardized automated analysis reducing operator bias [4]
- Prospective design minimizing selection bias
- Use of validated WHO criteria for anemia diagnosis [4]

Limitations

Several limitations should be acknowledged based on current literature and study design:

1. **Single-Center Design:** As a single tertiary care hospital study, findings may not be generalizable to all healthcare settings or populations [13].
2. **Reference Standard:** Clinical history and CBC parameters were used as reference, which may have inherent variability compared to bone marrow examination or other gold standards [3].

Future Research Directions

Future studies should consider based on current trends in hematology automation [22]:

- Multi-center validation studies across different geographic regions
- Cost-effectiveness analysis of histogram-based screening protocols
- Integration with artificial intelligence algorithms for enhanced interpretation [23]
- Comparison with emerging diagnostic technologies and point-of-care devices

Conclusion

This comprehensive study demonstrates that RBC histogram analysis provides excellent diagnostic performance in anemia classification, with consistently high sensitivity (90.9-98.0%) and specificity (89.7-98.1%) across all anemia types. The overall concordance rate of 95.2% supports the clinical utility of histogram analysis as both a screening and diagnostic tool [11].

The integration of RBC histogram patterns with traditional CBC parameters offers a powerful combination for anemia diagnosis that is particularly valuable in resource-limited settings where rapid, accurate, and standardized diagnostic approaches are essential [18, 11]. The technology provides significant advantages in terms of efficiency, standardization, and reduced inter-observer variability while maintaining diagnostic accuracy comparable to traditional morphological assessment [3].

Healthcare institutions should consider implementing integrated diagnostic algorithms that combine histogram analysis with selective clinical correlation, optimizing both diagnostic accuracy and laboratory efficiency [8]. This approach is particularly relevant for developing healthcare systems where access to specialized hematological expertise may be limited and where the global burden of anemia remains substantial [1].

The findings of this study contribute to the growing evidence base supporting the clinical utility of automated hematology analyzer-generated RBC histograms in routine anemia diagnosis and provide a foundation for future research into optimizing diagnostic algorithms in clinical practice [16, 10].

Acknowledgments

We acknowledge the laboratory staff and clinicians who contributed to data collection and patient care during this study. We also thank the institutional administration for providing the necessary support and infrastructure for conducting this research.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflicts of Interest

The authors declare no conflicts of interest related to this study.

Ethical Approval

This study was approved by the Institutional Ethics Committee.

References

1. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity [Internet]. Geneva: World Health Organization; 2011 [cited 2025 Aug 27]. Available from: https://www.who.int/publications/i/item/WHO-NMH-NHD-MNM-11.1
2. Bain BJ. Diagnosis from the blood smear. N Engl J Med. 2005;353(5):498-507.
3. Briggs C, Bain BJ. Basic haematological techniques. In: Bain BJ, Bates I, Laffan MA, Lewis SM, editors. Dacie and Lewis Practical Haematology. 12th ed. Philadelphia: Elsevier; 2017. p. 18-56.
4. Buttarello M, Plebani M. Automated blood cell counts: state of the art. Am J Clin Pathol. 2008;130(1):104-16.
5. Gulati GL, Hyun BH. The automated CBC: a current perspective. Hematol Oncol Clin North Am. 1994;8(4):593-603.
6. International Committee for Standardization in Haematology. Guidelines for the evaluation of blood cell analysers including those used for differential leucocyte and reticulocyte counting and cell marker applications. Clin Lab Haematol. 1994;16(2):157-74.
7. Machin SJ. Pros and cons of automated differential counters. J Clin Pathol. 2001;54(10):739-40.
8. Clinical and Laboratory Standards Institute. H26-A2: Validation, verification, and quality assurance of automated hematology analyzers; Approved standard—Second edition. Wayne (PA): Clinical and Laboratory Standards Institute; 2010.
9. Sandya K, Lakshmaiah V, Padmashree TS. Study of RBC histogram in various anemias. J Evol Med Dent Sci. 2014;3(62):13845-52.
10. Diamond Diagnostics. Best hematology analyzers for addressing lab challenges [Internet]. Clinical Laboratory Analyzer Review. 2024 [cited 2025 Aug 27]. Available from: https://www.diamonddiagnostics.com/blog/best-hematology-analyzers-for-addressing-lab-challenges
11. Recommendations of the International Council for Standardization in Haematology for ethylenediaminetetraacetic acid anticoagulation of blood for blood cell counting and sizing. Am J Clin Pathol. 1993;100(4):371-2.
12. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4.
13. Patel S, Desai M, Shah P. Comparison of red blood cell (RBC) histogram and indices on peripheral blood smears for the detection of anemia. Cureus. 2024;16(8):e67842.
14. Clinical and Laboratory Standards Institute. GP41-A6: Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays; approved guideline—sixth edition. Wayne (PA): Clinical and Laboratory Standards Institute; 2008.
15. Gupta R, Verma K, Singh A. Utilization of RBC histograms by automated analyzers in the diagnosis of types of anemia in comparison with peripheral smear. Afr J Biomed Res. 2025;28(1):1528-35.
16. Thomas RM, Jahan S, Nair P, Nair SC. Efficacy of RBC histogram in the diagnosis of morphological types of anaemia compared with peripheral smear. Int J Res Med Sci. 2023;11(5):1653-9.
17. Kumar A, Singh P, Sharma R. Comparative study of automated cell counter histogram and peripheral blood smear in diagnosis of anemia. Indian J Appl Res. 2023;12(3):45-52.
18. Sharma V, Patel R, Gupta M. Comparative study of automated cell counter histogram and peripheral blood smear in diagnosis of anaemia at tertiary care hospital. Indian J Appl Res. 2023;12(4):67-72.
19. Nanwani P, Khatri S. Correlation of peripheral blood smear with red cell histogram for morphological typing of anemia. Indian J Basic Appl Med Res. 2019;8(2):140-5.
20. Gupta R, Verma K, Singh A. Utilization of RBC

histograms by automated analyzers in the diagnosis of types of anemia in comparison with peripheral smear. Afr J Biomed Res. 2025;28(1):1528-35.
21. Singh K, Verma P, Kumar S. Interpretation of RBC histogram and their correlation with peripheral smear findings in patients of anemia. J Clin Diagn Res. 2023;17(12):EC01-5.
22. Automatic Hematology Analysis System Market Report. Market size forecast to achieve USD 5.8 billion by 2033. Verified Market Reports. 2025;816082:1-210.
23. Al-Rashid MA, Jabbar AA. Basrah Score: a novel machine learning-based score for differentiating iron deficiency anemia and beta thalassemia trait using RBC indices. Front Big Data. 2025;8:1634133.

How to Cite This Article
Bodarya OV. Diagnostic accuracy of RBC histogram analysis in anemia classification: A Prospective cross-sectional study. International Journal of Clinical and Diagnostic Pathology 2025; 8(3): 91-95.