A Comparative Diagnostic study of Red Blood Cell Indices, Red Blood Cell Histograms, and Peripheral Blood Smears for the Morphological Categorization of Adult Anemia

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Abstract— **Background: Accurate morphological classification is fundamental for directing the cost-effective diagnosis of anemia. While the peripheral blood smear (PBS) remains the traditional standard, automated analyzers provide quantitative red blood cell (RBC) indices and graphical RBC histograms. The optimal integration of these tools in clinical practice requires clearer definition.**

Objective: To perform a comparative diagnostic assessment of PBS, RBC indices, and RBC histograms for the morphological classification of anemia in adults.

Materials and Methods: In this prospective, cross-sectional study, 119 anemic adults were enrolled. Venous blood was analyzed for a complete blood count (CBC), RBC indices (MCV, MCH, RDW), and histograms. PBS examination was performed independently by two blinded pathologists. Statistical analysis evaluated associations using the Chi-square test and compared indices via ANOVA.

Results: A strong, statistically significant association ($\chi^2 = 142.7$, $p < 0.00001$) was found between RBC histogram patterns and PBS morphology, with an overall concordance of 92.4%. Normal Curve histograms primarily corresponded to Normocytic anemia, Left Shift to Microcytic Hypochromic, and Right Shift to Macrocytic anemia. The Broad/Bimodal histogram pattern was a specific indicator for complex cases. RBC indices varied significantly ($p < 0.0001$) across groups.

Conclusion: RBC histogram patterns show high concordance with PBS findings. When combined with RBC indices, the histogram is a reliable first-line screening tool. Discordant and Broad/Bimodal patterns effectively target samples requiring mandatory PBS review. We propose an integrated diagnostic algorithm that uses automated analysis for initial triage and strategically reserves PBS for complex cases, optimizing laboratory efficiency and diagnostic accuracy.

Index Terms— **Anemia, Diagnosis, Peripheral Blood Smear, Red Blood Cell Indices, RBC Histogram, Diagnostic Algorithm.**

I. INTRODUCTION

Anemia, a global public health challenge, is defined by a reduction in hemoglobin concentration, leading to impaired oxygen delivery. The World Health Organization estimates a prevalence of 24.8% worldwide, with significantly higher burdens in developing regions. Accurate initial morphological classification distinguishing microcytic, normocytic, and macrocytic types is the critical first step in guiding an efficient and targeted etiological investigation.

The diagnostic pathway relies on three complementary modalities. The Peripheral Blood Smear (PBS) examination is the historical gold standard, allowing direct visualization of RBC size, shape, and hemoglobin content. RBC Indices, including Mean Corpuscular Volume (MCV) and Red Cell Distribution Width (RDW), provide objective, quantitative data from automated hematology analyzers. The RBC Histogram, a graphical output of RBC volume distribution, offers immediate visual pattern recognition, such as a left shift indicating microcytosis.

While PBS is comprehensive, it is labor-intensive and subject to inter-observer variability. Automated indices and histograms are rapid and reproducible but

may not detect specific morphological abnormalities. Recent studies, such as those by Patel et al. (2024) and Rajurkar et al. (2024), have reaffirmed the value of histograms in anemia screening but also note instances of discordance with PBS, underscoring the need for clear integration protocols. This study aims to provide a contemporary comparative assessment and propose a data-driven algorithm for synergistically using these tools in a routine clinical setting.

II. MATERIALS AND METHODS

2.1 Study Design and Setting

A prospective, observational, cross-sectional study was conducted over eight months in the Department of Pathology at a tertiary care hospital, following approval from the Institutional Ethics Committee. Written informed consent was obtained from all participants.

2.2 Study Population

A total of 119 consecutive adult patients (>18 years) with anemia, as per WHO criteria (Hb <13 g/dL for men, <12 g/dL for non-pregnant women), were enrolled. Patients with a history of blood transfusion within the prior 120 days, known hemoglobinopathies, or active hemolysis were excluded.

2.3 Sample Collection and Laboratory Analysis

Venous blood was collected in K3-EDTA vacutainers and processed within 2 hours.

- Automated Hematological Analysis: A complete CBC with RBC indices (MCV, MCH, RDW) was performed on a modern automated analyzer. The accompanying RBC volume histogram was saved for each sample.
- Peripheral Blood Smear Examination: Standard wedge smears were Leishman-stained and examined independently by two certified pathologists blinded to the automated data. RBC morphology was classified as Normocytic Normochromic, Microcytic Hypochromic, or Macrocytic.
- RBC Histogram Classification: Histograms were categorized into four patterns: Normal Curve (symmetrical, narrow), Left Shift (peak shifted left), Right Shift (peak shifted right), and Broad/Bimodal (increased base width or dual peaks).

2.4 Statistical Analysis

Data were analyzed using SPSS software version 26.0. Continuous variables were expressed as mean ± standard deviation (SD). The Chi-square (χ^2) test assessed the association between histogram patterns and PBS morphology. One-way Analysis of Variance (ANOVA) was used to compare mean RBC indices across different morphology groups. A p-value <0.05 was considered statistically significant.

III. RESULTS

3.1 Demographic and Baseline Characteristics

The study included 119 participants with a mean age of 44.1 ± 14.3 years. The cohort comprised 62 (52.1%) females and 57 (47.9%) males. The mean hemoglobin concentration for the cohort was 9.8 ± 1.4 g/dL.

3.2 Concordance between RBC Histogram and PBS Morphology

A strong and statistically significant association was observed ($\chi^2 = 142.7$, $p < 0.00001$). The overall concordance between histogram pattern and PBS morphology was 92.4% (110/119 cases). Key discordant findings provided valuable clinical insights (Table 1).

Table 1: Association between RBC Histogram Pattern and PBS Morphology (n=119)

Histogram Pattern	Normocytic (PBS)	Microcytic Hypochromic (PBS)	Macrocytic (PBS)	Total
Normal Curve	48	2	0	50
Left Shift	3	49	1	53
Right Shift	0	0	8	8
Broad/Bimodal	2	5	1	8
Total	53	56	10	119

Note: Bold values indicate concordant cases.

Discordant findings included:

- Two cases with a normal histogram showed subtle hypochromia on PBS (suggestive of early iron deficiency).
- Three cases with a Left Shift histogram were classified as normocytic on PBS but had markedly low MCH, raising suspicion for thalassemia trait.
- All eight Broad/Bimodal histograms corresponded to complex PBS findings: five showed dimorphic populations, two marked anisocytosis, and one was related to technical artifact.

3.3 RBC Indices Across PBS Morphology Groups

RBC indices showed highly significant variation ($p < 0.0001$ for all) across the three primary PBS morphology groups, providing quantitative validation (Table 2).

Table 2: Red Blood Cell Indices (Mean ± SD) by PBS Morphology Group

PBS Morphology Group	MCV (fL)	MCH (pg)	RDW (%)	Characteristic Pattern
Normocytic. (n=53)	89.5 ± 4.2	29.1 ± 1.8	14.1 ± 1.5	Indices within reference limits.
Microcytic (n=56)	72.8 ± 3.9	22.4 ± 2.1	18.9 ± 2.3	Low MCV, Low MCH, High RDW (IDA pattern). Subgroup with thalassemia trait had normal RDW (13.5±0.8).
Macrocytic (n=10)	112.4 ± 5.1	35.2 ± 1.9	13.8 ± 1.2	High MCV, High MCH, Normal RDW.
ANOVA p-value	<0.0001	<0.0001	<0.0001	

IV. DISCUSSION

This study demonstrates a high degree of concordance (92.4%) between automated RBC histogram patterns and PBS examination for the primary morphological classification of anemia. This supports the findings of recent literature, such as the work by Varghese et al. (2023), which highlights the efficacy of histograms in anemia diagnosis. However, the clinically instructive 6.7% discordance rate and the identification of a distinct Broad/Bimodal pattern form the basis for a more nuanced, integrated diagnostic model.

4.1 Clinical Interpretation of Discordance and Pattern Specificity

The observed discordance is not a failure of either method but rather reveals their complementary strengths. The two cases with a "normal" histogram but hypochromic PBS underscore that histograms primarily reflect cell volume, while PBS assesses hemoglobinization. The three cases with a left-shift histogram but normocytic PBS and very low MCH are classic of thalassemia trait, where a uniform population of small, hemoglobin-deficient cells may not trigger a pronounced left shift, but indices are revealing.

Most significantly, the Broad/Bimodal histogram emerged with 100% positive predictive value for identifying samples requiring expert PBS review. This pattern acted as an automated flag for complex cases such as dimorphic anemia (e.g., iron deficiency during iron therapy), mixed deficiencies, or significant anisocytosis, which are easily missed by indices alone.

4.2 Proposal for a Synergistic, Data-Driven Diagnostic Algorithm

Based on our findings, we propose a refined diagnostic workflow that optimizes efficiency without compromising accuracy (Figure 1):

1. Automated First Pass: All samples undergo CBC analysis with generation of indices and RBC histogram.
2. Intelligent Triage:
   - Path 1 - Clear-Cut Cases: For the majority (~92%) with concordant Normal/Left/Right Shift patterns and supporting indices, the automated report can directly guide initial management (e.g., ordering iron studies for microcytic anemia with high RDW).
   - Path 2 - Mandatory PBS Review: PBS is reflexively performed for:
     - Any Broad/Bimodal histogram.
     - Any discordance between histogram pattern, RBC indices, and clinical context (e.g., left shift + normal RDW).
     - Specific analyzer flags for abnormal cells or distributions.

3. PBS as a Referee and Investigator: In this model, PBS transitions from a screening tool to a targeted investigator, resolving ambiguity, identifying specific pathognomonic features (e.g., schistocytes, spherocytes), and confirming complex morphologies.

This algorithm is supported by the work of Pursnani et al. (2025), who emphasized the role of histograms in guiding which cases necessitate detailed smear examination, thereby improving laboratory workflow.

4.3 Study Limitations and Future Directions

This study is limited by its single-center design. The morphological classification is a preliminary step; definitive diagnosis requires correlation with iron studies, vitamin assays, or hemoglobin electrophoresis. Future prospective, multi-center studies should validate this integrated algorithm's impact on diagnostic turnaround time, cost-effectiveness, and, ultimately, patient management outcomes.

V. CONCLUSION

RBC histogram patterns show high diagnostic concordance with PBS findings for classifying anemia. The combination of the visual histogram and quantitative RBC indices provides a robust, rapid, and objective first-line screening tool. Critically, discordant cases and the Broad/Bimodal histogram pattern serve as effective triggers for mandatory PBS review. We advocate for an integrated, synergistic diagnostic model where automated analysis performs initial high-volume triage, and PBS is strategically deployed as a powerful confirmatory and investigative tool for complex or flagged cases. This data-driven approach optimizes resource utilization in the hematology laboratory, ensuring both efficiency and diagnostic precision.

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