Original Article

DOI: 10.7860/NJLM/2020/42596:2377

The Utility of Automated Haematology Analyser Scattergrams in the Diagnosis of Malaria

Pathology Section

KEERTHI RAMACHANDRAN PILLAI[1], ARAVIND PALLIPADY[2], MUKTHA RAMESH PAI[3]

ABSTRACT

Introduction: The detection of malaria by peripheral smear examination is tedious and requires qualified staff. The diagnosis of malaria with flow cytometry-based haematology analyser scattergrams can become a vital diagnostic method and could help detect cases earlier, especially where there is no clinical suspicion. Hence, this study explores the possibilities of diagnosing malaria through hints provided by automated haematology analyser scattergrams.

Aim: To record and tabulate the scattergram abnormalities in malaria and assess the usefulness of Sysmex XN 1000 scattergrams in the diagnosis of malaria.

Materials and Methods: A total of 1000 samples of malaria patients received at the Central Diagnostic Laboratory, AJ Institute of Medical Sciences, Mangalore, Karnataka, India during the period July 2014 to June 2016 were enrolled for the study. The samples collected in the lavender-topped EDTA vacutainers were analysed by Sysmex XN 1000 and scattergrams obtained were studied for abnormalities associated with malaria. Peripheral smear diagnosis of malaria was considered as the gold standard. The statistical analysis was done by Chi-square test and Fisher's-exact test.

Results: *P.vivax* was the dominant species seen (60.8%). Greying of neutrophil and eosinophil populations was seen in 75% of vivax (p-value=0.019) and 23.2% of falciparum cases. Two eosinophil populations were seen in 71.6% of vivax (p-value <0.001) and 25.8% of falciparum malaria. Overlapping of eosinophil and neutrophil groups were seen in 73.7% of vivax (p-value <0.001). Two neutrophil populations were seen in 65.4% (p-value <0.001) and right shift of RBC ghost area was seen in 64.2% of vivax cases. The most common abnormality was two neutrophil populations: 69.1% in vivax malaria.

Conclusion: The study establishes that malarial parasites can cause scattergram abnormalities in automated haematology analysers which can aid in and increase the detection rate of malaria.

Keywords: Flow cytometry, Giemsa, Haemozoin, Parasite

INTRODUCTION

Malaria is a serious parasitic disease predominantly seen in the tropics with an estimated incidence of 300-500 million cases annually leading to the death of 1.1-2.7 million people as a result of severe malaria [1]. The diagnosis and identification of malaria through peripheral smear examination is considered the “imperfect gold standard”. Even though this procedure needs skill and is quite tedious where repetitive smear examinations are required, it is very effective when performed appropriately [2]. Other methods include quantitative buffy coat examination using fluorescent dyes, antigen coated dipstick tests, and polymerase chain reaction. However, these are high-priced and not customarily available [3].

Successful treatment and management in malaria patients requires an immediate and correct diagnosis. The clinical diagnosis of malaria is unreliable because the clinical presentation of malaria is varied, and it may be tough to differentiate it from other viral fevers and infections [1]. Automated haematology analysers are now routinely employed for basic blood investigations, thus scattergram abnormality assessment could possibly become a vital ancillary technique for the detection of malarial parasites. Scattergram abnormalities could help detect cases earlier, even in cases where there is no clinical suspicion. It could therefore, potentially reduce adverse outcomes related to malaria infections [4]. This study evaluates the different scattergram abnormalities seen in malaria using a larger study sample as previous studies have been done on a smaller sample size [3,5].

MATERIALS AND METHODS

Source of Data

The present two-year prospective study was undertaken at the Central Diagnostic Haematology Laboratory at AJ Institute of Medical Sciences (AJIMS), Mangalore, Karnataka, India from July 2014 to June 2016. The White Blood Cell (WBC) scattergrams were analysed and the cases with abnormalities were evaluated for presence of malarial parasites by peripheral smear examination. The automated haematology analyser (ISO/IEC-17025) scattergrams of 1000 malarial cases were studied.

Method of Data Collection

All the Complete Blood Count (CBC) profiles of malaria patients received at the Central Diagnostic Laboratory, from July 2014 to June 2016, were enrolled for the study. The whole blood samples (4 ml) were collected in the lavender-topped EDTA vacutainers. Sysmex XN-1000 analysed these samples and WBC scattergrams (WDF channel) were obtained. These scattergrams were studied for abnormalities associated with malaria and correlated with the peripheral smears. Peripheral smear diagnosis of malaria was considered as the gold standard.

Principle of the Sysmex XN 1000

The Sysmex XN 1000 automated haematology analyser utilises a combination of impedance and measure of radiofrequency conductance, 90° side-scatter (SSC) and 0° frontal-scatter (FSC) of laser light emitted by a semiconductor diode and detection of fluorescence due to polymethyne nucleic acid staining (90° side-fluorescence; SFL). The WDF channel (essentially a WBC differentiation function) uses surfactants in specific reagents which induce haemolysis and dissolution of red blood cells and platelets and also differentially damages the cell membrane of white blood cells. This causes the fluorescent dye in the specific reagent to cross the cell membrane and stain the nucleic acid. A 633nm laser beam excitation induces Side Fluorescent Light (SFL) and Side-Scattered Light (SSC), the intensity of which is measured and plotted on a 2-dimensional scattergram [6].

WBC Differential Fluorescence (WDF) scattergrams: Neutrophils and eosinophils scatter light the most and hence are plotted on the far right of the scattergrams due to their morphological complexities like nuclear lobulations and granules. Lymphocytes and monocytes scatter light the least as they have more regular, less complex nuclear morphology. Therefore, these form four distinct groups in different regions of the scattergrams.

Inclusion Criteria

1. Age-15 to 65 years
2. Males and females.
3. All samples included 4 mL of EDTA blood in lavender vacutainers.
4. All EDTA blood samples received as a part of CBC investigation.

Exclusion Criteria

1. Age <15 and >65 years
2. All samples less than 4 mL.
3. All samples which were haemolysed and had gross blood clots.

Informed consent: As the study was done on patients coming to the Central Diagnostic Laboratory at AJIMS, informed consent was considered as taken. The identities of the patients were not revealed during the study.

STATISTICAL ANALYSIS

The data was collected and statistical analysis was done by using SPSS software, Version 13.0. The tests used were Chi-square test and Fischer-exact test.

RESULTS

The total number of blood samples received were 68,536. Malaria investigations were done for 21,784 (31.8%) and of these 3050 (14%) cases were positive for malaria. Of the positive malaria cases, 1000 were included in this study and analysed for scattergram abnormalities.

Majority of the subjects affected by malaria were males, 84% (843) compared to 16% (157) of females. Therefore, the male: female ratio in our study was nearly 5:1. The age group of patients included in this study ranged from 15 to 65 years with a mean age of 29.5.

Of the 1000 cases included in the study, 60.8 % (608) had *P. vivax* malaria, followed by 38% (380) who had *P. falciparum* malaria. Only 1.2% (12) patients had mixed malaria i.e., both vivax and falciparum malaria.

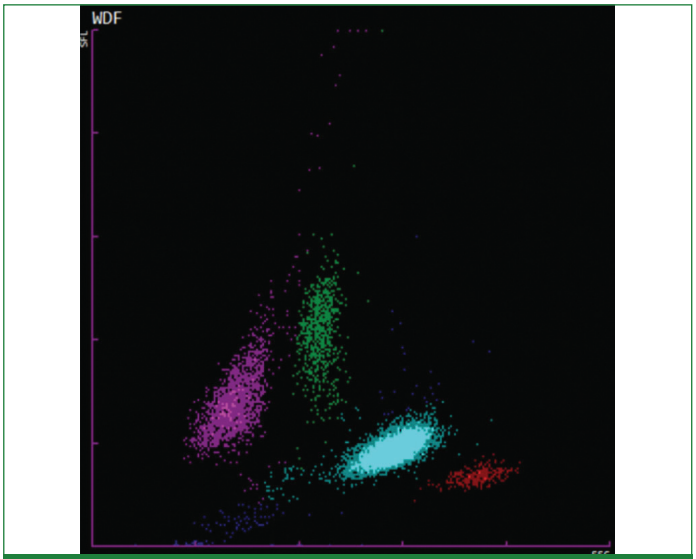
While analysing WBC scattergrams of Sysmex XN-1000, WDF channel, different abnormalities were noted in comparison with normal scattergrams [Table/Fig-1]. Many cases showed more than one abnormality. The abnormalities detected were:

a. Greying of neutrophil and eosinophil populations (G of NE)
b. Two eosinophil populations (Two E)
c. Overlapping of eosinophil and neutrophil populations (O of EN)
d. Two neutrophil populations (Two N)
e. Greying of lymphocyte and monocyte groups (G of LM)
f. Two lymphocyte populations (Two L) and
g. Right shift of RBC ghost area (Rt RBC).

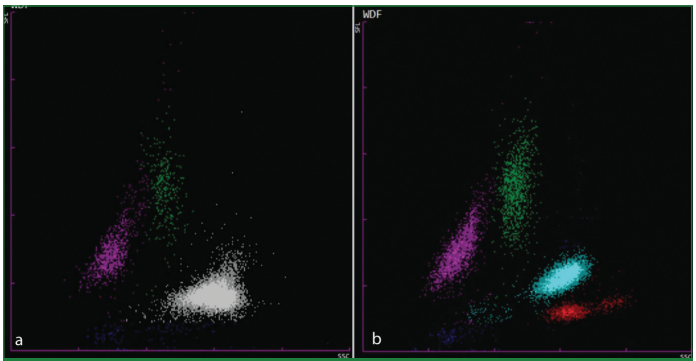
Scattergram Abnormalities

1. Greying of neutrophil and eosinophil populations: 5.6% (56) showed greying of neutrophil and eosinophil populations. Of these, 75% (42) were seen in vivax positive cases. The odds ratio calculated was 2.104 which was statistically significant with a p-value of 0.019. 23.2% (13) cases showing greying of both neutrophil and eosinophil had falciparum malaria. Only 1.8% (1) had mixed malaria [Table/Fig-2a].
2. Two eosinophil populations: 19% (190) cases showed scattergrams with two eosinophil populations. Of these, 71.6% (136) had vivax malaria. The odds ratio was 1.988 which is statistically highly significant with a p-value of <0.001. A 25.8% (49) were cases of falciparum malaria and 2.6% (5) were cases of mixed malaria [Table/Fig-2b].
3. Overlapping of eosinophil and neutrophil populations: 40.3% (403) showed overlapping of eosinophil and neutrophil groups. Of these, 73.7% (297) were vivax malaria cases with an odds ratio of 2.623. This is highly significant having a p-value <0.001 [Table/Fig-3a].
4. Two neutrophil populations: 64.2% (642) cases showed two neutrophil populations. Of these, 65.4% (420) were cases of vivax malaria. The odds ratio was 1.667 and p-value was <0.001 which was highly significant [Table/Fig-3b].
5. Greying of lymphocyte and monocyte: Only one (0.1%) falciparum malaria case showed greying of lymphocyte and monocyte populations [Table/Fig-4a].
6. Two lymphocyte populations: 3.1% (31) showed two lymphocyte populations. Of this, 48.4% (15) were vivax positive, 48.4% (15) were falciparum positive and 3.2% (1) were positive for mixed malaria [Table/Fig-4b].
7. Right shift of RBC ghost area: 41.6% (416) cases showed right shift of RBC ghost area. Of these, 64.2% (267) were vivax malaria, 34.6% (144) were falciparum malaria and 1.2% (5) were mixed malaria positive [Table/Fig-5a].

Many showed more than one abnormality [Table/Fig-5b].

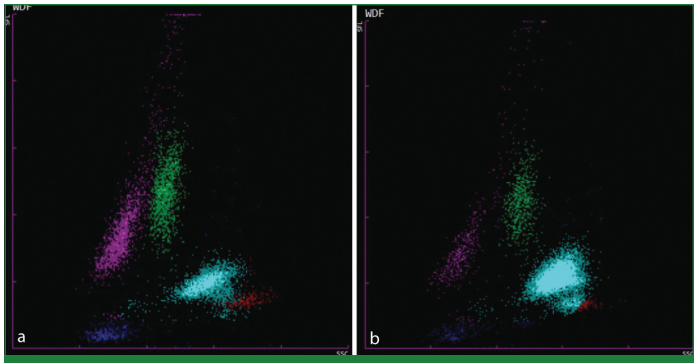


[Table/Fig-1]: Normal scattergram (WDF channel) showing different leucocyte populations. pink- lymphocytes; green- monocytes; light blue- neutrophils; red- eosinophils; dark blue- RBC ghost area.

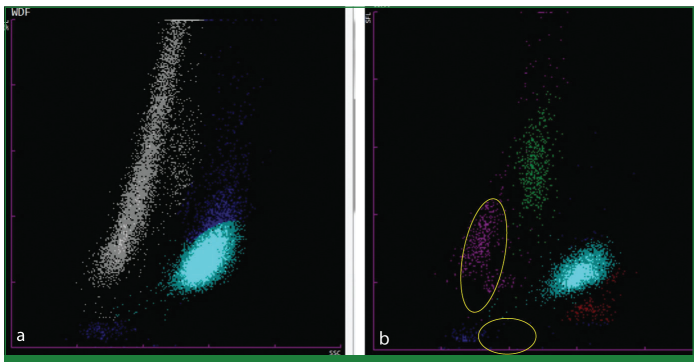


[Table/Fig-2]: a) Greying of neutrophil and eosinophil populations; b) Two eosinophil populations (red).

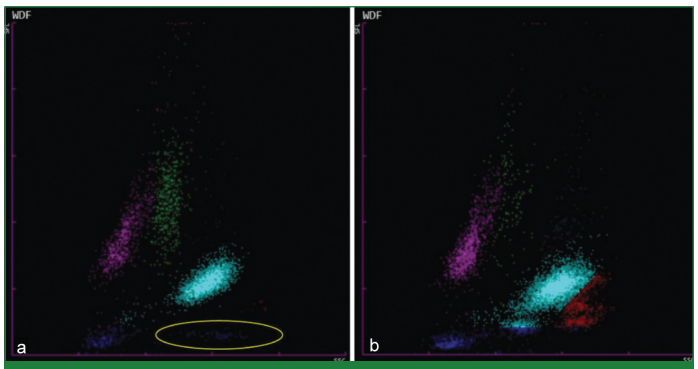
Of all the vivax cases (60.8%), 43.9% showed right shift of RBC ghost area, 2.5% showed two lymphocyte populations, 69.1% had two neutrophil populations, 48.8% had overlapping of eosinophil



[Table/Fig-3]: a) Overlapping of neutrophil (light blue) and eosinophil (red) populations; b) Two neutrophil (light blue) populations.



[Table/Fig-4]: a) Greying of lymphocyte and monocyte populations; b) Two lymphocyte populations (pink).



[Table/Fig-5]: a) Right shift of RBC ghost area (dark blue) due to increased side scatter of parasite and haemozoin containing RBCs and reticulocytes causing rightward shift; b) Multiple abnormalities i.e., two neutrophil groups (light blue), two eosinophil groups (red), overlapping of eosinophil and neutrophil groups and right shift of RBC ghost area.

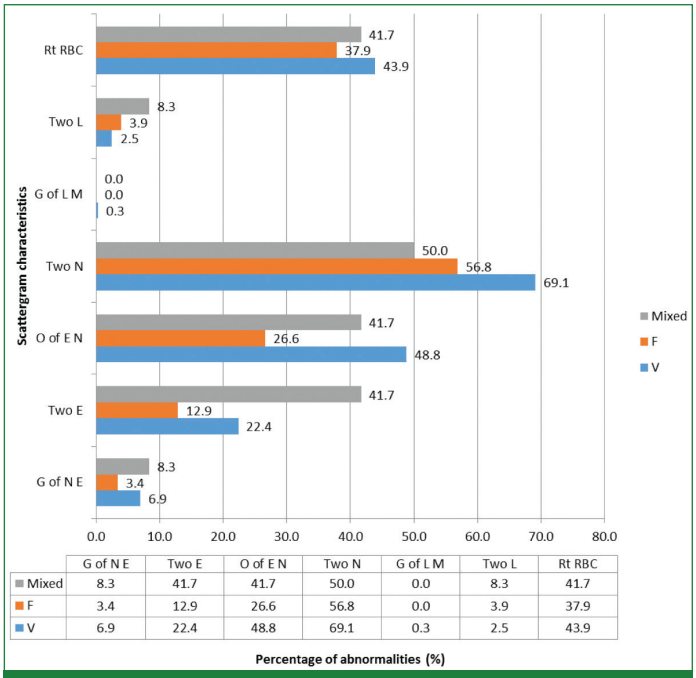
and neutrophil groups, 22.4% had two eosinophil groups and only 6.9% had greying of neutrophil and eosinophil populations. The different abnormalities seen in vivax, falciparum and mixed malaria are depicted in [Table/Fig-6].

DISCUSSION

Malaria diagnosis has relied on clinical observations and diagnostic tests, usually microscopic detection of *Plasmodium* species in Giemsa-stained blood smears and malarial antigen or antibody detection assay [3].

The drawback of these diagnostic tests is that they require skill, are unavailable routinely and can be quite expensive. There has been an increasing focus on the effectiveness of haematology analysers in the diagnosis of malaria as complete haemograms are one of the basic investigations done for febrile patients [3-5].

The majority of the cases in the present study were males as compared to females. The reason for this might be due to the fact that men are more prone to exposure to mosquitoes due to outdoor work, in the fields; construction sites etc., and therefore are more susceptible to malarial infection [7]. This is comparable to the previous studies done as shown in [Table/Fig-7] [3,5,8].



	G of N E	Two E	O of E N	Two N	G of L M	Two L	Rt RBC
Mixed	8.3	41.7	41.7	50.0	0.0	8.3	41.7
F	3.4	12.9	26.6	56.8	0.0	3.9	37.9
V	6.9	22.4	48.8	69.1	0.3	2.5	43.9

[Table/Fig-6]: Bar graph showing proportion of scattergram abnormalities (%) in malaria.
G of NE: Greying of neutrophil and eosinophils; Two E: Two eosinophil populations; O of EN: Overlapping of eosinophil and neutrophil populations; Two N: Two neutrophil populations; G of LM: Greying of lymphocyte and monocyte populations; Two L: Two lymphocyte populations; Rt RBC: Right shift of RBC ghost area; F: Falciparum; V: Vivax

Study	Taha K et al., [8]	Sharma S et al., [3]	Mubeen KH et al., [5]	Present study
Males	74.8%	55.4%	77.8%	84%
Females	25.2%	44.6%	22.2%	16%

[Table/Fig-7]: Comparison of gender distribution in malarial cases in various studies [3,5,8].

The present study showed a predominance of malaria in the age group 21-40 years, the mean being 29.5. This is comparable to studies by Taha K et al., Rasheed A et al., Shivalli S et al., etc., [8-10].

Though four species of malaria are identified in literature, only *P. vivax* and *P. falciparum* were encountered in the present study. This is comparable to other studies done in India as shown in [Table/Fig-8].

Study	Jain M et al., [12]	Narayana V et al., [13]	Sharma S et al., [3]	Mubeen KH et al., [5]	Present study
P. vivax	79.4%	93.8%	86.5%	88.9%	60.8%
P. falciparum	20.5%	5.9%	13.5%	11.1%	38%

[Table/Fig-8]: Comparison of different malarial species in various studies [3,5,12,13].

Species detection by thin smear preparation is the gold standard for the diagnosis of malaria. *P. vivax* shows trophozoites and schizonts which are easily recognisable. *P. falciparum* shows ring forms and gametocytes [11]. *P. vivax* was the dominant species encountered in the present study. This is comparable to Mubeen KH et al., Jain M et al., and Narayana V et al., etc., as shown in the table above [Table/Fig-8] [5,12,13].

Scattergram Abnormalities

While analysing WBC scattergrams, malaria cases showed different abnormalities as shown in the [Table/Fig-6]. Some of the cases, 546 (54.6%) showed more than one abnormality.

The most common abnormalities observed were two neutrophil populations, 642 (64.2%), followed by right shift of RBC ghost area, 416 (41.6%) and overlapping of eosinophil and neutrophil groups, 403 (40.3%). These abnormalities are caused when haemozoin containing white blood cells interfere with the machine's WBC detection system. Eosinophils and neutrophils are differentiated by the Sysmex XN-1000 analyser based on the variations in their

Abnormality	No of abnormalities in *P. vivax* (%)		No of abnormalities in *P. falciparum* (%)		Total no. of abnormalities (%)	
	Present study	Sharma S et al.,	Present study	Sharma S et al.,	Present study	Sharma S et al.,
Greying of neutrophil and eosinophil groups	75	47.05	23.2	18.50	5.6	41.10
Two eosinophil groups	71.6	12.7	25.8	29.60	19	16.20
Overlapping of eosinophil and neutrophil groups	73.7	5.70	25.1	18.50	40.3	16.20
Two neutrophil populations	65.4	15.70	33.6	11.10	64.2	14.70
Greying of lymphocyte and monocyte	--	4.90	0.1	22.20	0.1	8.50
Two lymphocyte populations	48.4	0.90	48.4	--	3.1	0.70
Right shift of RBC ghost area	64.2	--	34.6	--	41.6	--
Total abnormalities (N)	1177	102	539	27	1739	129

[Table/Fig-9]: Comparison of scattergram abnormalities in present study and that by Sharma S et al., [3].

light-scattering properties. The neutrophils which have ingested haemozoin exhibit amplified light scattering as a result of the birefringent quality of the pigment. Therefore, these neutrophils are falsely identified as eosinophils giving rise to two eosinophil populations and/or two neutrophil populations in the corresponding scattergram [14,15].

Two case series from South Korea have shown usefulness of abnormalities in WBC scattergrams in detecting malaria on Sysmex XE-2100. Huh HJ et al., in their study showed that 52.1% cases of malaria had abnormalities in their scattergram. A 21.5% had two eosinophil populations, 27.8% had two neutrophil populations and 48.6% had overlapping of neutrophil and eosinophil populations [15]. Yoo JH et al., also studied the scattergram abnormalities in malaria. They assessed 413 malaria cases. Only 15.7% cases showed abnormal WBC scattergrams like two eosinophil and two neutrophil populations [4].

Sharma S et al., observed that 83.8% showed scattergram abnormalities. A preponderance of greying of neutrophil and eosinophil groups (41.10%) followed by two eosinophil populations (16.20%) was observed. In the present study, greying of neutrophil and eosinophil populations were seen only in 5.6% (56), and two eosinophil populations in 19% (190). This could be because the sample size in the present study was 1000 when compared to their 148 malaria cases. Therefore, the present study could be statistically more accurate. Also, production of less haemozoin, presence of fewer and younger circulating parasites and less severity of infection might decrease the chances of detection leading to fewer scattergram abnormalities [3].

Other common abnormality observed by Sharma S et al., was overlapping of eosinophil and neutrophil populations (16.2%). Greying of lymphocyte and monocyte groups was seen in falciparum cases only 1 (0.1%). This is comparable to Sharma S et al., where they observed more falciparum cases (22.2%) with the above abnormality. This could be due to interference in their detection by rings forms of *P. falciparum* [3].

Right shift of RBC ghost area was observed in 416, 41.6% of the cases. This abnormality was also found in the study by Sharma S et al., who found 32.4% and 30.4% in vivax and falciparum respectively [Table/Fig-9].

In the present study, 64.2% (267) of vivax cases and 34.6% (144) of falciparum cases showed right shift of RBC ghost area. It has been hypothesised that the parasite and pigment containing RBCs and reticulocytes create more side scatter leading to rightward shift of the RBC cluster [12].

Campuzano-Zuluaga G et al., used scatterplots, histograms and quantitative data from automated haematology analysers to calculate predictive values for each malaria species [16].

Some scattergram abnormalities like greying of neutrophil and eosinophil groups (p-value-0.019); two eosinophil populations (p-value-<0.001); overlapping of eosinophil and neutrophil groups (p-value-<0.001) and two neutrophil populations (p-value-<0.001) were found to be statistically highly significant in vivax malaria when compared to falciparum malaria which was not significant. This is comparable to the specificity and sensitivity data obtained by Sharma S et al., Mubeen KH et al., and Jain M et al., [3,5,12]. This could be due to the lower number of falciparum cases in the present study as *P.vivax* is the dominant malarial species in that area.

LIMITATION

The present study addresses only the scattergrams provided by the Sysmex XN 1000 instrument. The efficacy of other automated haematology analyser in the diagnosis of malaria needs to be studied. Also, in the present study, the number of falciparum cases was low; therefore, larger studies from *P. falciparum* predominant areas are required to assess the utility of diagnosing falciparum positive malarial cases through WBC scattergrams.

CONCLUSION

Malaria is an important endemic disease in coastal Karnataka and other tropical areas and should be considered in all febrile patients. Several new techniques have been discovered for the detection and diagnosis of malaria but require technical expertise and involve increased expenditure etc. As complete blood counts are the most frequent requested investigation in febrile patients, the WBC scattergram abnormalities detected may alert the pathologist to the diagnosis of subclinical malaria. Therefore, these suspicious cases can be picked up and subsequently confirmed on peripheral smear.

AUTHORS DECLARATION

I hereby declare that this article is an original article and I am no way promoting the purchase and use of the Sysmex XN 1000 automated hematology analysers. I have no pecuniary or personal interest, direct or indirect, in the purchase, sale or use of Sysmex XN 1000 automated haematology analysers in the practice of pathology/hematology.

REFERENCES

[1] Lathia TB, Joshi R. Can hematological parameters discriminate malaria from non malarious acute febrile illness in the tropics? Indian J Med Sci. 2004;58(6):239-44.
[2] Maina RN, Walsh D, Gaddy C, Hongo G, Waitumbi J, Otieno L, et al. Impact of *Plasmodium falciparum* infection on haematological parameters in children living in West Kenya. Malar J. 2010; Suppl 3:S1-11.
[3] Sharma S, Sethi N, Pujani M, Kushwaha S, Sehgal S. Abnormal WBC scattergram: A clue to the diagnosis of malaria. Hematology. 2013;18(2):101-05.
[4] Yoo JH, Song J, Lee KA. Short Report: Automated detection of malaria-associated pseudoeosinophilia and abnormal WBC scattergram by the Sysmex XE-2100 hematology analyzer: a clinical study with 1,801 patients and real-time quantitative PCR analysis in vivax malaria-endemic area. Am J Trop Med Hyg. 2010;82:412-14.
[5] Mubeen KH, Devadoss CW, Rangan A, Monnappa G, Shetty P, VP Sunitha. Automated hematology analyzers in diagnosis of *Plasmodium vivax* malaria: an adjunct to conventional microscopy. Mediterr J Hematol Infect Dis. 2014;6(1):e2014034, DOI: 10.4084/MJHID.2014.034
[6] Kawauchi S, Takagi Y, Kono M, Wada A, Morikawa T. Comparison of the leukocyte differentiation scattergrams between the XN-Series and the XE-series of hematology analyzers. Sysmex Journal International. 2014;24(1):1-8.

[7] Finda MF, Moshi IR, Monroe A, et al. Linking human behaviours and malaria vector biting risk in south-eastern Tanzania. PLoS One. 2019;14(6):e0217414. Published 2019 Jun 3. doi:10.1371/journal.pone.0217414
[8] Taha K, El-Dein SZ, Idrees M, Makboul G, Baidas G. Haematological changes in malaria: relation to *Plasmodium* species. Kuwait Med J. 2007;39:262-67.
[9] Rasheed A, Saeed S, Khan SA. Clinical and laboratory findings in acute malaria caused by various plasmodium species. J Pak Med Assoc. 2009;59:220-23.
[10] Shivalli S, Pai S, Kibballi MA, D'Souza N. Construction site workers' malaria knowledge and treatment-seeking pattern in a highly endemic urban area of India. Malar J. 2016;15:168.
[11] Centre for Disease Control and Prevention [Internet]. Malaria [updated 2017 Dec 29; cited 2019 Sep 27]. Available from: https://www.cdc.gov/dpdx/malaria/.html
[12] Jain M, Gupta S, Jain J, Grover RK. Usefulness of automated cell counters in the detection of malaria in a cancer setup- Our experience. Indian J Pathol Microbiol. 2012;55(4):467-73.
[13] Narayana V, Das A, Suresh S, Chandrashekar R, Manohar VR. A retrospective epidemiological analysis of malaria in south Indian tertiary care hospital. Int Res J Pharm. 2014;5(10):786-88.
[14] Huh J, Jung J, Yoon H, Chung W. Pseudoeosinophilia associated with malaria infection determined in the Sysmex XE-2100 hematology analyzer. Ann Hematol. 2005; 84:400-02.
[15] Huh HJ, Oh GY, Huh JW, Chae SL. Malaria detection with the Sysmex XE-2100 hematology analyzer using pseudoeosinophilia and abnormal WBC scattergram. Ann Hematol. 2008;87:755-59.
[16] Campuzano-Zuluaga G, Alvarez-Sanchez G, Escobar-Gallo GE, Valencia-Zuluaga LM, Rios-Orrego AM, Pabon-Vidal A, et al. Design of malaria diagnostic criteria for the Sysmex XE-2100 hematology analyzer. Am J Trop Med Hyg. 2010;82:402-11.

PARTICULARS OF CONTRIBUTORS:
1. Junior Consultant, Department of Pathology, Asian Institute of Gastroenterology, Hyderabad, Telangana, India.
2. Associate Professor, Department of Pathology, AJ Institute of Medical Sciences, Mangalore, Karnataka, India.
3. Professor and Head, Department of Pathology, AJ Institute of Medical Sciences, Mangalore, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Keerthi Ramachandran Pillai,
Flat 102, Geeta's Grandeur, 24th St, Sriram Nagar Colony, Botanical Garden Road,
Kondapur, Hyderabad-500084, Telangana, India.
E-mail: pillai.keerthi88@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Aug 07, 2019**
Date of Peer Review: **Aug 16, 2019**
Date of Acceptance: **Oct 25, 2019**
Date of Publishing: **Jan 01, 2020**