BIOMEDICINE AND CHEMICAL SCIENCES
AN INTERNATIONAL PEER-REVIEWED JOURNAL

BIOMEDICINE AND CHEMICAL SCIENCES
Website: https://journals.irapa.org/index.php/BCS
e-ISSN: 2790-296X | p-ISSN:2957-5826

Original Article

Clinicopathological Study of Anemia Patterns and Their Correlation with Peripheral Blood Smear Findings

Corresponding Author:
Dr Bed Vishwasbhai Chhaganbhai
Assistant Professor, Department of Pathology, MPKB Medical College and Research Centre, Atkot, Gujarat, India

Article History:
Received on: Aug 06, 2026
Revised on: Aug 20, 2026
Accepted on: Sep 21, 2026
Published on: Sep 23, 2026

Citation: Dr Pooja Dilipsingh Solanki *et al*. Clinicopathological Study of Anemia Patterns and Their Correlation with Peripheral Blood Smear Findings. *Biomedicine and Chemical Sciences, 5 (3): 226-231, 2026*

Dr Pooja Dilipsingh Solanki[1], Dr Varun Bharatbhai Panchal[2], Dr Bed Vishwasbhai Chhaganbhai[3*]

[1]Assistant Professor, Department of General Medicine, Baroda Medical college, Vadodara, Gujarat, India

[2]JR/Tutor, Department of Pathology, Sardar Patel Medical College and Research Centre, Ahmedabad, Gujarat, India

[3]Assistant Professor, Department of Pathology, MPKB Medical College and Research Centre, Atkot, Gujarat, India

ABSTRACT

Background: Anemia is morphologically heterogeneous and automated red-cell indices do not always provide the information that can be seen on a well-prepared peripheral blood smear. The use of hematologic indices in conjunction with smear morphology may facilitate etiologic classification and help direct laboratory testing.

Objective: To describe clinicopathological features of anemia and to establish the relationship between automated red-cell indices, peripheral smear morphology and the final etiologic diagnosis.

Methods: Two hundred and forty consecutive adults with hemoglobin levels below sex-specific reference values were included in a cross-sectional clinicopathological study. Clinical data, complete blood count, red-cell indices, reticulocyte count and peripheral smear findings were noted. Iron profile, B12/folate, hemolysis tests, hemoglobin analysis, and other tests were done if clinically indicated. Automated indices and independently by smear morphology, anemia was classified as microcytic, normocytic, macrocytic or dimorphic.

Results: Microcytic anemia was the most common pattern (116/240, 48.3%), followed by normocytic (76/240, 31.7%), macrocytic (29/240, 12.1%), and dimorphic anemia (19/240, 7.9%). The most common etiologic diagnosis was iron deficiency (105/240, 43.8%). There was a strong association between microcytosis with hypochromia and low mean corpuscular volume and low ferritin ($p<0.001$). Macro-ovalocytes with hypersegmented neutrophils were strongly associated with vitamin B12/folate deficiency ($p<0.001$). There was agreement between automated index classification and smear-based morphology in 205 cases (85.4%) with a weighted kappa of 0.78. Peripheral smear review reclassified 21 cases with borderline indices (early IDA, mixed nutritional anemia, hemolysis). There was a positive correlation between RDW and anisocytosis grade ($r=0.64$, $p<0.001$).

Conclusion: Peripheral blood smear examination provides clinically valuable morphologic data in addition to automated red-cell indices, especially in borderline, mixed, macrocytic and hemolytic patterns. Interpretation is enhanced when combined with other tests.

Keywords: anemia; peripheral blood smear; red-cell indices; microcytosis; macrocytosis; morphology; iron deficiency.

INTRODUCTION

Anemia is one of the most common hematologic disorders in the world and is a common end point of a variety of nutritional, inflammatory, renal, marrow, hemolytic, and genetic disorders. It is still a significant problem worldwide in both age groups and regions, and the etiology of iron deficiency is variable, depending on the population and clinical context, with a large proportion being due to iron deficiency [1]. Anemia is a symptom and not a disease, so it is important to have a correct initial classification for effective investigation and treatment.

The modern CBC offers reproducible values for hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin, red-cell distribution width (RDW), and parameters related to the reticulocytes. These indices can be used to quickly classify into microcytic, normocytic, and macrocytic patterns and help to limit the differential diagnosis [2]. For microcytic anemia, for instance, iron deficiency, thalassemia, chronic inflammation and certain sideroblastic processes are important considerations. DeLoughery noted that morphology and iron studies must be used together as the MCV alone is not a reliable indicator of the major causes of microcytosis [3].

The peripheral blood smear is an important addition to the complete blood count, despite the advances in automation. Anisocytosis, poikilocytosis, hypochromia, target cells, elliptocytes, spherocytes, schistocytes, polychromasia, nucleated erythrocytes, macro-ovalocytes and red-cell inclusions can be identified by careful examination. Ford emphasized that red-cell morphology can influence the likelihood of a diagnosis of thalassemia in microcytosis, suggest hemolytic mechanisms in normocytic anemia, and differentiate megaloblastic from non-megaloblastic macrocytosis [4]. Therefore, automated parameters and morphology are complementary and not competing.

The purpose of the present study was to outline the clinicopathological spectrum of anemia in an adult hospital population and to assess the agreement between automated red-cell indices and peripheral blood smear classification. Another goal was to determine if there was a correlation between specific smear findings and common etiologic diagnoses and laboratory markers, to identify situations where manual smear review adds value to the diagnosis.

MATERIALS AND METHODS

The cross sectional clinicopathological study was carried out in the Department of Pathology in association with medical and surgical outpatient and in-patient services. Eligible were consecutive adults aged 18 years or older whose hemoglobin was below the laboratory reference value. Patients who had received red-cell transfusion in the last four weeks were excluded as the transfused cells might significantly affect the morphology and red-cell indices. Samples that had excessive clotting, were not processed within acceptable preanalytical time limits, or were missing clinical information were also excluded.

Age, sex, major symptoms, dietary history, gastrointestinal or menstrual blood loss, chronic inflammatory disease, renal disease, liver disease, medication history and previous hematologic diagnosis were documented for each participant. Venous blood collected in EDTA was analyzed on an automated hematology analyzer within four hours of collection. Hemoglobin, hematocrit, erythrocyte count, MCV, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, RDW, leukocyte count, platelet count, and reticulocyte count were recorded. The severity of anemia was classified as mild, moderate or severe based on locally agreed laboratory and clinical criteria.

Fresh EDTA blood was used to prepare peripheral smears which were stained with Leishman or Wright-Giemsa stain. The smears were independently examined by two pathologists or trained hematology observers, who were blinded to the final etiologic diagnosis. The following parameters were recorded: red-cell size, central pallor, anisocytosis, poikilocytosis, polychromasia, rouleaux, agglutination, nucleated erythrocytes, and characteristic cell forms. The predominant morphology was microcytic hypochromic, normocytic normochromic, macrocytic or dimorphic. The degree of anisocytosis and poikilocytosis were estimated semiquantitatively. Macro-ovalocytes and hypersegmented neutrophils were thought to be supportive of megaloblastic hematopoiesis, and schistocytes, spherocytes, polychromasia and nucleated red cells indicated hemolysis or marrow stress.

Further investigations were chosen based on clinical and morphologic data. Iron studies consisted of serum ferritin, serum iron, total iron-binding capacity, and transferrin saturation. Vitamin B12 and folate levels were determined in macrocytic or unexplained anemia. When hemolysis was suspected, bilirubin, lactate dehydrogenase, haptoglobin, and direct antiglulin testing were used, as well as reticulocyte response. Hemoglobin analysis was done for suspected thalassemia or

hemoglobinopathy. Renal, hepatic, thyroid, inflammatory and bone marrow investigations were performed as clinically indicated. A final etiologic category was assigned after integration of clinical and laboratory data.

Anemia pattern and etiology were analysed using descriptive statistics. Analysis of variance or Kruskal-Wallis test was used to compare continuous variables and chi-square test was used to compare categorical variables. The agreement of automated MCV classification with smear-based morphology was evaluated by weighted kappa. Spearman or Pearson correlation was used to assess relationships between RDW and anisocytosis grade and between MCV and smear-estimated cell size. The sensitivity, specificity or odds ratios were used to express the diagnostic association between characteristic smear findings and etiologic categories, as appropriate. A p value of < 0.05 was deemed statistically significant for two-tailed tests.

RESULTS

A total of 240 adults were included, comprising 139 women (57.9%) and 101 men (42.1%), with a mean age of 43.8 ± 16.7 years. Fatigue (72.1%), exertional dyspnea (38.8%), pallor (35.4%), and dizziness (28.3%) were the most frequently documented clinical features. On automated indices, 116 patients (48.3%) had microcytic anemia, 76 (31.7%) normocytic anemia, 29 (12.1%) macrocytic anemia, and 19 (7.9%) a mixed or dimorphic pattern. Table 1 shows the hematologic characteristics of these morphologic groups. Microcytic cases had the lowest mean MCV and mean corpuscular hemoglobin, whereas macrocytic cases had the highest MCV and a higher median RDW. Dimorphic anemia showed the widest RDW distribution.

Table 1. Hematologic profile according to anemia pattern

Parameter	Microcytic (n=116)	Normocytic (n=76)	Macrocytic (n=29)	Dimorphic (n=19)
Hemoglobin, g/dL	8.9 ± 1.8	9.6 ± 1.7	8.7 ± 1.9	8.3 ± 1.6
MCV, fL	68.4 ± 7.2	86.7 ± 4.6	108.9 ± 8.7	84.6 ± 12.8
MCH, pg	21.2 ± 3.1	29.1 ± 2.3	34.7 ± 3.8	27.2 ± 5.2
RDW, %	18.7 ± 3.2	15.1 ± 2.1	18.2 ± 3.5	22.4 ± 4.1
Reticulocytes, %	1.6 ± 0.9	2.1 ± 1.7	1.5 ± 0.8	2.0 ± 1.1
Severe anemia	34 (29.3%)	13 (17.1%)	9 (31.0%)	8 (42.1%)

Final clinicopathological diagnoses are summarized in Table 2. Iron deficiency anemia was the most frequent diagnosis (105/240, 43.8%), followed by anemia of chronic inflammation or chronic disease (50/240, 20.8%), megaloblastic anemia due to vitamin B12 and/or folate deficiency (30/240, 12.5%), hemolytic anemia (20/240, 8.3%), thalassemia trait or other hemoglobinopathy (16/240, 6.7%), and mixed nutritional or multifactorial anemia (19/240, 7.9%). Marked microcytosis and hypochromia were present in 83.8% of iron deficiency cases, while pencil cells or elliptocytes were identified in 61.0%. Target cells were significantly more frequent in thalassemia/hemoglobinopathy than in iron deficiency. Macro-ovalocytes and hypersegmented neutrophils occurred in 80.0% and 73.3% of megaloblastic cases, respectively. Polychromasia and nucleated red cells were enriched in hemolytic anemia.

Table 2. Peripheral smear findings by final etiologic diagnosis

Smear feature	Iron deficiency n=105	Chronic disease n=50	Megaloblastic n=30	Hemolytic n=20	Thal/Hbopathy n=16
Microcytosis/hypochromia	88 (83.8%)	18 (36.0%)	1 (3.3%)	2 (10.0%)	15 (93.8%)
Pencil cells/elliptocytes	64 (61.0%)	7 (14.0%)	4 (13.3%)	2 (10.0%)	5 (31.3%)
Target cells	12 (11.4%)	4 (8.0%)	2 (6.7%)	3 (15.0%)	13 (81.3%)
Macro-ovalocytes	2 (1.9%)	2 (4.0%)	24 (80.0%)	1 (5.0%)	0
Hypersegmented neutrophils	0	1 (2.0%)	22 (73.3%)	0	0
Polychromasia	3 (2.9%)	5 (10.0%)	2 (6.7%)	16 (80.0%)	3 (18.8%)
Schistocytes/spherocytes	0	1 (2.0%)	0	12 (60.0%)	0

Automated MCV-based and smear-based classifications agreed in 205 of 240 cases (85.4%), corresponding to a weighted kappa of 0.78 (Table 3). Smear review provided clinically relevant reclassification in 21 cases: eight patients with early iron deficiency had MCV values within the lower-normal range but definite hypochromia and anisopoikilocytosis; seven patients with combined iron and vitamin deficiency showed dimorphic populations despite near-normal mean MCV; and six patients with hemolysis had normocytic indices but prominent polychromasia, spherocytes, or fragmented cells. RDW correlated positively with anisocytosis grade ($r=0.64$, $p<0.001$), and MCV correlated with smear-estimated red-cell size

(r=0.72, p<0.001). Ferritin was significantly lower in microcytic hypochromic iron deficiency than in microcytic anemia of chronic disease (median 9 vs 72 ng/mL, p<0.001).

Table 3. Concordance and selected correlations

Analysis	Result	95% CI / statistic	p-value
Automated vs smear classification agreement	205/240 (85.4%)	Weighted κ = 0.78	<0.001
RDW vs anisocytosis grade	r = 0.64	Moderate-strong positive	<0.001
MCV vs smear-estimated cell size	r = 0.72	Strong positive	<0.001
Hypochromia predicting low ferritin	Sensitivity 84.8%	Specificity 78.2%	<0.001
Macro-ovalocytes + hypersegmentation for B12/folate deficiency	Sensitivity 70.0%	Specificity 96.7%	<0.001

DISCUSSION

This clinicopathological study illustrates the value of using automated indices in conjunction with peripheral smear morphology to improve the classification of anemia. Microcytic anemia was the most common form, and iron deficiency was the most common cause of anemia. There was good agreement between automated MCV-based categorization and smear classification, with the blood film providing extra information in about one tenth of the patients, especially when competing processes resulted in an apparently normal average cell size.

The high prevalence of iron deficiency is in line with its well known global significance. Camaschella highlighted that iron deficiency anemia is the most prevalent type of anemia in the world and that diagnosis should take into account the clinical source of iron loss, hemoglobin, cell indices, ferritin and transferrin saturation [5]. In the present study, iron deficiency was associated with a combination of low MCV, low MCH, high RDW, hypochromia and anisopoikilocytosis as expected. Supportive findings included pencil cells and elliptocytes, but these should not be used to exclude biochemical confirmation when possible.

The difference between iron deficiency and thalassemia trait is a good example of the importance of morphology in microcytosis. Both disorders can cause a low MCV, but the presence of target cells, a relatively normal red-cell count, and a disproportionate microcytosis should suggest thalassemia. The blood film is thus helpful in guiding the hemoglobin analysis when the automated indices are not clear. On the other hand, inflammatory anemia can be normocytic or slightly microcytic, and ferritin can be normal or high, as it is an acute-phase reactant. The clinical context, transferrin saturation, inflammatory markers and smear findings will still be required.

Macrocytic cases exhibited significant correlations with macro-ovalocytes, hypersegmented neutrophils and vitamin B12/folate deficiency. Aslinia et al. termed these features as classic clues to megaloblastic hematopoiesis and also mentioned that macrocytosis can be caused by alcohol, liver disease, medications, reticulocytosis, hypothyroidism, or marrow disorders [6]. A significant observation in the present study was the presence of several patients with combined nutritional deficiencies with a near normal mean MCV due to the presence of both microcytes and macrocytes. The smear showed the dimorphic population and broadened the diagnostic view to a more than one-index classification.

RDW was correlated with the semiquantitative anisocytosis, which confirmed the biological relationship between the automated red-cell volume variation and the visual anisocytosis. However, Buttarello noted that traditional red-cell indices such as MCV and RDW remain useful, and newer reticulocyte indices for the detection of iron-restricted erythropoiesis and monitoring treatment should be highlighted. Automated indices are objective and highly reproducible but are mathematically summarizing cell populations and can mask morphologically distinct subpopulations. The smear, on the other hand, shows those subpopulations directly [7].

This has been stated many times before: Blood-film value is not lost when the machine is automated. Peripheral smear examination was considered by Pierre to be a crucial step that guides the next step in the evaluation of anemia [8]. The current findings are in line with that in practice. Smear review reclassified cases in which the mean red-cell volume was not enough, such as early iron deficiency, mixed nutritional deficiency and hemolysis. The presence of polychromasia, spherocytes, and schistocytes was particularly significant as this may lead to immediate investigation for hemolysis, immune destruction, microangiopathy, or mechanical fragmentation.

Digital and computational assistance is also becoming more readily available for morphologic review. A methodological review of peripheral-smear image analysis reported advances in segmentation and automated red-cell classification, and highlighted problems with staining variation, overlapping cells, image quality, and generalizability to other laboratories [9]. The use of digital tools can eventually alleviate observer fatigue and standardise quantification, but in cases of complex morphology and/or when rare cells with clinical significance need to be identified, the expert microscopic review is still relevant.

The burden of anemia worldwide is etiologically complex and population level information should not be used to assume that all low hemoglobin levels are due to iron deficiency. Kassebaum et al. showed that there was significant geographic, age and sex variability in the causes of anemia [10]. Thus, a clinicopathological workflow must maintain diagnostic breadth. Normocytic anemia was seen in about one-third of the patients in the present cohort, such as chronic inflammation, renal disease, early nutritional deficiency, and hemolysis. The normal MCV should never be used as a negative clue to the clinically important anemia mechanisms.

There is much laboratory literature that supports the complementary role of morphology. A well-studied blood film can detect diagnostic patterns which are not adequately captured by numerical cell counts alone, Bain said [11]. Similarly, Tefferi et al. proposed a structured approach to the interpretation of the complete blood count (CBC) in which the red-cell indices, the clinical context, and the careful review of the smear are combined to interpret anemia [12]. Urrechaga et al. have reported that modern hypochromia and reticulocyte-based biomarkers are useful supplements to the traditional indices for the diagnosis of iron-restricted erythropoiesis [13]. Previous studies by Fairbanks demonstrated that the peripheral-film morphology is not sufficient for the diagnosis of iron deficiency alone [14]. In a similar study, Jen et al. concluded that the smear is most useful when used in conjunction with laboratory and clinical findings and not as a standalone test [15]. The present results therefore indicate that there is a place for a combination of automated indices for reproducible screening and microscopy for discordant, mixed, and morphologically distinctive cases.

There are some limitations to this study. It was conducted in a hospital setting, and so is not a measure of the prevalence in the community. Confirmatory testing was not done in all participants, and was conducted based on clinical indication, which may lead to verification bias. Even with a dual-observer review, the subjective aspect of smear grading cannot be avoided. Only selected cases underwent bone marrow examination and this was not a part of routine etiologic classification. The study also was conducted in adults and reference intervals and morphology are different in children and should be evaluated separately. The advantages are that it includes cases consecutively, incorporates clinical and biochemical data, allows for independent smear review, and provides for formal evaluation of the concordance between automated and morphologic classification.

CONCLUSION

In adults with anemia, the peripheral blood smear is still a valuable ancillary test to automated hematology analysis. Microcytic hypochromic anemia, mainly caused by iron deficiency, was the most common pattern, macrocytic and dimorphic morphologies were important clues to vitamin deficiency and mixed disease. There was good overall agreement between automated indices and smear classification, with the addition of valuable information in borderline and mixed cases by the manual morphology. A combination of these can help to enhance etiologic triage, limit unnecessary testing, and help to identify those who should be assessed specifically for nutritional deficiency, hemolysis, hemoglobinopathy, or marrow disease.

REFERENCES

1. Kassebaum NJ, Jasrasaria R, Naghavi M, et al. A systematic analysis of global anemia burden from 1990 to 2010. Blood. 2014;123(5):615-624. DOI: 10.1182/blood-2013-06-508325. PMID: 24297872.
2. Buttarello M. Laboratory diagnosis of anemia: are the old and new red cell parameters useful in classification and treatment, how? Int J Lab Hematol. 2016;38 Suppl 1:123-132. DOI: 10.1111/ijlh.12500. PMID: 27195903.
3. DeLoughery TG. Microcytic anemia. N Engl J Med. 2014;371(14):1324-1331. DOI: 10.1056/NEJMra1215361. PMID: 25271605.
4. Ford J. Red blood cell morphology. Int J Lab Hematol. 2013;35(3):351-357. DOI: 10.1111/ijlh.12082. PMID: 23480230.
5. Camaschella C. Iron-deficiency anemia. N Engl J Med. 2015;372(19):1832-1843. DOI: 10.1056/NEJMra1401038. PMID: 25946282.
6. Aslinia F, Mazza JJ, Yale SH. Megaloblastic anemia and other causes of macrocytosis. Clin Med Res. 2006;4(3):236-241. DOI: 10.3121/cmr.4.3.236. PMID: 16988104.
7. Kaferle J, Strzoda CE. Evaluation of macrocytosis. Am Fam Physician. 2009;79(3):203-208. PMID: 19202968.

8. Pierre RV. Red cell morphology and the peripheral blood film. Clin Lab Med. 2002;22(1):25-61. DOI: 10.1016/S0272-2712(03)00066-0. PMID: 11933577.
9. Navya KT, Prasad K, Singh BMK. Analysis of red blood cells from peripheral blood smear images for anemia detection: a methodological review. Med Biol Eng Comput. 2022;60(9):2445-2462. DOI: 10.1007/s11517-022-02614-z. PMID: 35838854.
10. Camaschella C. Iron deficiency: new insights into diagnosis and treatment. Hematology Am Soc Hematol Educ Program. 2015;2015:8-13. DOI: 10.1182/asheducation-2015.1.8. PMID: 26637694.
11. Bain BJ. Diagnosis from the blood smear. N Engl J Med. 2005;353(5):498-507. DOI: 10.1056/NEJMra043442. PMID: 16079373.
12. Tefferi A, Hanson CA, Inwards DJ. How to interpret and pursue an abnormal complete blood cell count in adults. Mayo Clin Proc. 2005;80(7):923-936. DOI: 10.4065/80.7.923. PMID: 16007898.
13. Urrechaga E, Borque L, Escanero JF. Biomarkers of hypochromia: the contemporary assessment of iron status and erythropoiesis. Biomed Res Int. 2013;2013:603786. DOI: 10.1155/2013/603786. PMID: 23555091.
14. Fairbanks VF. Is the peripheral blood film reliable for the diagnosis of iron deficiency anemia? Am J Clin Pathol. 1971;55(4):447-451. DOI: 10.1093/ajcp/55.4.447. PMID: 5554197.
15. Jen P, Woo B, Rosenthal PE, Bunn HF, Loscalzo A, Goldman L. The value of the peripheral blood smear in anemic inpatients: the laboratory's reading versus a physician's reading. Arch Intern Med. 1983;143(6):1120-1125. DOI: 10.1001/archinte.1983.00350060042005. PMID: 6860043.