

Utility of Abnormal WBC Scattergram & Pseudo Eosinophilia with Thrombocytopenia in Presumptive Diagnosis of Malaria

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Abstract

Background: Malaria remains a major global health problem, and while Giemsa-stained peripheral blood smear microscopy is the diagnostic gold standard, it is limited by interobserver variability and resource constraints in many endemic settings. Automated haematology analysers, routinely used for complete blood counts, may provide valuable presumptive diagnostic clues through characteristic haemogram changes and abnormal WBC scattergram patterns.

Aim: To evaluate the utility of abnormal WBC scattergram patterns and pseudoeosinophilia, in combination with thrombocytopenia and other haemogram abnormalities, as indicators for the presumptive diagnosis of malaria.

Materials and Methods: This was a descriptive study in retrospective analysis of all malaria positive cases. It was conducted over four months (August–November 2023) at Dr SN Medical College and associated hospitals, Jodhpur. A total of 1,200 EDTA blood samples from patients clinically suspected of malaria were analysed using the Sysmex XS-800i automated haematology analyser, and corresponding Giemsa-stained thick and thin peripheral blood smears were examined microscopically for Plasmodium species. Pseudoeosinophilia was defined as a spurious increase in eosinophil count on the analyser not corroborated by manual differential counts, thrombocytopenia as platelet count $<150,000/\mu\text{L}$, and abnormal WBC scattergram patterns were recorded as per predefined criteria.

Results: Of 1,200 samples, 61 (5.1%) were positive for Plasmodium vivax malaria on peripheral smear, with patient ages ranging from 10 to 65 years. Among malaria-positive cases, 86.8% showed thrombocytopenia, 42.6% anaemia, 40.9% pseudoeosinophilia and 65.5% abnormal WBC scattergram findings; pancytopenia and bicytopenia were observed in 9.8% and 37.7% of cases, respectively. The most frequent scattergram abnormalities included “graying” and overlap of neutrophil and eosinophil clusters and double neutrophil or eosinophil populations. In most cases, higher degrees of parasitaemia correlated with more pronounced scattergram abnormalities and pseudoeosinophilia.

Conclusion: Thrombocytopenia, pseudoeosinophilia and abnormal WBC scattergram patterns on the Sysmex XS-800i are frequently associated with P. vivax malaria and, when interpreted together, substantially enhance the presumptive detection of infection. In malaria-endemic areas, careful review of haemograms and WBC scattergrams can serve as a rapid, cost-effective screening adjunct to guide targeted smear examination and improve early laboratory recognition of malaria.

Keywords: Malaria, CBC, WBC scattergram, Giemsa stain.

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Introduction

Malaria remains a critical global public health challenge and is caused by protozoan parasites belonging to the genus Plasmodium. Light microscopy of Giemsa-stained peripheral blood smears is currently recognized as the gold standard for detection of malaria parasite, [1] but it is significantly limited by interobserver variability.

Although newer antigen-antibody based techniques are rapid and sensitive, their high cost and lack of routine availability in many settings necessitate more accessible screening methods. [2] In malaria-endemic areas, there is a need for rapid, sensitive and cost-effective screening methods. Because a complete blood count (CBC) is a

baseline investigation for nearly all patients presenting with fever, automated hematology analyzers have emerged as an essential tool for the presumptive diagnosis of malaria. [3] Automated haematology analysers can offer fast, sensitive and cost-effective assessment of suspected malaria cases.[2,3] Many haematological parameters change in malaria, but none is entirely specific.

The diagnostic utility of automated haematology analyzers in malaria detection relies on identifying hemozoin, a birefringent crystal produced as a byproduct of parasite-mediated hemoglobin digestion, which depolarizes laser light to generate bizarre and unusual scattergram patterns. [4] When host white blood cells phagocytose hemozoin, the resulting increased side-light scattering causes neutrophils to be misclassified as eosinophils—a phenomenon termed pseudo eosinophilia—while also manifesting as specific abnormal WBC scattergram patterns including double neutrophil zones, "graying" of neutrophil and eosinophil clusters, and prominent events above the X-axis. [5-7] Beyond these scattergram irregularities, malaria infection frequently triggers broader hematological changes including anemia and leukopenia; among these cytopenic findings, thrombocytopenia has emerged as the strongest predictor of malaria. Integration of these hematological abnormalities with abnormal WBC scattergram patterns significantly enhances detection sensitivity and increases the overall parasite detection rate. [5,6]

The present study evaluates the utility of abnormal WBC scattergram patterns and pseudo eosinophilia, in combination with thrombocytopenia, as helpful indicators for the diagnosis of malaria.

Aims

- To evaluate the usefulness of automated cell counters by analysing WBC scattergrams and instrument flagging in malaria-positive cases.
- To compare other haematological parameters that may help identify malaria parasites on peripheral blood smear, even in the absence of clinical suspicion

Material and Method

Study Design: This was a descriptive study in which retrospective analysis of all malaria positive cases was done during the study period. It was conducted at Dr. SN medical college and associated groups of Hospitals.

Study Duration: Total 4 months (August 2023 to November 2023)

Inclusion and Exclusion Criteria: All the blood samples for CBC and PBFs received at the department for suspected (clinical diagnosis) of Malaria were included. Samples without any proper

clinical history and hemolysed samples were excluded.

Sample Collection and Processing

Specimen: Venous blood samples were collected in EDTA tubes. Samples were processed on the Sysmex XS-800i automated hematology analyzer within 8 hours of collection.

Automated Analyzer Analysis: The study evaluates specific flags and parameters generated by the Sysmex XS-800i:

Most common flag sign of automated hematoanalyser in malaria

1. Abnormal WBC scattergram
2. Atypical lymphocytes
3. Immature granulocytes
4. Thrombocytopenia
5. Abnormal platelet distribution
6. Platelets clumps
7. Anaemia
8. Eosinophilia

Identification of Pseudo eosinophilia: This is defined as a **spurious increase** in the eosinophil count provided by the analyzer that is not corroborated by manual microscopic counts. It is calculated as a difference of between automated and manual eosinophil counts.

Thrombocytopenia Assessment: The automated platelet count is recorded, with thrombocytopenia generally defined as a count below 150000 plates / cumm.

WBC-DIFF scattergrams for abnormal patterns. Common malarial abnormalities include the "graying" of neutrophil and eosinophil clusters, a reduced or merged space between these populations, and abnormal events occurring above the X-axis.

Peripheral Blood Film (PBF): Thick and thin smears were prepared from the same EDTA samples. Smears were stained using **Giemsa stain**.

Microscopy: Expert pathologists reviewed the slides under oil immersion (100x magnification) to confirm the presence of Plasmodium species and identify various parasite stages like trophozoites, schizonts, or gametocytes. A slide was considered negative only after examining at least 200 fields

Evaluation of Diagnostic Utility: The presence of abnormal scattergram patterns, pseudo eosinophilia, and thrombocytopenia is correlated with the microscopic findings from the PBF

Result

In the present study, out of 1200 blood samples 61 cases were diagnosed as Plasmodium Vivax malaria. Patients age group of this study is 10 yrs to

65 yrs. Cases having a variation of more than 5% in eosinophils count were categorized as spurious/pseudoeosinophilia. Complete haematological parameters were assessed in all malaria-positive cases and abnormal findings were recorded. Parasitaemia was quantified on peripheral smear examination, and in most cases the degree of parasitaemia was directly proportional to the degree of WBC scattergram abnormality.

In this study, 86.8% of malaria-positive cases showed thrombocytopenia, 40.9% showed

pseudoeosinophilia and 65.5% showed abnormal WBC scattergram findings. [Table no. 1] The WBC scattergram findings in malaria positive patients are shown in Image no. 2 (a – f). Among the haematological abnormalities evaluated, thrombocytopenia (86.8% of cases) emerged as the strongest predictor of malaria.

This study showed that high degree of parasitemia found in PBF examination were associated with abnormal WBC scattergram and pseudoeosinophilia. [Table no. 2].

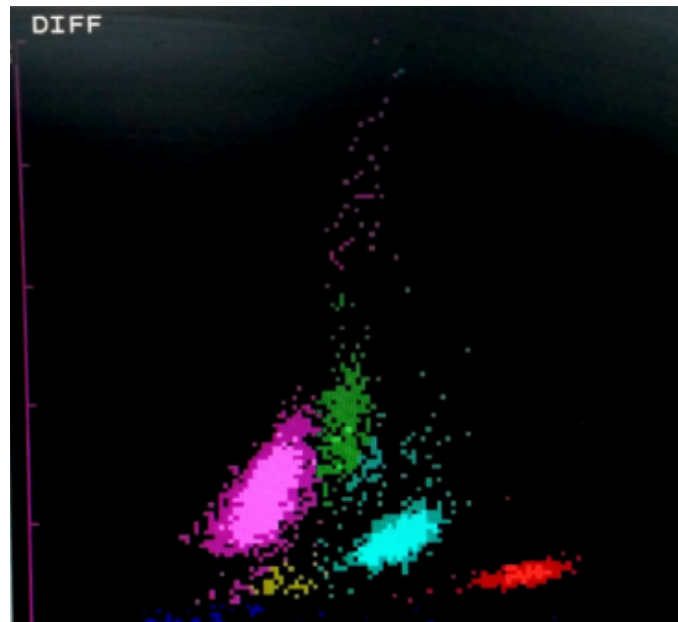


Figure 1: Normal WBC scattergram

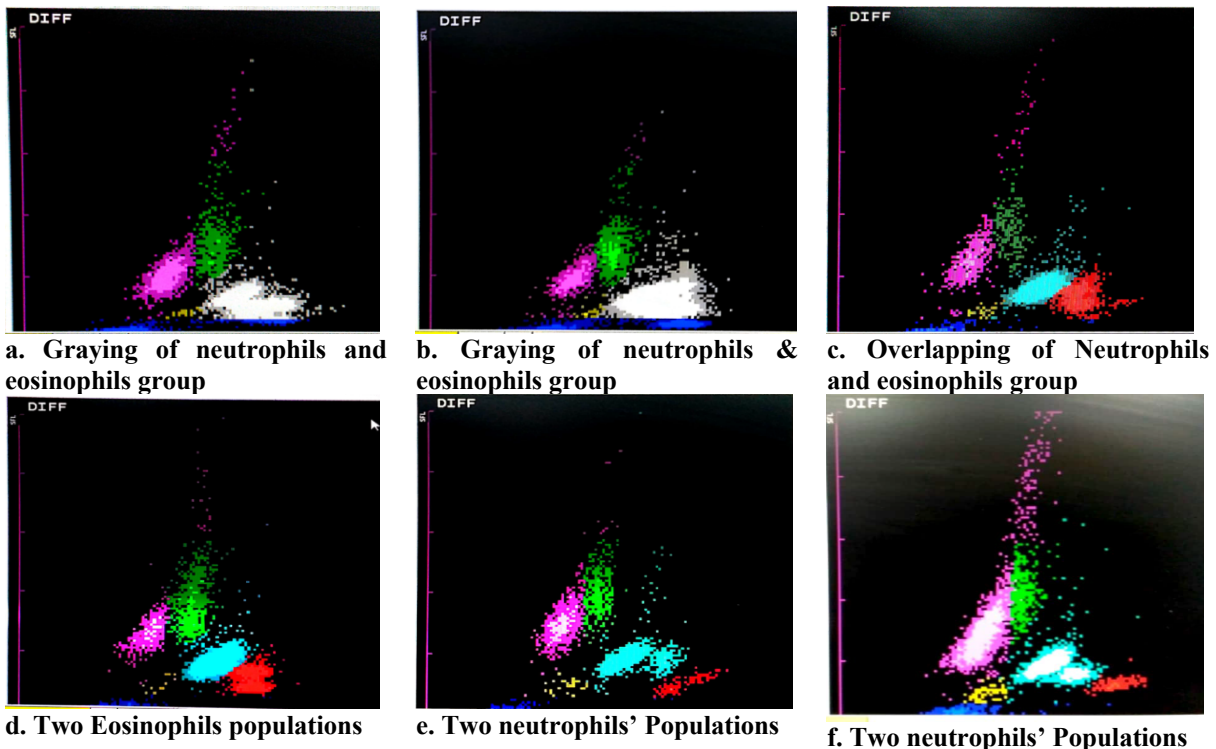


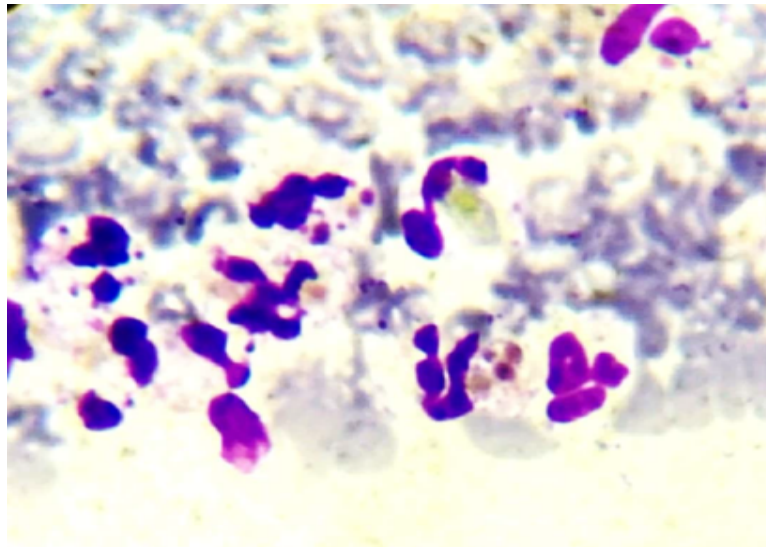
Figure 2 (a to f) : WBC Scattergram Findings in Malaria Positive cases.

Table 1: Abnormalities in hemogram in present study.

Hemogram abnormality	No of malaria cases
Pancytopenia	6 (9.8 %)
Bicytopenia	23 (37.7%)
Anaemia	26 (42.6%)
Thrombocytopenia	53 (86.8%)
Pseudoeosinophilia	25 (40.9%)
Leucocytosis	15 (24.5%)
Leucopenia	3 (4.9 %)
No abnormality	3 (4.9%)

Table 2: Abnormality in WBC scattergram in present study.

Abnormality of WBC scattergram	Number of cases of abnormal scattergram (40)
Graying of neutrophils and eosinophils group	24 (60%)
Overlapping of neutrophils and eosinophils	10 (25%)
Two populations of eosinophils	3 (7.5%)
Two populations of neutrophils	3 (7.5%)

**Figure 3: Hemozoin containing neutrophils on peripheral blood smear.**

Discussion

The Sysmex XS-800i classifies leucocytes based on three signals: forward-scattered light, side-scattered light and fluorescence intensity. It uses an organic acid reagent that binds specifically to eosinophil granules, allowing differentiation from neutrophils by higher side-scatter intensity. Sysmex analysers can indicate possible malaria infection through pseudoeosinophilia and abnormal WBC scattergram patterns. [5]

Malaria parasites digest haemoglobin and detoxify free haem by converting it to a stable, birefringent crystal known as hemozoin. [4] During schizont rupture, hemozoin is released from infected red cells and is subsequently phagocytosed by host WBCs. As a birefringent crystal, hemozoin increases side-scatter properties of neutrophils that contain it, resulting in misclassification of these neutrophils as eosinophils and causing pseudoeosinophilia on the analyser. [4]

Spurious eosinophilia detected in malaria patients has been reported as a reasonable indicator of the degree of parasitaemia. Huh et al. [5] found pseudoeosinophilia in 38.9% and abnormal WBC scattergram in 52.1% of 144 malaria cases, similar to the present study (pseudoeosinophilia 40.9%, abnormal WBC scattergram 65.5%). Yoo et al. [7] studied 413 malaria cases and reported pseudoeosinophilia in 39% and abnormal WBC scattergram patterns, including double neutrophil and double eosinophil populations, in 15.7%.

The present study showed that most malaria cases had cytopenias affecting at least one blood cell lineage, with thrombocytopenia (86.6%) and anaemia (42.6%) being the most common. Previous studies have also documented a high incidence of thrombocytopenia and anaemia in malaria. [8-10] When abnormal WBC scattergram findings are considered alongside these haematological

abnormalities, the detection rate of malaria can be significantly improved.

Conclusion

Attention to differential count, low platelet and WBC scattergram provided by Sysmex Xs 800i would be a valuable tool in malaria diagnosis even in the absence of clinical request. If the pathologist is aware of these WBC scattergram abnormalities, they will be more careful to look for the presence of parasite in blood smears so sensitivity can be increased.

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