Scholars Journal of Applied Medical Sciences
Abbreviated Key Title: Sch J App Med Sci
ISSN 2347-954X (Print) | ISSN 2320-6691 (Online)
Journal homepage: www.saspublishers.com

OPEN ACCESS

Pathology

Malaria Associated Pseudoeosinophilia Determined in Automated Hematology Analyzer Sysmex XN-1000

Dr. Atul Tiwari[1*], Dr. Ashish Gupta[2], Dr. M. L. Yadav[3]

[1]Resident, Department of Pathology, Mahatma Gandhi Medical College and Hospital, Jaipur, Rajasthan, India
[2]Senior Consultant, Department of Hematology and Molecular Pathology, Mahatma Gandhi Medical College and Hospital, Jaipur, Rajasthan, India
[3]Professor, Department of Pathology, Mahatma Gandhi Medical College and Hospital, Jaipur, Rajasthan, India

DOI: 10.36347/sjams.2019.v07i12.048 | **Received:** 09.12.2019 | **Accepted:** 22.12.2019 | **Published:** 28.12.2019

***Corresponding author:** Dr. Atul Tiwari

Abstract **Original Research Article**

Malaria being one of the most common parasitic disease worldwide, places a significant burden on the health care system. Hemozoin is known to be an end-product of hemoglobin digestion by malaria parasite. It is a birefringent crystal, and thus hemozoin-containing white blood cells (WBCs) may show the atypical light scattering pattern. The purpose of this study was to investigate pseudoeosinophilia associated with malaria infection using a Sysmex XN-1000 hematology analyzer (Sysmex Corporation, Kobe, Japan). Sysmex XN-1000 automated hematology analyzer was used to analyze EDTA anticoagulated samples of 25 patients with malaria infection. Leishman stained peripheral smears were also examined. Out of 25 cases studied, 24% showed erroneously high eosinophil counts and atypical eosinophil distributions in the WBC scattergram, which was due to the presence of hemozoin-containing neutrophils. In three patients, their erroneously high eosinophil counts declined as the parasitemia decreased with treatment. In conclusion, hematologists should consider the possibility of pseudoeosinophilia as a result of hemozoin-containing WBCs and confirm the WBC differential count by microscopy.

Keywords: Malaria, eosinophil, pseudoeosinophilia, Sysmex XN-1000, hemozoin.

INTRODUCTION

Malaria is one of the most common parasitic diseases present worldwide. It places a significant burden on the health care system and its endemic in various countries including India [1]. Diagnosis of malaria is time consuming and challenging as majority of the laboratories still use conventional microscopic identification of malaria parasite on Giemsa stained thick and thin smears. There is necessity for a convenient, sensitive and cost-effective method to effectively screen all samples, especially when the workload is high, so as not to miss any malaria case [2, 3]. Newer techniques like RDT, Quantitative buffy coat (QBC) and Polymerase chain reaction (PCR) have been introduced for malaria detection. Despite this, Giemsa stained peripheral smear examination remains the gold standard test for malaria parasite detection. RDTs are very convenient and are less labor intensive and can be performed by relatively unskilled technicians but have some limitations [4]. QBC and PCR tests are not available in all laboratories and are not cost effective at present [4].

There is a constant search for alternate methods to detect malaria. One such methods is the use of automated analyzers to detect malaria. As complete blood count (CBC) is one of the basic investigations invariably done on any febrile patient, simultaneously noting abnormalities in WBC scattergrams can be helpful in early detection and decreases the turnaround time of a laboratory [1, 4, 5].

Interestingly, we found a few cases of malaria-infected patients with markedly elevated erroneously high eosinophil counts, as determined by Sysmex XN-1000 hematology analyzer (Sysmex Corporation, Kobe, Japan). The purpose of this study was to investigate pseudoeosinophilia associated with malaria infection using Sysmex XN-1000 hematology analyzer.

MATERIAL AND METHODS

Our study group was composed of 25 patients with malaria infection between July 2019 and October 2019 at Mahatma Gandhi Medical College and Hospital, Jaipur, Rajasthan, India.

For complete blood analysis, we used a Sysmex XN-1000 hematology analyzer. This instrument differentiates WBCs using side fluorescence and side-scattered light.

A diagnosis of malaria was made by examining the Leishman stained peripheral blood smear. Parasitemia was determined by counting the number of parasites per 100 WBCs in a thin blood smear. This figure was then converted to the number of parasites per microliter of blood.

RESULTS

Of 25 malaria-infected patients, 6 (24%) showed erroneously high eosinophil counts and atypical WBC scattergrams (Table-1, Figure 1 & 2). They were all infected with *Plasmodium vivax*.

Three patients after treatment initiation showed a reduction in erroneous eosinophil counts as parasitemia decreased (Table-2).

Table-1: Comparison of neutrophil and eosinophil counts between Sysmex XN-1000 and microscopy in six malaria infected patients

Case No.	Age/Sex	Sysmex XN-1000 (%)		Microscopy (%)	
		Neutrophil	Eosinophil	Neutrophil	Eosinophil
1	22/M	69.8	11.2	81	0
2	16/M	54.3	32.1	80	1
3	17/M	54.4	42.9	91	0
4	65/M	43.4	52.5	89	1
5	65/F	65.5	27.2	90	0
6	30/M	76.9	13.7	89	0

Table-2: Comparison of eosinophil counts between Sysmex XN-1000 and microscopy in four malaria patients after initiation of treatment

Case No.	Age/Sex	Date	Parasitemia (µL)	Eosinophil (%)	
				Sysmex XN-1000	Microscopy
2	16/M	Day 0	4900	32.1	1
		Day 1	1340	10.9	0
		Day 2	440	1.2	0
		Day 3	Negative	1.8	0
3	17/M	Day 0	5130	42.9	0
		Day 1	3350	12.3	0
		Day 2	650	1.1	0
		Day 3	Negative	1.3	0
4	65/M	Day 0	5600	52.5	1
		Day 1	3760	14.3	0
		Day 2	1280	5.2	0
		Day 3	Negative	1.4	0

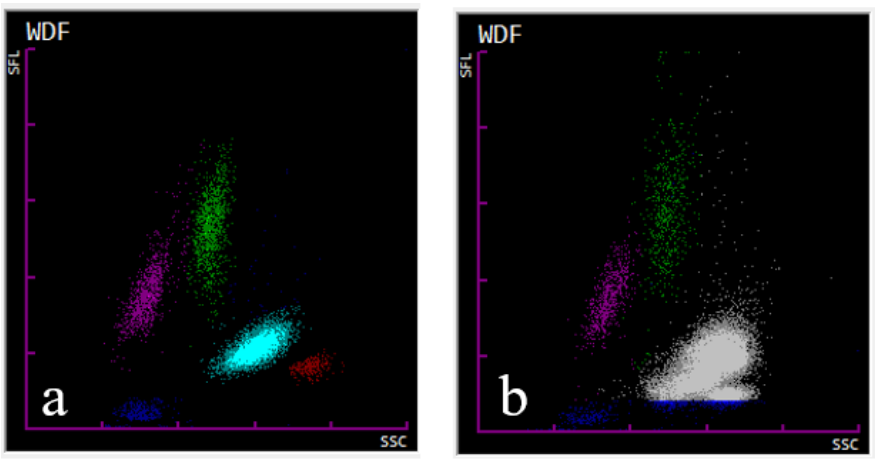
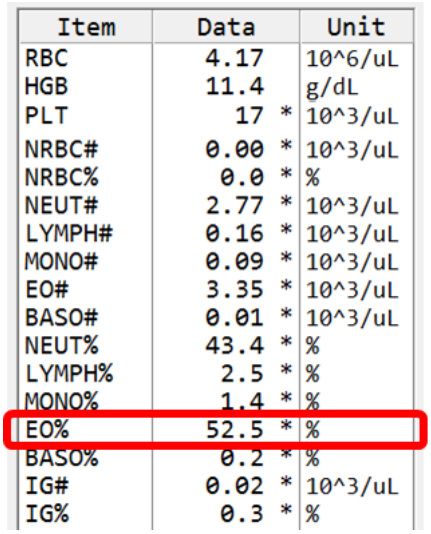


Fig-1: Scattergram generated by a Sysmex XN-1000 hematology analyzer. (a) Sample from a patient without malaria infection, showing two distinct clusters of neutrophils and eosinophils. (b) Sample from a patient with malaria infection (Case 4), showing atypical distribution therefore creating graying of neutrophils and eosinophils clusters.



Item	Data		Unit
RBC	4.17		10^6/uL
HGB	11.4		g/dL
PLT	17	*	10^3/uL
NRBC#	0.00	*	10^3/uL
NRBC%	0.0	*	%
NEUT#	2.77	*	10^3/uL
LYMPH#	0.16	*	10^3/uL
MONO#	0.09	*	10^3/uL
EO#	3.35	*	10^3/uL
BASO#	0.01	*	10^3/uL
NEUT%	43.4	*	%
LYMPH%	2.5	*	%
MONO%	1.4	*	%
EO%	52.5	*	%
BASO%	0.2	*	%
IG#	0.02	*	10^3/uL
IG%	0.3	*	%

Fig-2: Pseudoeosinophilia reported by Sysmex XN-1000 hematology analyzer in Case 4

DISCUSSION

Sysmex analyzer works on the principle of flow cytometry and uses a semi-conductor laser to give three types of optical data about the cells. Forward scatter light (FSL) which indicates cell size, side scatter (SSC) indicates the complexity of internal structure such as granules and side fluorescence light (SFL) indicates the nuclear content [5].

Hemozoin is a crystalline brown pigment. It is produced when free heme is liberated during hemoglobin catabolism by malaria parasite. Hemozoin is phagocytosed by neutrophils and monocytes. These cells allow the identification of malaria infection by automated methods by producing abnormal scattergrams [3].

Several authors have described detection of the malaria parasite employing the depolarizing property of hemozoin using a hematology analyzer of Cell-Dyn series (Abbott Diagnostics) [6-10]. Very few studies have been done scattergram abnormalities caused by hemozoin pigment using a Sysmex XN-1000 hematology analyzer. We found that the Sysmex XN-1000 analyzer can detect hemozoin containing neutrophils by examining atypical eosinophil distribution in WBC scattergrams.

The Sysmex XN-1000 analyzer differentiates eosinophils from neutrophils based on the side light scattering differences. Since hemozoin is a birefringent crystal, hemozoin-containing neutrophils may show considerable side light scattering and they may be incorrectly classified as eosinophils, which may be the cause of pseudoeosinophilia.

While the Cell-Dyn instrument can also detect the hemozoin-containing monocytes using depolarizing properties, the Sysmex XN-1000 analyzer cannot differentiate hemozoin-containing monocytes, based on sider scatter.

Therefore, hematologists, should consider the possibility of pseudoeosinophilia as a result of presence of hemozoin-containing neutrophils and confirm differential WBC counts by microscopy if an atypical scattergram is encountered in malaria-infected patients.

It has been suggested that the kinetics of hemozoin-containing WBCs may vary between populations and that it could be related to severity of infection and host immunity [11-14]. One report described that the percentage of hemozoin-containing WBCs varied and ranged from 58% to 100%, according to age and severity of the disease severity [13]. It also mentioned that there is no correlation between the number of hemozoin-containing WBCs and parasitemia [13]. In agreement with these reports, not all malaria infected patients in our study group showed atypical eosinophil clusters in WBC scattergrams, corresponding to hemozoin-containing neutrophils. We found that 24% of the patients had an erroneously high eosinophil count. These findings suggest that, the detection of hemozoin-containing WBCs depends ultimately on the various factors that influence the kinetics of hemozoin-containing WBCs in each individual.

It has been reported that hemozoin-containing WBCs were detected in some patients after parasite clearance, as determined using a Cell-Dyn hematology analyzer [13, 14]. In the present study, three patients showed a reduction in their erroneously high eosinophil counts as the parasitemia decreased. However, our study group was small and further evaluation is needed.

CONCLUSION

In conclusion, hematologists should be aware that samples containing malaria parasites may give erroneously high eosinophil counts, and thus they should examine WBC scattergrams carefully before confirming the results. Our study group was too small to point out sensitivity, specificity, positive and negative predictive value for the detection of malaria infection by the Sysmex XN-1000 analyzer and further examination is needed with larger samples.

ACKNOWLEDGEMENTS

We would like to thank Mr. Narendra Kumar Mahawar, Lab Technician at Mahatma Gandhi Medical College and Hospital, Jaipur for his efforts in the selection of cases.

REFERENCES

1. Sharma S, Sethi N, Pujani M, Kushwaha S, Sehgal S. Abnormal WBC scattergram: a clue to the diagnosis of malaria. Hematology. 2013;18(2):101-105.

2. Jain M, Gupta S, Jain J, Grover RK. Usefulness of automated cell counter in detection of malaria in a cancer set up-Our experience. Indian Journal of Pathology Microbiology. 2012;55(4):467.
3. Briggs C, Costa AD, Tech ND, Freeman L, Tech ND, Aucamp I, Tech ND, Ngubeni B, Tech ND, Machin SJ. Development of an automated malaria discriminant factor using VCS technology. American journal of clinical pathology. 2006 Nov 1;126(5):691-698.
4. Bailey JW, Williams J, Bain BJ, Parker-Williams J, Chiodini PL. Guideline: the laboratory diagnosis of malaria. General Haematology Task Force of the British Committee for Standards in Haematology. Br J Haematol. 2013;163(5):573-80.
5. Mohapatra S, Samantaray JC, Arulselvi S, Panda J, Munot K, Saxena R. Automated detection of malaria with haematology analyzer Sysmex XE-2100. Indian J Med Sci. 2011;65(1):26-31.
6. Suh IB, Kim HJ, Kim JY, Lee SW, An SS, Kim WJ, Lim CS. Evaluation of the Abbott Cell-Dyn 4000 hematology analyzer for detection and therapeutic monitoring of Plasmodium vivax in the Republic of Korea. Tropical Medicine & International Health. 2003 Dec;8(12):1074-81.
7. Scott CS, Van Zyl D, Ho E, Meyersfeld D, Ruivo L, Mendelow BV, Coetzer TL. Automated detection of malaria-associated intraleucocytic haemozoin by Cell-Dyn CD4000 depolarization analysis. Clinical & Laboratory Haematology. 2003 Apr;25(2):77-86.
8. Hänscheid T, Pinto B, Cristino J, Grobusch M. Malaria diagnosis with the haematology analyser Cell-Dyn 3500™: What does the instrument detect? Clinical Laboratory Haematology. 2000;22(5):259-61.
9. Mendelow BV, Lyons C, Nhlangothi P, Tana M, Munster M, Wypkema E, Liebowitz L, Marshall L, Scott S, Coetzer TL. Automated malaria detection by depolarization of laser light. British journal of haematology. 1999 Mar;104(3):499-503.
10. Grobusch MP, Hänscheid T, Krämer B, Neukammer J, May J, Seybold J, Kun JF, Suttorp N. Sensitivity of hemozoin detection by automated flow cytometry in non-and semi-immune malaria patients. Cytometry Part B: Clinical Cytometry: The Journal of the International Society for Analytical Cytology. 2003 Sep;55(1):46-51.
11. Amodu OK, Adeyemo AA, Olumese PE, Gbadegesin RA. Intraleucocytic malaria pigment and clinical severity of malaria in children. Trans R Soc Trop Med Hyg. 1998;92(1):54-6.
12. Day NP, Pham TD, Phan TL, Dinh XS, Pham PL, Ly VC, Tran TH, Nguyen TH, Bethell DB, Nguyan HP, White NJ. Clearance kinetics of parasites and pigment-containing leukocytes in severe malaria. Blood. 1996 Dec 15;88(12):4694-700.
13. Metzger WG, Mordmuller BG, Kremsner PG. Malaria pigment in leucocytes. Trans R Soc Trop Med Hyg. 1995;89(6):637-8.
14. Phu NH, Day N, Diep PT, Ferguson DJ, White NJ. Intraleucocytic malaria pigment and prognosis in severe malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1995;89(2):200-4.