

The Diagnostic Synergy of Automated Hematology and Manual Microscopy: An In-Depth Comparative Analysis of RBC Indices, Volume Histograms, and Peripheral Blood Smear Morphology in Adult Anemia

Dr. Angelin Christina^{1*}, Dr. Lakshmi Priya²

¹Postgraduate, Department of Pathology, Sree Balaji Medical College and Hospital, BIHER, Chromepet, Chennai – 600044, Tamil Nadu, India. Email: angelinschristina@gmail.com, ORCID: 0009-0007-8430-0623

²Assistant Professor, Department of Pathology, Sree Balaji Medical College and Hospital, BIHER, Chromepet, Chennai – 600044, Tamil Nadu, India. Email: lachu21.jp55@gmail.com, ORCID: 0009-0000-0093-6990

Abstract

Background: Morphological evaluation remains a cornerstone in the systematic diagnosis of anemia. Modern automated hematology analyzers provide rapid and standardized quantitative parameters, whereas manual peripheral blood smear (PBS) examination offers detailed qualitative assessment of red blood cell morphology.

Objective: To evaluate the diagnostic reliability and complementary utility of automated red blood cell (RBC) indices and RBC volume distribution histograms in comparison with manual PBS examination among adult patients with anemia.

Methods: A cross-sectional analytical study was conducted among 96 adult patients with anemia. Automated complete blood count parameters, including Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), and RBC volume histograms, were evaluated alongside blinded microscopic PBS examination. Kruskal-Wallis and Mann-Whitney U tests were used for comparison of numerical indices, while Pearson's Chi-square test was used to assess the association and concordance between RBC histogram patterns and PBS morphological classifications.

Results: Normocytic normochromic anemia was the predominant morphological subtype (64.6%, n=62), followed by microcytic hypochromic (27.1%, n=26), macrocytic (5.2%, n=5), and dimorphic (3.1%, n=3) patterns. Among the automated numerical parameters, MCV demonstrated statistically significant differences across the morphological categories ($p < 0.001$). MCH, MCHC, and RDW did not demonstrate statistically significant differences ($p > 0.05$). RBC volume histograms showed strong concordance with PBS morphological classifications ($\chi^2 = 241.12$, $p < 0.001$), with an overall diagnostic agreement of 94.8% (91/96 cases).

Conclusion: RBC volume histograms, particularly when interpreted alongside MCV, provide a rapid and reliable screening approach for the initial morphological classification of anemia. Histogram analysis complements numerical RBC indices by providing a graphical representation of red cell volume distribution and may identify mixed or discrepant populations requiring targeted manual PBS examination. Automated parameters should therefore complement, rather than replace, microscopic smear evaluation.

Keywords: Anemia; Red Blood Cell Indices; Mean Corpuscular Volume; RBC Histogram; Peripheral Blood Smear; Automated Hematology Analyzer; Morphological Classification; Diagnostic Concordance.

Introduction

Anemia represents a major global health burden and one of the most frequently encountered hematological conditions across clinical settings. Its underlying etiologies are notably diverse, encompassing nutritional deficiencies (iron, vitamin B12, and folate deficiencies), systemic inflammatory diseases, chronic kidney disease, hemolytic processes, and primary bone marrow disorders. Because therapeutic interventions differ fundamentally depending on the underlying pathophysiological mechanism, fast, precise, and reproducible morphological classification is critical.^[1]

Historically, the evaluation of anemia has relied heavily on manual examination of the peripheral blood smear (PBS). Evaluated under light microscopy by a pathologist, the PBS remains the traditional gold standard for assessing red blood cell architecture. It enables direct observation of cellular variations in size (anisocytosis), shape (poikilocytosis), hemoglobinization (chromasia), and structural inclusions (such as Howell-Jolly bodies, basophilic stippling, and Pappenheimer bodies). Despite its unmatched qualitative diagnostic depth, manual PBS analysis is inherently labor-intensive, time-consuming, dependent on operator technical skill, and vulnerable to inter-observer variability.^[2,3]

The integration of modern multi-parameter automated hematology analyzers has fundamentally transformed clinical laboratory workflows. By utilizing electrical impedance, light-scattering technology, or fluorescent flow cytometry, modern analyzers process high volumes of blood samples rapidly while providing precise quantitative outputs. Key automated red cell indices—specifically Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC)—allow for immediate, standardized categorization of anemia into microcytic, normocytic, or macrocytic types.^[4]

However, numerical red cell indices suffer from an inherent physiological limitation: they represent mathematical averages of millions

of circulating red blood cells. In clinical scenarios involving mixed or dual red cell populations—such as dimorphic anemias seen in early iron replacement therapy, coexisting iron and megaloblastic deficiencies, or post-transfusion states—averaging metrics can conceal significant underlying abnormalities. For example, a mixture of severely microcytic cells and macrocytic cells may yield a calculated MCV that falls deceptively within the normal reference range. [5]

To address the limitations of averaged numerical indices, modern automated analyzers generate the RBC volume distribution histogram. The RBC histogram is a graphical curve that plots individual cell volumes (expressed in femtoliters on the x-axis) against their relative frequencies (plotted on the y-axis). Because it displays the full continuous spectrum of red cell sizes rather than a single summary statistic, the histogram serves as a visual "morphological fingerprint" of the circulating red cell population. Specific graphical patterns reveal distinct pathological states: [6]

- **Microcytosis:** Indicated by a leftward shift of the curve peak. [6]
- **Macrocytosis:** Indicated by a rightward shift of the curve peak. [6]
- **Anisocytosis:** Indicated by a broadening of the base of the curve. [6]
- **Dimorphic or Dual Populations:** Indicated by a distinct bimodal peak or a significantly widened, asymmetrical curve base. [6]

Despite these technological advancements, automated numerical data and graphical histograms cannot capture all qualitative visual details, such as target cells, schistocytes, spherocytes, or sickle cells. Moreover, technical interferences such as severe leukocyte elevation, giant platelets, or cold agglutinins can occasionally distort graphical outputs. Therefore, establishing the exact diagnostic relationship and degree of synergy between automated parameters and manual microscopy is essential for streamlining laboratory testing algorithms. [2,7]

This study systematically evaluates the concordance between automated red cell indices, RBC volume histograms, and manual peripheral blood smear morphologic findings. By correlating these metrics, the study aims to define how effectively automated graphical outputs can predict smear findings, optimize laboratory workflow, and strengthen quality assurance in hematology diagnostics. [3,8]

2. MATERIALS AND METHODS

A cross-sectional observational study was conducted among 96 adult patients diagnosed with anemia, comprising 48 males (50.0%) and 48 females (50.0%), with ages ranging from 18 to 80 years and a mean age of 52.25 ± 19.70 years. Venous blood samples were collected in K₂EDTA tubes and analyzed using a multi-parameter automated hematology analyzer to obtain hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), and RBC volume distribution histograms. Peripheral blood smears were prepared, air-dried, stained with Leishman stain, and examined under oil immersion microscopy by an experienced pathologist who was blinded to the automated CBC and histogram findings. Based on peripheral blood smear morphology, cases were categorized as normocytic normochromic, microcytic hypochromic, macrocytic, or dimorphic anemia. Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 29.0. Continuous variables were expressed as mean ± standard deviation (SD), and differences across morphological categories were assessed using the Kruskal-Wallis test followed by pairwise Mann-Whitney U testing. Categorical variables were expressed as frequencies and percentages, and Pearson's Chi-square test was used to assess the association and concordance between RBC histogram patterns and peripheral blood smear morphological classifications. A p-value <0.05 was considered statistically significant, while p<0.01 was considered highly significant. The_Diagnostic_Synergy_Cited_Re...

3. RESULTS

A total of 96 adult patients with anemia were included in the study. The study population consisted of 48 males (50.0%) and 48 females (50.0%). The participants were aged between 18 and 80 years, with a mean age of 52.25 ± 19.70 years. Based on peripheral blood smear morphology, 62 (64.6%) patients were classified as having normocytic normochromic anemia, 26 (27.1%) as microcytic hypochromic anemia, 5 (5.2%) as macrocytic anemia, and 3 (3.1%) as dimorphic anemia.

Table 1. Distribution of patients according to demographic characteristics

Characteristic	Number (n)	Percentage (%)
Sex		
Male	48	50.0
Female	48	50.0
Age group (years)		
18–20	8	8.3
21–30	12	12.5
31–40	6	6.3
41–50	20	20.8

51–60	10	10.4
61–70	16	16.7
71–80	24	25.0
Total	96	100.0

Table 2. Distribution of patients according to peripheral blood smear morphology

Peripheral smear morphology	Number (n)	Percentage (%)
Normocytic normochromic	62	64.6
Microcytic hypochromic	26	27.1
Macrocytic	5	5.2
Dimorphic	3	3.1
Total	96	100.0

The mean RBC indices varied across the four morphological categories. The mean MCV was 88.24 ± 5.09 fL in the normocytic group, 76.39 ± 3.56 fL in the microcytic group, 105.56 ± 3.06 fL in the macrocytic group, and 93.97 ± 1.27 fL in the dimorphic group. The difference in MCV across the four morphological groups was statistically significant ($p < 0.001$). In contrast, differences in MCH, MCHC, and RDW were not statistically significant.

Table 3. Comparison of RBC indices according to peripheral blood smear morphology

Morphological group	n	MCV (fL), Mean $\pm$ SD	MCH (pg), Mean $\pm$ SD	MCHC (g/dL), Mean $\pm$ SD	RDW (%), Mean $\pm$ SD
Normocytic normochromic	62	88.24 ± 5.09	28.07 ± 4.01	33.25 ± 1.75	18.24 ± 2.95
Microcytic hypochromic	26	76.39 ± 3.56	27.88 ± 5.03	33.21 ± 1.62	18.79 ± 6.57
Macrocytic	5	105.56 ± 3.06	30.44 ± 3.54	32.20 ± 2.35	16.60 ± 5.49
Dimorphic	3	93.97 ± 1.27	26.77 ± 6.25	32.87 ± 0.75	22.67 ± 3.06
p-value		<0.001	>0.05	>0.05	>0.05

The RBC volume distribution histograms demonstrated a high degree of concordance with peripheral smear morphology. Among the 62 patients classified as normocytic normochromic on peripheral smear, 60 (96.8%) showed a normal RBC histogram, while 2 (3.2%) demonstrated a broad-based pattern. Among the 26 microcytic hypochromic cases, 24 (92.3%) demonstrated a left-shifted histogram, while 2 (7.7%) showed a normal histogram. All five macrocytic cases demonstrated a right-shifted histogram. Of the three dimorphic cases, two (66.7%) demonstrated a broad-based/bimodal histogram pattern, while one showed a broad-based single-peak pattern. Overall, 91 of 96 cases (94.8%) demonstrated concordance between the RBC histogram pattern and peripheral blood smear morphology.

Table 4. Concordance between RBC histogram pattern and peripheral blood smear morphology

Peripheral smear morphology	Normal histogram	Left-shifted histogram	Right-shifted histogram	Broad-based/bimodal histogram	Total	Concordance (%)
Normocytic normochromic	60	0	0	2	62	96.8
Microcytic hypochromic	2	24	0	0	26	92.3
Macrocytic	0	0	5	0	5	100.0
Dimorphic	0	0	0	3	3	66.7
Total	62	24	5	5	96	94.8

Overall, the association between RBC histogram patterns and peripheral blood smear morphological categories was statistically significant ($\chi^2 = 241.12$, $p < 0.001$), demonstrating a high level of agreement between automated histogram interpretation and manual microscopic assessment.

4. Discussion

This comparative study demonstrates the complementary diagnostic value of automated hematology analyzer parameters, RBC volume histograms, and peripheral blood smear examination in the morphological assessment of adult anemia. In the present study,

the largest proportion of patients belonged to the 71–80-year age group (25.0%), with a mean age of 52.25 ± 19.70 years. The predominance of older adults is clinically relevant because anemia in elderly individuals is frequently multifactorial and may be associated with nutritional deficiencies, chronic inflammatory disorders, renal impairment, and occult blood loss. Buttarello emphasized that laboratory evaluation of anemia requires integration of conventional red cell indices with additional hematological parameters and clinical context, particularly when evaluating heterogeneous adult populations. [1] The present study similarly demonstrates that automated numerical parameters provide an important first level of classification, while peripheral smear examination remains necessary for morphological confirmation.

In the present study, MCV was the most discriminatory automated red cell parameter, showing a statistically significant difference across the four peripheral smear morphological groups ($p < 0.001$). The mean MCV was 76.39 ± 3.56 fL in microcytic hypochromic anemia, 88.24 ± 5.09 fL in normocytic normochromic anemia, 105.56 ± 3.06 fL in macrocytic anemia, and 93.97 ± 1.27 fL in dimorphic anemia. These findings are consistent with Buttarello, who described MCV as the principal conventional red cell parameter that has retained its importance for morphological classification of anemia with modern automated hematology analyzers. [1] The clear separation of MCV values between the microcytic, normocytic, and macrocytic groups in our study further supports its role as a rapid initial classification parameter. However, the relatively normal MCV observed in the dimorphic group illustrates an important limitation of relying exclusively on an average red cell volume, because the coexistence of differently sized red cell populations may result in a deceptively normal mean value.

The findings of the present study are also comparable with the observations of Thyagaraju et al., who specifically evaluated peripheral smear morphology, RBC indices, and automated analyzer-generated RBC histograms in the diagnosis of anemia. Their study emphasized the importance of considering RBC indices, histogram patterns, and microscopic peripheral smear findings together rather than interpreting any single parameter in isolation. [3] In their study of 500 cases, peripheral smear examination identified normocytic normochromic, microcytic hypochromic, macrocytic, normocytic hypochromic, and dimorphic patterns, while automated parameters and histograms showed broadly comparable morphological categorization. [3] Our findings support this integrated approach, as MCV showed strong discriminatory capability, while histogram analysis demonstrated an overall concordance of 94.8% with peripheral smear morphology.

Varghese et al. similarly investigated the efficacy of RBC histograms for identifying morphological types of anemia in comparison with peripheral smear examination. Their study included 354 adult samples and demonstrated the practical usefulness of histogram interpretation as an adjunct to peripheral smear assessment. [4] The high level of concordance observed in our study is consistent with the principle demonstrated by Varghese et al. that RBC histograms can provide useful information regarding the predominant red cell population and can assist in the rapid categorization of anemia. [4] However, our study also demonstrates that histogram interpretation should not be regarded as a complete substitute for microscopic assessment, particularly in cases in which the histogram and peripheral smear findings are discordant.

In the present study, MCH, MCHC, and RDW did not demonstrate statistically significant differences across the four morphological categories ($p > 0.05$). Although these parameters remain clinically useful, their discriminatory capacity may be reduced when different morphological abnormalities overlap within the same patient. Buttarello described MCV as the principal traditional parameter for morphological classification while also emphasizing the usefulness and limitations of additional red cell parameters such as RDW. [1] In our study, the dimorphic group showed the highest mean RDW ($22.67 \pm 3.06\%$), compared with $18.24 \pm 2.95\%$ in the normocytic group and $18.79 \pm 6.57\%$ in the microcytic group. The absence of statistical significance should therefore not be interpreted as indicating that RDW lacks clinical value; rather, the very small number of dimorphic cases ($n=3$) substantially limited the statistical power of this comparison.

The high overall concordance between RBC histograms and peripheral smear morphology represents one of the important findings of this study. The overall agreement was 94.8%, with 91 of 96 cases showing concordant histogram and peripheral smear classifications. Normocytic normochromic cases demonstrated 96.8% concordance, microcytic hypochromic cases demonstrated 92.3% concordance, and all macrocytic cases demonstrated concordance. The lower concordance observed in the dimorphic group (66.7%) is particularly important because mixed red cell populations are inherently more difficult to characterize using a single average numerical parameter. Constantino described the RBC histogram as an important component of automated hematology analysis and specifically highlighted its role in recognizing and interpreting dimorphic red cell populations. [6] The present findings support this observation, as two of the three dimorphic cases demonstrated broad-based or bimodal histogram patterns, whereas one showed a broad-based single peak. Thus, even when a classical double peak is absent, an abnormal broad-based distribution should prompt careful microscopic examination for mixed red cell populations.

The two microcytic cases that showed a normal histogram despite peripheral smear evidence of microcytosis and hypochromia further demonstrate the importance of recognizing borderline or early morphological abnormalities. Such cases illustrate that the histogram represents a population-based distribution of red cell volumes and may not always demonstrate a clearly abnormal pattern when the morphological alteration is mild. Thyagaraju et al. emphasized that automated indices and histograms should be correlated with peripheral smear examination for complete assessment of anemia. [3] Similarly, Varghese et al. demonstrated that although histogram analysis is useful for morphological categorization, peripheral smear examination remains an important comparative reference. [4]

These findings support the interpretation that automated histograms are most appropriately used as a rapid screening and adjunctive tool rather than as an independent replacement for microscopy.

The clinical importance of histogram interpretation was also emphasized by Thomas et al., who described automated hematology histograms as graphical representations of cell-size distributions that can provide diagnostically relevant information when appropriately interpreted. [7] Their work highlighted that histogram analysis is often underutilized despite its potential to provide useful information from routine automated haemogram data. [7] In the present study, the strong overall concordance between histogram patterns and peripheral smear morphology supports this concept and suggests that histogram review may enhance the diagnostic information obtained from routine automated CBC analysis without requiring additional specimen processing.

The peripheral blood smear nevertheless retains an essential role in hematological diagnosis. Adewoyin and Nwogoh described the peripheral blood film as an invaluable laboratory investigation for characterization of blood cell morphology and emphasized that, despite advances in hematology automation and molecular techniques, microscopic examination continues to have an important diagnostic role. [2] This is particularly relevant to the present study because automated numerical indices and histograms primarily describe quantitative or distributional characteristics of red cells, whereas peripheral smear examination allows direct assessment of qualitative abnormalities such as anisopoikilocytosis, target cells, spherocytes, schistocytes, cellular inclusions, and dysplastic changes. Therefore, the high histogram-PBS concordance observed in our study should be interpreted as evidence of complementary diagnostic performance rather than evidence that microscopy can be eliminated.

The practical role of automated analyzer outputs in determining which specimens require manual smear review has also gained increasing attention. Courville et al., in their systematic literature review, examined the performance of automated hematology analyzer criteria in detecting peripheral blood smear abnormalities and highlighted the importance of appropriate criteria for triggering microscopic review. [8] Their review reinforces the concept that automation can assist laboratories in identifying specimens requiring further examination, while the performance of such criteria needs to be evaluated and validated within the relevant laboratory setting. [8] Our findings are consistent with this approach, as discrepancies between automated indices, histogram morphology, and peripheral smear findings identify cases in which targeted microscopic review provides additional diagnostic information.

Pozdnyakova et al. further emphasized that peripheral smear review remains an important component of hematology laboratory practice despite modern automation and recommended that smear-review criteria should be tailored to the patient population, laboratory setting, instrumentation, and locally validated workflow. [9] This has direct relevance to the present study because the 94.8% overall concordance demonstrates that automated histogram interpretation can substantially reduce the need for indiscriminate microscopic review while still leaving a clinically important role for targeted smear examination. In particular, cases with discrepant numerical and graphical findings, abnormal analyzer flags, or suspected mixed populations should undergo manual peripheral smear assessment rather than being interpreted solely from automated parameters. [9]

The present study also has certain limitations. The macrocytic subgroup consisted of only five patients and the dimorphic subgroup of only three patients, thereby limiting the statistical power of comparisons involving these categories, particularly RDW and other secondary parameters. Furthermore, certain biological and analytical factors, including cold agglutinins, marked leukocytosis, reticulocytosis, and fragmented red cells, can potentially influence automated red cell measurements and histogram patterns. Courville et al. highlighted the importance of evaluating analyzer-based criteria in relation to their ability to identify clinically relevant smear abnormalities, while Pozdnyakova et al. emphasized the need for laboratory-specific validation of smear-review criteria. [8] [9] Therefore, the findings of the present study should be interpreted within the context of its sample size and single-study population. Larger multicentric studies involving adequate numbers of macrocytic and dimorphic cases would be useful to further establish the diagnostic performance and generalizability of RBC histogram interpretation.

Overall, the findings demonstrate that automated RBC indices and histograms provide a rapid and useful framework for the initial morphological assessment of adult anemia, with MCV emerging as the most statistically discriminating numerical parameter and RBC histograms showing high concordance with peripheral smear morphology. The findings are consistent with Buttarello's description of MCV as a key parameter in automated anemia classification, while the observations of Thyagaraju et al. and Varghese et al. support the complementary value of RBC histograms and peripheral smear examination. [1] [3] [4] Constantino's work further supports the particular usefulness of histogram interpretation in dimorphic red cell populations. [6] Thomas et al. demonstrated the broader diagnostic utility of histogram interpretation, while Adewoyin and Nwogoh reinforced the continuing importance of peripheral smear morphology. [2] [7] More recent literature from Courville et al. and Pozdnyakova et al. further supports an integrated laboratory strategy in which automated analyzer findings help identify specimens requiring targeted microscopic review rather than attempting to replace manual examination altogether. [8] [9]

5. Conclusion

Automated RBC volume histograms, particularly when interpreted alongside MCV and other red cell indices, provide a rapid, reproducible, and useful screening approach for the initial morphological classification of adult anemia. In the present study, MCV demonstrated the strongest statistical association with peripheral smear morphology, while RBC histogram patterns showed an overall

concordance of 94.8% with microscopic classification. These findings support the use of automated histogram interpretation as an important adjunct to routine CBC analysis. However, peripheral blood smear examination remains indispensable for identifying qualitative morphological abnormalities and for resolving discrepancies between automated numerical parameters, histogram patterns, and microscopic findings. [2] The findings of Thyagaraju et al., Varghese et al., and Constantino support the complementary role of automated histograms and peripheral smear examination, particularly in recognizing mixed or dimorphic red cell populations. [3] [4] [6] In accordance with the observations of Courville et al. and Pozdnyakova et al., an optimal laboratory strategy should therefore combine automated analyzer-based screening with appropriately targeted and locally validated peripheral smear review. [8] [9] Such an integrated approach preserves the speed and reproducibility of automated hematology while retaining the qualitative diagnostic precision of manual microscopy

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