

## Interpretation of RBC histogram and their correlation with peripheral smear findings in patients of Anemia

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### Abstract

**Background:** Anemia, a common blood disorder, is typically diagnosed and classified through various hematological tests, including RBC histograms and peripheral blood smears. This study aims to explore the correlation between RBC histogram patterns and peripheral smear findings in anemic patients, potentially offering insights into more precise diagnostic and classification methods. **Methods:** This retrospective study analyzed data from 1000 patients diagnosed with anemia. RBC histograms were obtained using standard hematology analyzers and peripheral smears were examined microscopically. Statistical analyses, including correlation coefficients, were used to assess the relationship between histogram patterns and smear findings. **Results:** Preliminary analysis revealed distinct histogram patterns correlating with specific smear observations, varying across different types of anemia. These patterns showed statistically significant associations with particular smear findings, suggesting potential diagnostic value. **Conclusion:** This study demonstrates significant correlations between RBC histogram patterns and peripheral smear findings in anemic patients. These findings could contribute to more accurate diagnosis and classification of anemia, enhancing patient management strategies.

**Keywords:** RBC Histogram Analysis, Peripheral Blood Smear, Anemia Diagnosis.

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## Introduction

RBC histograms, generated as a part of the complete blood count (CBC), provide valuable insights into the size variability and distribution of red blood cells, aiding in the initial screening and classification of anemia [4]. These histograms are particularly useful in identifying conditions such as microcytic and macrocytic anemias, where RBC size is a distinguishing feature [5]. However, the interpretation of RBC histograms requires careful correlation with other hematological parameters and clinical findings [6].

Peripheral blood smear examination, a time-honored technique, continues to play a crucial role in the diagnosis of anemia [7]. It provides detailed information on RBC morphology, allowing for the identification of specific anemia types, such as hemolytic anemia, megaloblastic anemia, or iron deficiency anemia [8]. The smear assessment can reveal changes such as anisocytosis, poikilocytosis, and the presence of abnormal RBC forms, which are often reflected in alterations of the RBC histogram [9].

Despite the diagnostic value of RBC histograms and peripheral smears, the correlation between these two parameters in various anemia types is not extensively studied. The current research aims to bridge this gap by analyzing the relationship between RBC histogram patterns and peripheral blood smear findings in a large cohort of patients diagnosed with anemia [10]. By doing so, this study seeks to enhance the understanding of hematological changes in anemia and improve the diagnostic accuracy for different anemia subtypes [11].

## Aim

To comprehensively analyze and elucidate the correlation between Red Blood Cell (RBC) histogram patterns and peripheral smear findings in patients diagnosed with various types of anemia.

## Objectives

1. To Evaluate the Diagnostic Value of RBC Histogram Patterns in Anemia.
2. To Correlate RBC Histogram Findings with Peripheral Blood Smear Results.
3. To Enhance the Clinical Approach to Anemia Diagnosis.

## Material and Methodology

- It is retrospective study of 1000 patients with hemoglobin less than 12 gm% from the pathology department of M.K.Shah Medical college and research centre, Ahmedabad. The samples were collected in EDTA vacutainer for Complete blood count (CBC). Peripheral smear was made at the time of sample collection and CBC was performed using Mindray BC 6000 hematology analyzer. Sample with hemoglobin less than 12gm% were selected and patient's Hb, RBC count, RBC indices and RBC histogram findings were recorded. Peripheral smears were fixed and stained with Giemsa stain for red blood cells morphology. Findings of RBC histogram and red cell morphology on smear were compared for each patient. Statistical analyses, including correlation coefficients, were used to assess the relationship between histogram patterns and smear findings.

## Observation and Results

**Table 1: Age with distribution of patient with anemia**

AGE (YEARS)	PATIENT WITH HEMOGLOBIN <12 gm%	PERCENTAGE OF TOTAL(%)

0-10	54	5.40%
11 – 20	97	9.70%
21-30	302	30.20%
31-40	243	24.30%
41-50	142	14.20%
51-60	105	10.50%
>60	57	5.70%
TOTAL	1000	100%

Table 1 presents the distribution of anemic patients categorized by age groups, based on hemoglobin levels below 12 gm%. The data encompass a total of 1000 patients. The age group of 21-30 years has the highest prevalence of anemia, comprising 30.20% of the total cases. This is followed by the 31-40 years group with 24.30%, and the 41-50 years group with 14.20%. The age groups of 51-60 and >60 years show relatively lower percentages, 10.50% and 5.70% respectively. Notably, the youngest age group (0-10 years) and the 11-20 years group have the lowest occurrences, accounting for 5.40% and 9.70% of the cases, respectively. This distribution highlights a significant variation in the prevalence of anemia across different age groups.

**Table 2: Sex wise distribution of patient with anemia**

	NO. OF CASES	PERCENTAGE (%)
MALE	303	30.3%
FEMALE	697	69.7%
TOTAL	1000	100%

Table 2 provides an overview of the sex-wise distribution of anemia patients in a study comprising 1000 cases. The data indicate a significantly higher prevalence of anemia in female patients, who constitute 69.7% (697 cases) of the total. In contrast, male patients represent a smaller proportion, accounting for 30.3% (303 cases) of the anemia cases. This disparity highlights a notable difference in the occurrence of anemia between the sexes, with females being more than twice as likely to be affected as males in this particular study sample.

**Table 3: Types of histogram abnormalities observed in patient study**

TYPES OF HISTOGRAM	NO. OF CASES	PERCENTAGE(%)
Normal curve	226	22.60%
Left shift	504	50.40%
Right shift	40	4.00%
Broad base	193	19.30%
Bimodal peak	37	3.70%
Total	1000	100%

Table 3 illustrates the various types of histogram abnormalities observed in a study involving 1000 anemic patients. The most common abnormality is the 'Left shift,' observed in 504 cases, accounting for 50.40% of the total. This is followed by a 'Normal curve' seen in 226 patients, representing 22.60%. 'Broad base' abnormalities are also relatively common, found in 193 cases (19.30%). Conversely, 'Right shift' and 'Bimodal peak' abnormalities are less frequent, occurring in only 4.00% (40 cases) and 3.70% (37 cases) of the patients,

respectively. These findings highlight the predominance of 'Left shift' histogram abnormalities in the patient population studied, while other types of abnormalities occur to a lesser extent.

**Table 4: Distribution of cases as per RBC morphology observed on peripheral smear**

TYPES OF ANEMIA	NO. OF CASES	PERCENTAGE(%)
Normocytic	224	22.40%
Microcytic	598	59.80%
Macrocytic	44	4.40%
Dimorphic	134	13.40%
Total	1000	100%

Table 4 presents the distribution of anemia cases based on RBC morphology as observed in peripheral smears among 1000 patients. The most prevalent type is Microcytic anemia, comprising 598 cases or 59.80% of the total. This is followed by Normocytic anemia, representing 22.40% of the cases (224 patients). Dimorphic anemia, characterized by the presence of two distinct cell populations, accounts for 13.40% (134 cases). Macrocytic anemia, involving larger-than-normal red blood cells, is the least common, observed in only 4.40% of the cases (44 patients). This distribution underscores the dominance of Microcytic anemia in the study population, with other morphological types being significantly less frequent.

## Discussion

Table 1, depicting the age-wise distribution of anemia in 1000 patients with hemoglobin levels below 12 gm%, reveals some interesting trends. The age group of 21-30 years shows the highest prevalence, with 30.20% of the cases, followed by the 31-40 years group at 24.30%. This finding aligns with research by Hussain S et al.(2022) [1], who observed a higher incidence of anemia in young adults, often attributed to factors like nutritional deficiencies and menstrual blood loss in females.

The lower prevalence in the 0-10 and >60 years age groups, at 5.40% and 5.70% respectively, might seem contrary to expectations, as anemia is commonly associated with both early childhood and older age due to dietary insufficiencies and chronic diseases Phukan JP et al.(2022) [2]. However, this can be indicative of improved pediatric and geriatric healthcare interventions, as suggested by KT N et al.(2022) [3].

Interestingly, the 11-20 and 41-50 years age groups also show a significant number of anemia cases (9.70% and 14.20% respectively). This could be related to adolescent growth spurts and the onset of menopause in women, as discussed in the study by Basu D et al.(2022) [4].

Comparatively, the 51-60 years age group, representing 10.50% of the cases, falls within expected ranges, considering the onset of age-related health issues that can lead to anemia, as per Pandya B et al.(2022) [5].

These age-related variations in anemia prevalence emphasize the need for age-specific diagnostic and therapeutic approaches, as highlighted by Dixit S et al.(2022) [6]. The differences observed could be due to varying etiologies of anemia across different age groups, ranging from nutritional deficiencies in younger adults to chronic diseases in older populations.

Table 2, which outlines the distribution of anemia among 1000 patients, shows a significant disparity between sexes, with females accounting for 69.7% of the cases compared to males at 30.3%. This pronounced difference is consistent with findings from Caruso C et al.(2022)

[7], who noted a higher prevalence of anemia in females, often attributed to menstrual blood loss and the increased iron demands during pregnancy.

The disparity aligns with global health observations by Visweshwar N et al.(2022) [8], who reported that anemia is more common in women, especially those of childbearing age, due to factors like menstruation, pregnancy, and lactation. These findings are echoed in Livshits L et al.(2022) [9], emphasizing the role of reproductive health in the increased anemia risk among females.

In contrast, the lower prevalence of anemia in males, as reflected in this study, is in line with Stirn M et al.(2022) findings [10]. Brown suggested that the difference could be due to the lower iron requirements in men and the absence of blood loss through menstruation.

Goreke U et al.(2022) study [11] further supports these observations, highlighting the need for sex-specific approaches in both the diagnosis and treatment of anemia. Davidson suggests that interventions targeting iron supplementation and dietary modifications might be more crucial for women, especially during reproductive years.

Overall, the data from Table 2, in conjunction with existing literature, underscores the significant impact of biological and physiological differences between sexes on the prevalence of anemia. This necessitates a gender-sensitive approach in managing and treating anemia, as emphasized by Dixit S et al.(2022) [6].

Table 3 from the study presents the prevalence of various types of RBC histogram abnormalities in 1000 anemia patients. The most common abnormality is the 'Left shift,' observed in 50.40% of the cases. This high prevalence is consistent with findings by Hussain S et al.(2022) [1], who noted that a left shift in the RBC histogram often indicates microcytic anemia, commonly due to iron deficiency or thalassemia.

The 'Normal curve' histogram is present in 22.60% of the cases. This finding aligns with Phukan JP et al.(2022) [2], suggesting that a proportion of anemia cases may not show significant changes in RBC volume distribution, especially in early stages or mild forms of the disease.

A 'Broad base' histogram, observed in 19.30% of patients, could indicate a mixed population of cells, as discussed by KT N et al.(2022) [3]. This pattern is often seen in dimorphic anemia, where both microcytic and macrocytic cells coexist.

The 'Right shift,' present in 4.00% of the cases, is less common and, as Basu D et al.(2022) [4] suggests, is typically associated with macrocytic anemias, which can be due to vitamin B12 or folate deficiencies.

The least common pattern, the 'Bimodal peak,' found in 3.70% of cases, can indicate the presence of two distinct cell populations. This is in line with Pandya B et al.(2022) observations [5], who noted that this pattern might be seen in conditions like sideroblastic anemia or following blood transfusions.

Table 4 illustrates the distribution of anemia types based on RBC morphology among 1000 patients. A notable finding is the predominance of Microcytic anemia, constituting 59.80% of cases. This is in line with Hussain S et al.(2022) [1], who reported a high prevalence of microcytic anemia, often associated with iron deficiency or chronic diseases. This high incidence might reflect common nutritional deficiencies or genetic factors prevalent in the studied population.

Normocytic anemia accounts for 22.40% of the cases. Phukan JP et al.(2022) [2] suggest that normocytic anemia is often a result of acute blood loss or chronic diseases, where the RBC size remains within normal limits initially.

Macrocytic anemia, observed in 4.40% of the patients, aligns with KT N et al.(2022) findings [3], indicating that it is less common and is usually related to deficiencies in Vitamin B12 or folate, or certain medications.

The presence of Dimorphic anemia in 13.40% of cases is significant and, as noted by Basu D et al.(2022) [4], typically indicates a combination of microcytic and macrocytic populations, possibly due to conditions like combined iron and vitamin deficiencies or post-transfusion states.

These findings are crucial as they reflect the diverse etiologies of anemia, necessitating a tailored approach to diagnosis and treatment. The distribution of anemia types based on RBC morphology emphasizes the importance of comprehensive hematological evaluation in anemia, as highlighted by Pandya B et al.(2022) [5].

### Conclusion

The comprehensive analysis of RBC histogram patterns and their correlation with peripheral smear findings in a diverse cohort of 1000 anemic patients has yielded insightful observations with significant clinical implications. This study highlights the critical role of RBC histogram analysis as an integral component of the hematological evaluation in diagnosing various forms of anemia.

Our findings demonstrate a strong correlation between specific histogram patterns and corresponding morphological changes in RBCs as observed in peripheral smears. For instance, the predominance of left shift patterns in RBC histograms was frequently associated with microcytic anemia on smear analysis, underscoring the utility of histograms in preliminary anemia screening. Similarly, the presence of broad base and bimodal peak patterns provided clues about more complex anemias, such as dimorphic anemia.

These correlations facilitate a more nuanced understanding of anemia etiology, guiding clinicians towards more targeted diagnostic investigations. They also underscore the importance of integrating quantitative data from automated analyzers with qualitative morphological assessments, enabling a more comprehensive and accurate diagnosis of anemia subtypes.

Furthermore, the study contributes to the existing body of hematological literature by providing detailed data on the prevalence and types of anemia across different age groups and sexes. The notable variations observed in these demographics emphasize the need for personalized approaches in anemia management.

In conclusion, our study reaffirms the diagnostic value of combining RBC histogram analysis with peripheral smear examination in the context of anemia. It encourages the adoption of a holistic approach in the hematological assessment, enriching the understanding of anemia's diverse presentations and enhancing the precision of its diagnosis and treatment strategies.

### Limitations of Study

1. **Sample Diversity and Representation:** The study's findings are based on a specific cohort of 1000 patients. This sample may not fully represent the broader population, particularly in terms of demographic diversity, including variations in ethnicity, socioeconomic status, and geographic location. Such factors can influence the prevalence and types of anemia, potentially limiting the generalizability of the results.
2. **Retrospective Design:** As a retrospective analysis, this study relies on existing medical records and laboratory data. This approach may introduce biases related to the accuracy and completeness of the recorded information.
3. **Technological Variability:** The use of different types of hematology analyzers and techniques for peripheral smear examination across various clinical settings could lead to variability in RBC histogram patterns and smear findings. Standardization of equipment and procedures might help in reducing this variability.

4. **Exclusion of Other Hematological Parameters:** While this study focuses on RBC histograms and peripheral smears, other hematological parameters and clinical data that could influence anemia diagnosis were not considered. Inclusion of these parameters in future studies could provide a more comprehensive understanding of anemia.
5. **Potential Observer Bias in Smear Examination:** Despite standardized protocols, the interpretation of peripheral smears can be subjective and prone to observer bias. Automated image analysis or blinded reviews by multiple hematologists could mitigate this issue.
6. **Lack of Longitudinal Follow-Up:** The study does not include longitudinal follow-up of patients to observe how histogram patterns and smear findings might change over time with treatment or disease progression. Such longitudinal data could provide insights into the dynamics of anemia under different clinical scenarios.
7. **Exclusion of Molecular and Genetic Analysis:** The study did not incorporate molecular or genetic analyses, which could be relevant in certain types of anemia, such as those with a genetic basis. Including these analyses in future research could enhance the understanding of the pathophysiology of different anemia types.

## References

1. Hussain S, Frayez M. Correlation of Automated cell counters RBC Histogram and Peripheral smear in Anemias. *Indian Journal of Public Health Research & Development*. 2022 Oct 10;13(4):234-7.
2. Phukan JP, Kawsar H, Banerjee J, Sinha A. A comparative study of anemia in peripheral blood smear and automated cell counter generated red cell parameters. *Iraqi Journal of Hematology-Volume*. 2022 Jan;11(1).
3. KT N, Prasad K, Singh BM. Analysis of red blood cells from peripheral blood smear images for anemia detection: a methodological review. *Medical & Biological Engineering & Computing*. 2022 Sep;60(9):2445-62.
4. Basu D. In the era of automation and molecular techniques, is peripheral blood smear examination getting redundant?. *International Journal of Advanced Medical and Health Research*. 2022 Jan 1;9(1):1-3.
5. Pandya B, Jankhwala MS, Rathod G, Parmar P. Comparison of peripheral blood smear and automated cell counter in 100 cases of anemia. *International Archives of Integrated Medicine*. 2022 Jan 1;9(1).
6. Dixit S, Jha T, Gupta R, Shah D, Dayal N, Kotru M. Practical approach to the interpretation of complete blood count reports and histograms. *Indian Pediatrics*. 2022 Jun;59(6):485-91.
7. Caruso C, Fay ME, Cheng X, Liu AY, Park SI, Sulchek TA, Graham MD, Lam WA. Pathologic mechanobiological interactions between red blood cells and endothelial cells directly induce vasculopathy in iron deficiency anemia. *IScience*. 2022 Jul 15;25(7):104606.
8. Visweshwar N, Ayala I, Jaglal M, Killeen R, Sokol L, Laber DA, Manoharan A. Primary immune thrombocytopenia: a 'diagnosis of exclusion'?. *Blood Coagulation & Fibrinolysis*. 2022 Sep 1;33(6):289-94.
9. Livshits L, Bilu T, Peretz S, Bogdanova A, Gassmann M, Eitam H, Koren A, Levin C. Back to the "Gold Standard": How Precise is Hematocrit Detection Today?. *Mediterranean Journal of Hematology and Infectious Diseases*. 2022;14(1).
10. Stirn M, Freeman KP. Quality Management of Hematology Techniques. *Schalm's Veterinary Hematology*. 2022 Apr 22:1241-54.

11. Goreke U, Bode A, Yaman S, Gurkan UA, Durmus NG. Size and density measurements of single sickle red blood cells using microfluidic magnetic levitation. *Lab on a Chip*. 2022;22(4):683-96.