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Original Research Article

Diagnostic utility of white blood cell histogram analysis in the subtyping of macrocytic anemia

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ABSTRACT

Background: Macrocytic anemia is widely categorized into megaloblastic and non-megaloblastic forms, each requiring specific diagnostic and treatment approaches. Automated hematology analyzers generate WBC histograms that may provide a rapid and objective adjunct for the diagnosis of macrocytic anemia.
Methods: This cross-sectional study comprised 120 patients with macrocytic anemia (MCV>100 fL) attending a tertiary care hospital during 12 months. Complete blood count, WBC histogram analysis, peripheral smear examination, reticulocyte count, and serum vitamin B12 estimation were done. WBC histogram findings were associated with vitamin B12 insufficiency, reticulocyte count, and nuclear hypersegmentation. Diagnostic performance was tested utilizing sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and receiver operating characteristic (ROC) curve analysis.
Results: The mean age of the study population was 33.93±20.16 years, with a little male predominance (52.5%). Megaloblastic anemia accounted for 65% of cases, while 35% were non-megaloblastic. WBC histogram >250 fL revealed a sensitivity of 62.8%, specificity of 61.9%, PPV of 75.4%, NPV of 47.3%, and an AUC of 0.62 for detecting vitamin B12 deficiency. Against nuclear hypersegmentation, sensitivity and specificity were 58.6% and 66.7%, respectively, with a PPV of 89.2% (AUC 0.63). Overall, WBC histogram demonstrated moderate diagnostic accuracy (AUC 0.57–0.63) and substantial correlation with vitamin B12 deficiency and nuclear hypersegmentation.
Conclusions: WBC histogram analysis offers a speedy, affordable, and objective adjunct for screening and subtyping macrocytic anemia. Although it cannot replace peripheral smear examination, it may help prioritize future investigations, particularly in resource-limited situations.

Keywords: Macrocytic anemia; WBC histogram, Megaloblastic anemia, Vitamin B12 deficiency, Peripheral blood smear, Automated hematology analyzer

INTRODUCTION

Macrocytic anemia is a clinically relevant subtype of anemia characterized by a mean corpuscular volume (MCV) >100 fL and is broadly classified into megaloblastic and non-megaloblastic types.[1] Megaloblastic anemia, most commonly resulting from vitamin B12 or folate deficiency, is characterized by defective DNA synthesis, macro-ovalocytes, and hypersegmented neutrophils, whereas non-megaloblastic macrocytosis is associated with conditions such as liver disease, chronic alcohol use, hypothyroidism, myelodysplastic syndromes, and certain medications. Accurate differentiation is essential because management varies considerably, and delayed diagnosis may lead to irreversible neurological complications or missed systemic and hematological disorders.[2] In India, nutritional deficiencies and vegetarian dietary practices contribute substantially to the burden of megaloblastic anemia.[3]

Automated hematology analyzers have transformed the evaluation of anemia by providing complete blood count parameters together with red blood cell (RBC) and white blood cell (WBC) histograms.[4] While RBC histograms demonstrate alterations in red cell size distribution, WBC histograms may exhibit characteristic changes related to neutrophil hypersegmentation in megaloblastic anemia. Previous studies have demonstrated good correlation between histogram findings and peripheral smear examination; however, peripheral smear evaluation remains the reference standard for morphological confirmation.[5]

Evaluation of WBC histogram patterns may provide a rapid, objective, and cost-effective approach for subtyping macrocytic anemia. Therefore, the present study was undertaken to evaluate WBC histogram patterns in macrocytic anemia, determine their role in differentiating megaloblastic and non-megaloblastic anemia, and assess their sensitivity and specificity in comparison with peripheral smear findings.[6]

METHODS

This cross-sectional study was conducted at the Department of Pathology, in a tertiary care hospital of India, over a period of 12 months after getting approval from the Institutional Ethics Committee. Written informed permission was acquired from all individuals. Patients admitted for complete blood count (CBC) with hemoglobin <13 g/dl in males and <12 g/dl in females and mean corpuscular volume (MCV) >100 fL were included, while patients with mixed anemia or receiving vitamin B12 supplements were excluded. A sample size of 120 patients was included based on the availability of eligible cases during the study period utilizing convenience sampling.

Venous blood samples were taken in EDTA tubes for hematological investigations and plain tubes for serum vitamin B12 determination. Complete blood count, including MCV, red cell distribution width (RDW), and WBC histograms, was performed using the Zybio Z3 automated hematology analyzer. Peripheral blood smears stained with Leishman stain were analyzed for red cell morphology and hypersegmented neutrophils. Reticulocyte counts were assessed by New Methylene Blue supravital staining, and serum vitamin B12 levels were analyzed by automated chemiluminescent immunoassay (CLIA). WBC histogram patterns were analyzed for variations in neutrophil volume distribution and linked with peripheral smear results, vitamin B12 levels, RDW, and reticulocyte count to classify macrocytic anemia into megaloblastic and non-megaloblastic subtypes.

Primary outcome

Primary outcome was vitamin B12 deficiency.

Secondary outcomes

Secondary outcomes was peripheral smear and Reticulocyte count.

Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics version 23. Categorical data were reported as frequencies and percentages, whereas continuous variables were summarized using appropriate descriptive statistics. The diagnostic performance of WBC histogram was examined by calculating sensitivity, specificity, positive predictive value, negative predictive value, and total diagnostic accuracy. Receiver operating characteristic (ROC) curve analysis was done to measure diagnostic performance, and a p-value <0.05 was regarded statistically significant.

RESULTS

A total of 120 patients with macrocytic anemia were included in the study. The mean age was 33.93±20.16 years (median: 28 years; range: 1–85 years). The biggest proportion of participants belonged to the <20 years age group (31.7%), followed by 20–29 years (20.8%) and 30–39 years (15.8%). There was a small male majority, with 63 (52.5%) males and 57 (47.5%) females. (Table 1)

Table 1: Baseline demographic characteristics of the study population (n=120).

Characteristics	Values
Age (years), mean±SD	33.93±20.16
Median age (years)	28
Age range (years)	1–85
Male, N (%)	63 (52.5)
Female, N (%)	57 (47.5)

Among the study population, 78 (65.0%) patients had megaloblastic anemia, while 42 (35.0%) had non-megaloblastic anemia based on serum vitamin B12 levels. WBC histogram values >250 fL were observed in 65 (54.2%) cases, whereas 55 (45.8%) had values <250 fL. A broad-based RBC histogram was present in 46 (38.3%) patients, and a right-shifted platelet histogram was observed in 78 (65.0%) patients (Table 2).

Table 2: Distribution of macrocytic anemia and hematological histogram findings.

Characteristics	Value N (%)
Subtype of macrocytic anemia	
Megaloblastic anemia	78 (65.0)
Non-megaloblastic anemia	42 (35.0)
WBC histogram	
>250 fL	65 (54.2)
<250 fL	55 (45.8)
RBC histogram	
Broad base	46 (38.3)
Platelet histogram	
Right shift	78 (65.0)

Using serum vitamin B12 deficiency as the reference standard, a WBC histogram >250 fL demonstrated a sensitivity of 62.8%, specificity of 61.9%, positive predictive value (PPV) of 75.4%, negative predictive value (NPV) of 47.3%, and an AUC of 0.62 (p=0.009). When compared with a reticulocyte count <0.5%, the WBC histogram showed a sensitivity of 65.8%, specificity of 48.6%, PPV of 39.7%, NPV of 73.5%, and an AUC of 0.57 (p=0.14). For identifying patients with reticulocyte counts >2.5%, a WBC histogram <250 fL demonstrated a sensitivity of 75.0%, specificity of 51.4%, PPV of 14.3%, NPV of 95.0%, and an AUC of 0.63 (p=0.15). Against nuclear hypersegmentation on peripheral smear, a WBC histogram >250 fL yielded a sensitivity of 58.6%, specificity of 66.7%, PPV of 89.2%, NPV of 25.5%, and an AUC of 0.63 (p=0.035) (Table 3) (Figure 1).

Table 3: Diagnostic performance of WBC histogram compared with Vitamin B12 deficiency and reticulocyte count.

Reference standard	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	P value
Vitamin B12 deficiency	62.8	61.9	75.4	47.3	0.62	0.009
Reticulocyte count <0.5%	65.8	48.6	39.7	73.5	0.57	0.14
Reticulocyte count >2.5%	75.0	51.4	14.3	95.0	0.63	0.15

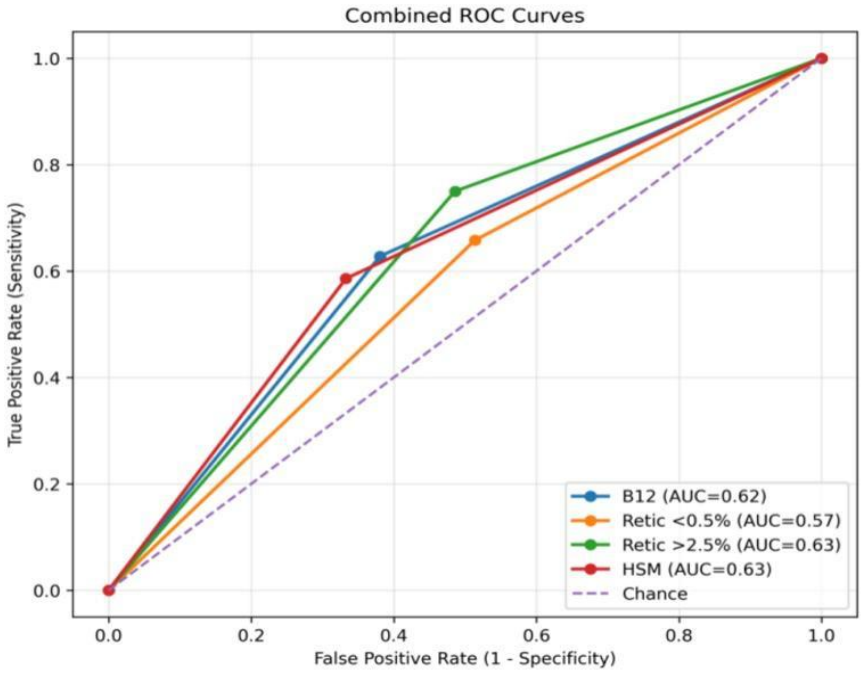


Figure 1: Combined ROC Curves: WBC Histogram (>250 fL) vs other factors

For reticulocyte count >2.5%, a WBC histogram <250 fL was deemed a positive result. Overall, WBC histogram revealed moderate diagnostic accuracy (AUC 0.57–0.63) for subtyping macrocytic anemia. It demonstrated the highest PPV for predicting nuclear hypersegmentation (89.2%) and the highest NPV for excluding reticulocytosis >2.5% (95.0%). (Figure 2) Significant connections were detected with serum vitamin B12 deficiency and nuclear hypersegmentation, although correlations with reticulocyte count were not statistically significant (Table 4).

Among all parameters, the highest AUC was observed for reticulocyte >2.5 percent and nuclear hypersegmentation (0.63), while the lowest was for reticulocyte <0.5 percent (0.57). (Figure 3) Overall, WBC histogram demonstrated moderate diagnostic performance, supporting its role as an adjunct rather than a standalone diagnostic tool (Figure 1).

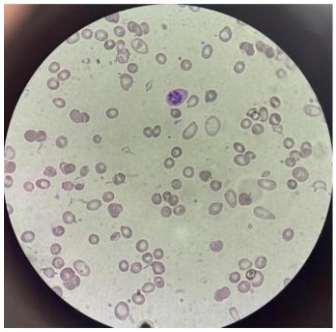


Figure 2: A Leishman-stained peripheral smear showing macro ovalocytes, schistocytes and hypersegmented neutrophils (100x).

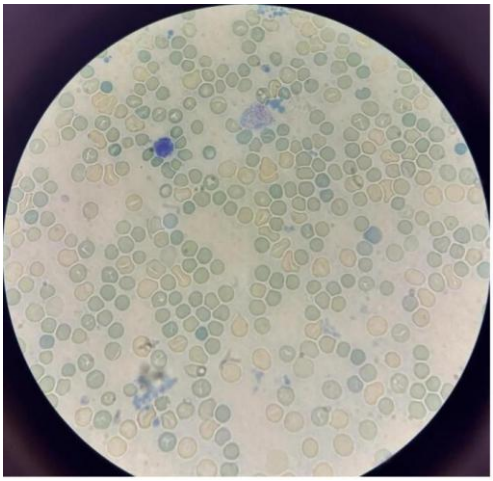


Figure 3: A new methylene blue stained smear showing reduced reticulocyte count (100x).

Table 4: Diagnostic performance of WBC histogram for predicting nuclear hypersegmentation.

Parameters	Value
Sensitivity (%)	58.6
Specificity (%)	66.7
PPV (%)	89.2
NPV (%)	25.5
AUC	0.63
P value	0.035

DISCUSSION

Macrocytic anemia includes a complex set of illnesses needing correct distinction between megaloblastic and non-megaloblastic variants because their etiologies and therapy differ greatly. In the present study, the majority of patients were younger than 30 years, with a mean age of 33.93±20.16 years, and a little male predominance (52.5%). These findings differ from those of Varghese et al, who observed a majority of elderly patients, but are comparable to the younger groups described by Nida Mehreen et al and Pursnani et al, where dietary inadequacies were identified as the primary cause of macrocytic anemia.[7,8,9] Regional eating practices, socioeconomic circumstances, and healthcare accessibility may explain for these demographic variances.

Megaloblastic anemia constituted 65% of all macrocytic anemia cases in the present study, while 35% were non-megaloblastic. This distribution is comparable with the findings of Hawaldar R et al and Rao PS et al, who likewise observed megaloblastic anemia as the main subtype, mostly related to vitamin B12 and folate insufficiency.[10,11] These data reinforce the persistent importance of nutritional inadequacy as the major cause of macrocytic anemia in poor nations and underline the necessity for early detection of vitamin B12 deficiency.

Unlike most prior research that focused largely on RBC histogram analysis, the present study analyzed WBC histogram patterns in addition to RBC and platelet histograms. A WBC histogram shift >250 fL was seen in 54.2% of patients, while a broad-based RBC histogram and right-shifted platelet histogram were recorded in 38.3% and 65% of cases, respectively. Earlier investigations by Sinha et al and Thyagaraju et al indicated that RBC histogram abnormalities correspond well with peripheral smear results, whereas Pursnani et al reported better diagnostic accuracy using histogram analysis.[9,12,13] The present work extends previous data by showing that WBC histogram variations may represent anomalies in leukocyte nuclear maturation linked with faulty DNA synthesis.

The diagnostic performance of WBC histogram revealed modest accuracy when compared with recognized diagnostic markers. Using serum vitamin B12 insufficiency as the reference standard, WBC histogram demonstrated a sensitivity of 62.8%, specificity of 61.9%, and an AUC of 0.62. Similar observations have been reported by Hawaldar et al and Rao et al, who established the utility of automated hematology analyzer-derived leukocyte parameters in identifying vitamin B12 insufficiency.[10,11] Although the diagnostic performance was moderate, our findings support the significance of WBC histogram as a rapid and inexpensive screening tool, particularly in resource-limited situations.

Correlation with reticulocyte count and peripheral smear further underlined the complementing importance of WBC histogram analysis. WBC histogram revealed modest sensitivity for recognizing hypoproliferative conditions and a strong negative predictive value (95%) for excluding hyperproliferative anemia. Comparison with nuclear hypersegmentation indicated a sensitivity of 58.6%, specificity of 66.7%, and a high positive predictive value of 89.2%. These findings are comparable with those reported by Francis et al, Salafranca et al, and Bhatia et al, who underlined neutrophil hypersegmentation as an essential morphological sign of faulty DNA synthesis and an early diagnostic of vitamin B12 deficiency.[14-16]

Overall, the present study supports earlier findings that automated hematology analyzer-derived parameters are helpful adjuncts in the evaluation of macrocytic anemia. While Thomas et al, Thyagaraju et al, Mehreen et al, and Pursnani et al primarily examined RBC histogram or analyzer-derived indices, the present study specifically studied WBC histogram patterns and indicated reasonable diagnostic performance (AUC 0.57–0.63).[8,9,14,17] Although WBC histogram cannot replace standard biochemical and morphological examinations, its rapid availability, low cost, and objective character make it a useful screening and supportive tool. Further multicentric research with bigger sample sizes are necessary to define uniform WBC histogram criteria and validate their involvement in the routine subtyping of macrocytic anemia.

CONCLUSION

WBC histogram analysis provides a quick, objective, and cost-effective adjunct in the evaluation of macrocytic anemia. It revealed moderate diagnostic accuracy in subtyping macrocytic anemia and showed substantial connection with vitamin B12 deficiency and peripheral smear results. Although it cannot replace standard diagnostic approaches, WBC histogram can serve as a useful screening tool to identify individuals requiring further investigations such as vitamin B12 estimation, reticulocyte count, and bone marrow examination, particularly in resource-limited situations. WBC histogram is a rapid screening adjunct that complements conventional investigations.

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