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Original Research Article

Towards smear-free anemia diagnosis: evidence from a high-volume laboratory

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ABSTRACT

Background: Objectives of the study were to evaluate whether peripheral smear (PS) examination is essential in all cases of moderate anemia, or if automated hematology analyzer parameters—particularly RBC indices, red cell distribution width (RDW), and reticulocyte count can provide reliable diagnostic information.
Methods: This retrospective audit was conducted in the hematology laboratory of a tertiary care teaching hospital over six months. A total of 707 consecutive cases of moderate anemia (hemoglobin 8–10 g/dl) were included. Cases were classified by automated RBC indices into microcytic, macrocytic, normocytic normochromic (NCNC), or dimorphic categories. Peripheral smears were reviewed independently by two hematopathologists. RDW and reticulocyte count were analyzed for additional diagnostic utility. Concordance between analyzer-based and smear-based classifications was evaluated using contingency tables and Chi-square testing (χ^2=504, df=24, $p<0.001$). One-way ANOVA with post-hoc Tukey analysis assessed differences in RDW values across anemia subtypes.
Results: Microcytic anemia demonstrated the highest concordance between analyzer and smear findings (83.8%), followed by macrocytic (56%), dimorphic (40.8%), and NCNC anemia (17.5%). Hemolytic anemia showed no concordance. Elevated RDW values effectively flagged hidden mixed morphologies, while reticulocyte counts were useful in distinguishing regenerative states. Dimorphic anemia exhibited significantly higher RDW compared to both microcytic and macrocytic anemia ($p<0.05$).
Conclusion: Selective smear review, guided by RBC indices, RDW, and reticulocyte counts, ensures diagnostic accuracy while reducing workload. Universal smear policies are impractical in high-volume laboratories, and targeted approaches provide an evidence-based balance between accuracy and efficiency.

Keywords: Anaemia, Red cell indices, Peripheral smear, Reticulocytes, Haematology analyzer

INTRODUCTION

Anaemia remains a leading cause of global morbidity and mortality, affecting an estimated 1.6 billion people worldwide.[1] Correct classification is critical for diagnosis and management. Automated haematology analysers provide standardized red blood cell (RBC) indices including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) which are fast and reproducible.[2] However, analysers depend on averages and may fail to detect mixed or evolving morphologies.[3]

Peripheral smear examination continues to be the morphological gold standard, detecting anisocytosis, polychromasia, parasites, dual populations, or haemolytic features.[4] Nonetheless, universal smear review is impractical in high-volume tertiary centres with heavy workloads.[5] Modern consensus including the International Council for Standardization in Haematology (ICSH) guidelines supports combining analyser flags, red cell distribution width (RDW), reticulocyte counts, and targeted smear review to maintain diagnostic accuracy while optimizing efficiency.[5,6] This study aimed to evaluate whether peripheral smear examination is

mandatory in all cases of moderate anaemia or if automated haematology parameters—particularly RBC indices, RDW, and reticulocyte count—can provide sufficient diagnostic information. By assessing the concordance between analyser-based classifications and peripheral smear findings, the study seeks to identify subsets of moderate anaemia where smear review may be safely omitted. The goal is to propose an evidence-based, selective smear review protocol that balances diagnostic accuracy with operational efficiency in high-volume laboratory settings.

Aim and objectives

This study aims to evaluate whether peripheral smear (PS) examination is essential in all cases of moderate anaemia or if automated haematology analyser parameters—specifically RBC indices, RDW, and reticulocyte count—can reliably guide morphological classification.

Objectives of the study were to assess the concordance between analyser-based classification and peripheral smear findings in moderate anaemia, to evaluate the utility of RDW and reticulocyte count in identifying mixed or regenerative anaemia patterns, and to identify subsets of moderate anaemia where peripheral smear review may be safely omitted without compromising diagnostic accuracy.

METHODS

This retrospective audit was conducted in the haematology laboratory of University College of Medical sciences and Guru Teg Bahadur hospital, a tertiary care teaching hospital over a six-month period (January 2024 to June 2024). A total of 707 consecutive cases of moderate anaemia, (8-10 g/dl) as defined by WHO criteria, were included. Only cases with complete haematology analyser data for red cell indices (MCV, MCH, MCHC), RDW, and reticulocyte count, along with an adequately prepared and interpretable peripheral smear, were considered eligible. Cases with mild (Hb>10 g/dl) or severe anaemia (Hb<8 g/dl), incomplete analyser data, or inadequate smear quality were excluded. Samples showing analytical or instrumental errors, or obtained from patients with known haematological malignancies, recent blood transfusion within three weeks, or those receiving cytotoxic therapy were also excluded to avoid confounding of red cell indices. Each case was classified based on MCV from the automated haematology analyser into microcytic (<80 fl), normocytic normochromic (NCNC; 80–100 fl), or macrocytic (>100 fl) categories. Dimorphic anaemia was flagged by the analyser when scatterplots or elevated RDW suggested dual red cell populations. Peripheral smear examination was performed on Wright-stained blood films by two independent hematopathologists blinded to the analyser data. Additional parameters, including red cell distribution width (RDW-CV, %) and reticulocyte count (%), were extracted from the master dataset. Concordance between automated classifications and smear-based morphological diagnosis was assessed using cross-tabulated contingency matrices. Discordance rates and reasons were recorded to evaluate the diagnostic utility of automated parameters in predicting morphological patterns.

Statistical analysis

Data were analysed using IBM statistical package for the social sciences (SPSS) statistics version 25.0 (IBM Corp., Armonk, NY) and Python (SciPy and Stats Models libraries). Descriptive statistics (mean, standard deviation) were computed for all quantitative parameters including RDW-CV and reticulocyte count across anaemia subtypes as defined by haematology analyser classifications. Concordance between automated classifications and peripheral smear findings was evaluated using cross-tabulated contingency tables, and the Chi-square (χ^2) test was applied to determine statistical significance of association (χ^2=504, df=24, p<0.001).

One-way ANOVA was performed to compare RDW-CV values across the five anaemia categories.

RESULTS

Study cohort

A total of 707 consecutive cases of moderate anaemia were included in the analysis. Based on automated haematology analyser indices, the majority were classified as microcytic hypochromic anaemia, followed by dimorphic anaemia normocytic normochromic anaemia. RBC indices threshold is based on our laboratory criteria. Peripheral smear was treated as the gold standard for morphological diagnosis. Moderate anaemia cases were recorded on analyser as well as peripheral smear (Table 1).

Table 1: Case distribution of moderate anemia based on analyzer and peripheral smear findings (n=707).

Anemia type	N (analyzer)	% (analyzer)	N (peripheral smear)	% (peripheral smear)
MCHC (microcytic)	357	50.5	377	53.3
Macrocytic	45	6.4	43	6.1
NCNC (normocytic)	57	8.1	187	26.4
Dimorphic	243	34.4	71	10.0
Hemolytic	5	0.7	7	1.0
Others/poor smear	–	–	22	3.1
Total	707	100.0	707	100.0

Concordance between analyzer and peripheral smear

The overall concordance between analyser-based classification and PS diagnosis varied by anaemia subtype. Microcytic hypochromic anaemia demonstrated the highest agreement, with 83.8% of analyser-flagged cases confirmed on smear. In contrast, concordance was markedly lower for macrocytic anaemia (56%), dimorphic anaemia (40.8%), and NCNC anaemia (17.5%). Haemolytic anaemia showed no concordance, as all analyser-flagged cases were reclassified on smear (Table 2). The overall association between analyser and smear findings was statistically significant (χ^2=504, df=24, p<0.001) (Table 3).

Table 2: Concordance between analyser classification and peripheral smear findings.

Analyzer type	N (analyzer)	Confirmed on PS (N)	Concordance (%)	Top discordant PS categories
MCHC	357	299	83.8	Dimorphic (29); NCNC (18)
Macrocytic	45	24	56.0	NCNC (5); dimorphic (8)
NCNC	57	33	17.5	Dimorphic (130); macrocytic (3)
Dimorphic	243	29	40.8	NCNC (130); MCHC (60)
Hemolytic	5	0	0.0	Reclassified as MCHC/NCNC

Table 3: Association between analyser classification and smear findings (Chi-square test).

Test	χ^2	df	P value	N
Chi-square (analyzer versus PS categories)	504	24	<0.001	707

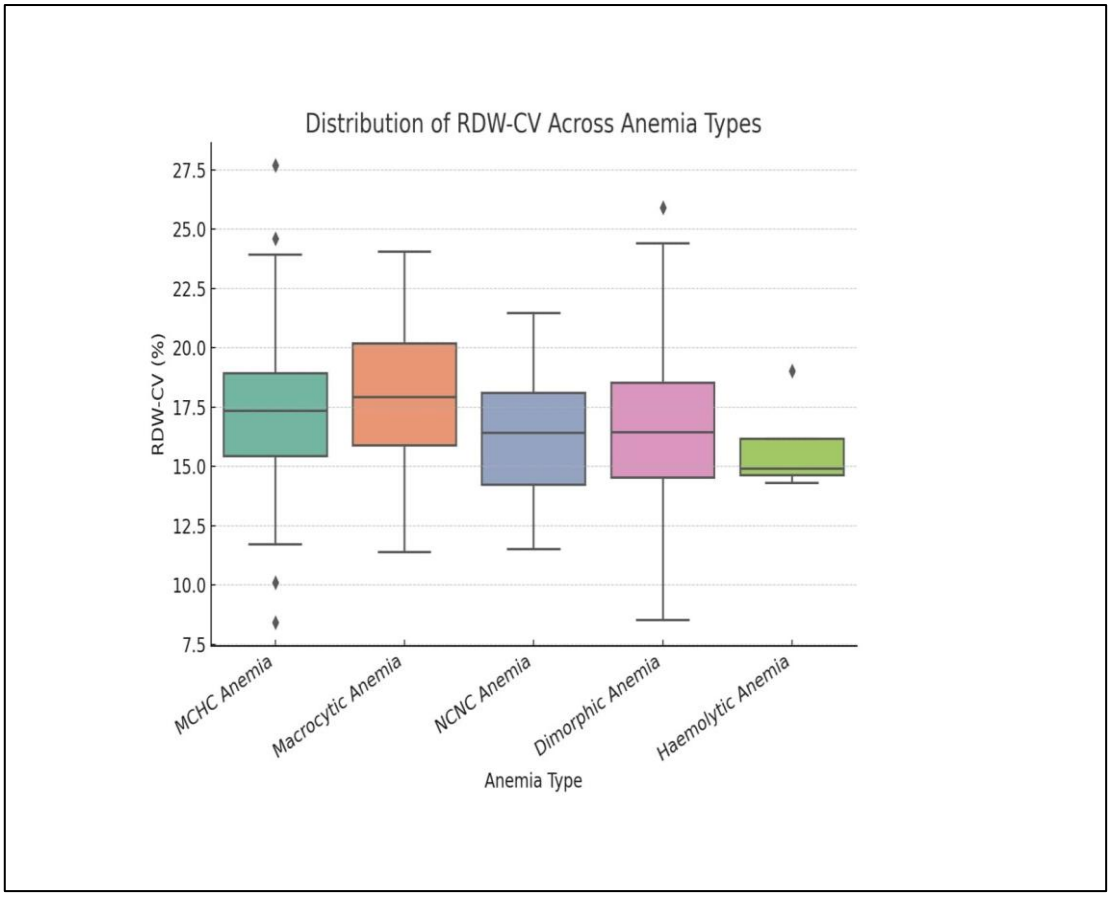

Figure 1: Distribution of RDW-CV across anemia types.

RDW and reticulocyte analysis

Mean RDW values differed across anaemia types, with the highest values observed in haemolytic (17.7±2.2%) and macrocytic anaemia (17.6±2.9%) (Table 4). One-way ANOVA confirmed significant intergroup variation in RDW (p<0.001).

Table 4: RDW-CV and reticulocyte percentage across analyser-defined anaemia types.

Analyzer type	RDW-CV (mean±SD)	Reticulocytes % (mean±SD)
MCHC	17.23±2.72	2.52±0.88
Macrocytic	17.64±2.94	2.59±1.33
NCNC	16.49±2.41	2.40±0.85
Dimorphic	16.65±3.01	2.47±0.91
Hemolytic	17.70±2.18	2.50±0.63

Post-hoc Tukey analysis revealed that dimorphic anaemia had significantly higher RDW compared to both MCHC anaemia (mean difference=0.70; 95% CI: 0.07–1.34; p=0.020) and macrocytic anaemia (mean difference=1.46; 95% CI: 0.22–2.69; p=0.011). No statistically significant differences were noted between other pairwise groups.

The boxplot demonstrates significant intergroup variation (ANOVA, p<0.001), with dimorphic anaemia showing higher RDW values compared to MCHC and macrocytic anaemia (Tukey HSD, p<0.05) (Figure 1).

Reticulocyte percentages did not differ significantly between anaemia types (mean range: 2.4–2.6%, p>0.05), suggesting limited discriminatory value in moderate anaemia when analysed in isolation (Table 4).

DISCUSSION

An accurate morphological classification of anaemia remains fundamental to the workup of haematological disorders and directly influences diagnostic and treatment pathways.[1] While automated haematology analysers have dramatically increased efficiency and standardization in high-volume laboratories, this audit highlights persistent diagnostic gaps when relying solely on RBC indices. Our real-world data show that integrating peripheral smear review with additional analyser parameters such as RDW and reticulocyte count remains essential for comprehensive anaemia workup, especially in moderate anaemia where hidden mixed patterns are common.[2,3]

In our series, microcytic hypochromic anaemia demonstrated the highest concordance (83.8%) between analyser classification and smear findings. This aligns with widely published evidence that MCV is reliable for classic iron deficiency anaemia (IDA) because affected red cells are uniformly small and hypochromic.[1,4] However, the notable 16% discordance is clinically significant, as these mismatches were predominantly due to hidden mixed states such as iron deficiency coexisting with emerging B12 or folate deficiency, or early treatment response leading to partial correction.[1] It is well recognized that the MCV, as an average, can mask these transitional states.[2] In this subgroup, a high RDW was an excellent indicator of hidden heterogeneity in cell size, echoing Garg et al recommendations that RDW should always be interpreted alongside MCV flags to detect dual populations.[5,6] Pure microcytic anaemia typically shows a low or normal reticulocyte count. Therefore, an unexpected elevation in reticulocyte count in a microcytic case should prompt suspicion of concurrent blood loss or recovery (underlying haemolysis), which must be confirmed on smear.[1,4]

Macrocytic anaemia demonstrated a lower concordance of 56%, supporting prior literature that MCV alone cannot differentiate between megaloblastic anaemia and regenerative macrocytosis.[1,2] This is especially relevant because regenerative macrocytosis may occur in response to recent haemorrhage or haemolysis, which needs to be morphologically distinguished from classic megaloblastic changes. Our dataset confirmed that macrocytic cases with elevated RDW often represented mixed anaemia — a feature well described by Bain and Hoff brand.[1,2] In such cases, reticulocyte count offered additional clarity: a high reticulocyte count pointed to a regenerative response, while megaloblastic macrocytosis is typically reticulocytopenia until therapy begins.[2] Peripheral smear review proved crucial here by revealing hallmark macro-ovalocytes and hyper segmented neutrophils indicative of B12 or folate deficiency.[1]

Normocytic normochromic anaemia presented the greatest diagnostic challenge, with only 17.5% of analyser-classified cases matching final smear morphology. This major discordance is consistent with the fact that MCV can remain normal when mixed deficiencies balance each other out, masking an underlying dimorphic state.[1,3,5] Studies like those by Briggs et al have emphasized that normocytic indices should never be interpreted in isolation because they frequently conceal iron-B12 dual deficiencies, early marrow dysplasia, or a haemolytic response.[3] In our audit, many NCNC cases showed high RDW or an unexpected reticulocytosis. These findings were critical, as they flagged subtle heterogeneity and active marrow response, which the smear confirmed as mixed or evolving macrocytic states. Such cases underline the indispensable role of the smear in verifying true morphology and ruling out overlooked conditions like early haemolysis.[2,4]

The dimorphic anaemia category illustrates the complexity of relying solely on analyser scatterplots and RDW. While the analyser flagged 71 cases as dimorphic, only 41% were confirmed on smear. This overcall mirrors reports by Asghar et al, who found that RDW alone often misclassifies simple anisocytosis as true dimorphism.[7] Our audit reiterates that while RDW is sensitive for size variation, it cannot specify whether distinct cell populations exist. Only a skilled smear review can visualize two clearly separate red cell populations or confirm the presence of microcytic and macrocytic cells side by side.[1,2] This finding reinforces the ICSH's long-standing guidance that RDW should not replace direct morphology for diagnosing dimorphic states.[5,6]

Perhaps most striking was the haemolytic anaemia subgroup. Here, analyser-based classification

demonstrated zero concordance with smear findings. The analyser flags haemolysis indirectly through high RDW and elevated reticulocyte counts, which indicate increased marrow turnover.[1] However, only the smear can directly visualize hallmark features such as schistocytes, spherocytes, Heinz bodies, or malaria parasites.[2,4] Our audit showed that all five analyser-flagged haemolytic cases were reclassified on smear as microcytic or normocytic without haemolytic morphology. This result aligns with the WHO's position and ICSH recommendations that morphological examination remains the gold standard for diagnosing haemolytic anaemia and should never be omitted.[4,5]

Limitations

This study was retrospective and limited to cases of moderate anaemia, potentially underrepresenting the full spectrum of anaemic disorders. The reliance on a single-institution dataset may restrict generalizability to different population profiles or laboratory setups. Cases with severe anaemia and known haematological disorders were excluded, which could limit the diagnostic diversity. Furthermore, the interpretation of peripheral smears, though performed independently by two haematopathologists, remains observer-dependent. Finally, the audit did not include biochemical correlation (iron studies, B12, folate), which could have provided additional confirmation of the morphological subtypes.

CONCLUSION

This real-world audit confirms that selective smear protocols, combined with smart use of RBC indices, RDW, and retic count, preserve diagnostic accuracy while managing workload. Blanket smear policies are unsustainable; evidence-based triage ensures hidden mixed anaemia or haemolysis is not overlooked.

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