

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

Jong-Ha Yoo, Jaewoo Song, Kyung-A Lee, Young-Kyu Sun, Young-Ah Kim, Tae Sung Park,* and Jong Rak Choi*

Department of Laboratory Medicine, National Health Insurance Corporation, Ilsan Hospital, Goyang, Korea;

Department of Laboratory Medicine, Yonsei University College of Medicine, Seodaemun-gu, Seoul, Korea;

Department of Laboratory Medicine, Kyung Hee University School of Medicine, Seoul, Korea

Abstract. Recently, the XE-2100 hematology analyzer was investigated in a rather small patient group; pseudo-eosinophilia or abnormal white blood cell (WBC) scattergrams reported by this instrument were considered as significantly valuable diagnostic parameters in detecting vivax malaria. This study was conducted not only to assess the usefulness of pseudo-eosinophilia or abnormal WBC scattergram in vivax malaria-endemic areas with large patient groups ($N = 1,801$) but also to investigate the correlation of parasitemia and platelet count with pseudo-eosinophilia and abnormal WBC scattergrams. Of the 1,801 analyzed patients, 413 (22.9%) were found to have malaria by Wright–Giemsa stained blood smears. Overall, either pseudo-eosinophilia or abnormal WBC scattergram was detected in 191 of 413 malaria patients and 4 of 1,388 patients without malaria (sensitivity = 46.2%, specificity = 99.7%). We suggest that clinical hematology laboratories using the XE-2100 analyzer should be aware of such specific parameters, even with the absence of a clinical request.

Malaria diagnosis has relied on clinical observations and diagnostic tests, usually microscopic detection of *Plasmodium* spp. of Giemsa-stained blood smears and malaria-antigen or antibody-detection assay. The disadvantage of these diagnostic tests is that they are performed only if there is a clinical request. However, complete blood count (CBC) is almost always requested without exception as part of the routine investigation in febrile patients. There are some reports using routine automated hematology analyzers for presumptive diagnosis of malaria infection. Most reports are studies regarding abnormal depolarizing patterns of the Cell-Dyn hematology analyzer (Abbott Diagnostics, Santa Clara, CA).^{1–6} Recent studies from South Korea investigated pseudo-eosinophilia and abnormal white blood cell (WBC) scattergrams of vivax malaria-infected patients on a Sysmex XE-2100 hematology analyzer (Sysmex Corporation, Kobe, Japan).^{7,8} Because the sensitivity and specificity of these methods were apparently high, its application to early detection can lead to a significant reduction in patients' morbidity and mortality.^{2,8}

Previous studies had limitations of a rather small patient number and their inaccurate evaluation of parasitemia. The aim of this study was to assess the usefulness of pseudo-eosinophilia and abnormal WBC scattergrams for the diagnosis of malaria and to investigate correlation of parasitemia and platelet count with pseudo-eosinophilia and abnormal WBC scattergram.

This study was conducted in the department of laboratory medicine of a large teaching hospital (Ilsan Hospital) located at a vivax malaria-endemic area in Korea during 36 months between January 2006 and December 2008. After obtaining informed consent, a total of 1,801 ethylenediaminetetraacetic acid (EDTA)-anticoagulated samples were submitted for both CBC analysis and malaria smears. Real-time polymerase chain reaction (RT-PCR) assay was conducted for determination of parasitemia level. Malaria patient group was composed of

413 patients that were initially diagnosed with *Plasmodium vivax* infection (276 males and 137 females). A diagnosis of malaria was made by examining Wright–Giemsa stained thick and thin blood smears. The tests were performed no more than 5 hours after blood drawing. This study was reviewed and approved by the Research Ethics Board of the National Health Insurance Corporation at Ilsan Hospital.

All samples were analyzed with the Sysmex XE-2100 hematology analyzer. The Sysmex XE-2100 measures WBCs by flow cytometry using a semiconductor red-diode laser to detect forward- and side-scattered light information. Red-cell lysis was performed using a reagent that selectively suppressed the degranulation of basophils, resulting in their separation from other forms of WBCs. The differential count (DIFF) channel uses side scatter and fluorescence intensity after staining nuclear DNA/RNA with specific dyes, and it is the source of information for counts of neutrophils, lymphocytes, monocytes, and eosinophils. An organic acid reagent binds specifically to the granules of eosinophils and allows them to be discriminated from neutrophils based on their higher side-scatter signal intensities. In addition, in the DIFF channel, a different total WBC count (sum of five-part DIFF) is presented together with the ratio of both WBC counts (WBC-DIFF/WBC-basophil [BASO]). When this ratio is not close to one, results of the differential WBC analyses are not displayed (error message means abnormal WBC scattergram). Manual WBC differential counting was also performed by microscopic examination of Wright–Giemsa-stained smears. Atypical features were categorized as pseudo-eosinophilia (a gap of more than 5% in eosinophil counts between the Sysmex XE-2100 analyzer and microscopic examination) and abnormal WBC scattergram.⁸

Parasitemia was calculated by RT-PCR technology, essentially as described previously.⁹ A malaria RG PCR kit (QIAGEN, Hamburg, Germany) was used with the Rotor-Gene Q (QIAGEN, Hamburg, Germany). Malaria patients were subgrouped as a low parasitemia group (< 500 copies/ μL) and a high parasitemia group (≥ 500 copies/ μL).⁸

Statistical tests were performed using the Statistical Package for the Social Sciences (SPSS) for Windows, Release 12.0 (SPSS Inc., Chicago, IL). Comparisons of parameters were performed with Student's *t* test, and correlation analyses

*Address correspondence to Tae Sung Park, Department of Laboratory Medicine, Kyung Hee University School of Medicine, 1 Hoegi-dong, Dongdaemun-gu, Seoul 130-702, Korea. E-mail: 153jesus@hanmail.net or Jong Rak Choi, Department of Laboratory Medicine, Yonsei University College of Medicine, 250 Seongsanno, Seodaemun-gu, Seoul 120-752, Korea. E-mail: cjr0606@yuhs.ac.

were performed using Pearson's correlation test. A value of $P < 0.05$ was considered statistically significant, and values were expressed as mean \pm standard deviation (SD).

Of the 1,801 analyzed patients, 413 (22.9%) were found to have malaria by Wright–Giemsa-stained thick and thin blood smears. All patients suffered from *P. vivax* malaria, of which 130 had low parasitemia and 283 had high parasitemia. Table 1 presents findings of the Sysmex XE-2100 analyzer for 1,801 analyzed patients. Using pseudo eosinophilia for the diagnosis, the sensitivity and specificity were 39.0% and 100%. Using WBC abnormal scattergrams for the diagnosis, the sensitivity and specificity were 15.7% and 99.7%. Overall, either pseudo eosinophilia or WBC abnormal scattergrams were observed in 191 of 413 malaria patients and in 4 of 1,388 patients without malaria (sensitivity = 46.2%, specificity = 99.7%). In the subgroup of malaria patients with parasitemia, either pseudo eosinophilia or abnormal WBC scattergram was observed in 41 of 130 patients with low parasitemia (31.5%) and 150 of 283 patients with high parasitemia (53.0%).

In 161 malaria patients with pseudo eosinophilia, the mean neutrophil counts by Sysmex XE-2100 analyzer and microscopic examination were 58.5% and 71.3%, respectively. The mean eosinophil counts by Sysmex XE-2100 analyzer and microscopic examination were 16.7% and 0.8%, respectively (Table 2).

The patients without pseudo eosinophilia or abnormal WBC scattergrams showed significantly lower parasite counts (2354.3 ± 3542.1 copies/ μ L versus 10682.3 ± 3458.2 copies/ μ L) and higher platelet count (71.6 ± 36.3 $10^3/\mu$ L versus 51.4 ± 25.6 $10^3/\mu$ L) than the patients with pseudo eosinophilia or abnormal WBC scattergram ($P < 0.05$; Table 3). It was evident that pseudo eosinophilia was significantly correlated with the parasite count ($P < 0.001$; Figure 1).

Automated hematology analyzers are capable of detecting specific abnormalities in the blood of patients with malaria. The Cell-Dyn (CD) 3500 (Abbott Diagnostics) is the first automated analyzer to detect malaria hemozoin in monocytes according to their abnormal depolarizing pattern.^{5,6} In addition, hemozoin-containing neutrophils are probably misidentified as eosinophils, because other features of neutrophils are shared with eosinophils.¹⁰ Hemozoin is produced by maturing malarial parasites and is the end product of hemoglobin digestion, consisting of a polymer of heme groups.^{2,11}

Several authors have described pseudo eosinophilia or abnormal WBC scattergrams as a result of hemozoin-containing neutrophils using the Sysmex XE-2100 analyzer.^{7,8,12} The Sysmex XE-2100 analyzer differentiates eosinophils from neutrophils based on side light-scattering differences. The neutro-

TABLE 1

Findings with the Sysmex XE-2100 analyzer for 1,801 patients with or without malaria infection

	Total patients	No. (%) of patients		
		Pseudo eosinophilia	WBC abnormal scattergram	1–2 abnormalities
<i>P. vivax</i> overall	413	161 (39.0)	65 (15.7)	191 (46.2)
Low parasitemia	130	35 (27.0)	14 (10.8)	41 (31.5)
High parasitemia	283	126 (44.5)	51 (18.0)	150 (53.0)
None	1,388	0 (0)	4 (0.3)	4 (0.3)
Sensitivity (%)		39.0	15.7	46.2
Specificity (%)		100	99.7	99.7
PPV (%)		100	94.2	97.9
NPV (%)		84.6	79.9	86.2

PPV, positive predictive value; NPV, negative predictive value.

TABLE 2

Comparison of differential count between results with the Sysmex XE-2100 analyzer and results with microscopic examination in 161 patients with pseudo eosinophilia

	Differential count [mean \pm SD (%)]		Mean difference
	Sysmex XE-2100	Microscopic exam	
Neutrophil	58.5 \pm 61.1	71.3 \pm 14.8	12.9
Lymphocyte	22.1 \pm 12.7	21.4 \pm 13.4	0.8
Monocyte	8.2 \pm 5.0	6.1 \pm 4.7	2.0
Eosinophil	16.7 \pm 10.2	0.8 \pm 1.1	16.0
Basophil	0.6 \pm 0.8	0.1 \pm 0.4	0.5

phils may show considerable side light scattering because of the birefringent character of hemozoin, and therefore, hemozoin-containing neutrophils may be incorrectly classified as eosinophils or two atypical eosinophil populations and two neutrophil populations.^{7,8} Furthermore, because hemozoin in WBC seems to be a sensitive indicator of prognosis, an automated hematology analyzer may offer a new way to assess disease severity.³ In this study, the mean difference in eosinophil counts between the Sysmex XE-2100 analyzer and microscopic examination was 16.0%, and the mean difference in neutrophil counts was 12.9%. This difference could be explained by the incorrect classification of hemozoin-containing neutrophils as eosinophils in malaria patients.

In this study, the overall sensitivity (46.2%) and specificity (99.7%) of the Sysmex XE-2100 analyzer for the detection of malaria were lower than in the previous study of South Korea (69.4% and 100%, respectively).⁸ In several studies, the percentage of hemozoin-containing WBCs varied according to populations, age, and disease activity.^{13–15} A remarkable reduction in sensitivity in this study was probably caused by the presence of younger and fewer circulating parasites, severity of infection, and host immunity factors,^{16–18} which produced less hemozoin and were below the analyzer detection limits. Our study group was larger (1,801 patients) than that of the previous study of South Korea (463 patients),⁸ but further evaluation will be needed with larger samples.

Our results showed that samples with pseudo eosinophilia or abnormal WBC scattergrams had significantly higher parasite counts and lower platelet counts than the samples without pseudo eosinophilia or abnormal WBC scattergrams ($P < 0.05$). Because the half-life (6–7 hours) of neutrophils is short,¹⁴ recent parasite counts could be reflected by pseudo eosinophilia or abnormal WBC scattergrams on the Sysmex XE-2100 analyzer. In addition, the Sysmex XE-2100 analyzer could be used as a clinical follow-up parameter.

TABLE 3

Comparison of parasite counts and platelet counts between samples with and without atypical features on the Sysmex XE-2100 analyzer in 413 malaria-infected patients

	Parasite count mean \pm SD (copies/ μ L)		<i>P</i> value	Platelet count mean \pm SD ($10^3/\mu$ L)		<i>P</i> value
Pseudo eosinophilia						
Positive (<i>N</i> = 161)	4,384.5 \pm 6,371.2	0.003		56.2 \pm 24.5	0.003	
Negative (<i>N</i> = 252)	1,863.4 \pm 1,567.6	0.003		79.4 \pm 44.7	0.003	
WBC abnormal scattergram						
Positive (<i>N</i> = 65)	15,735.2 \pm 19,635.5	0.002		42.7 \pm 33.3	0.000	
Negative (<i>N</i> = 348)	2,578.9 \pm 4,506.3	0.002		73.1 \pm 41.7	0.000	
Either or both abnormalities						
Positive (<i>N</i> = 191)	10,682.3 \pm 3,458.2	0.000		51.4 \pm 25.6	0.001	
Negative (<i>N</i> = 222)	2,354.3 \pm 3,542.1	0.000		71.6 \pm 36.3	0.001	

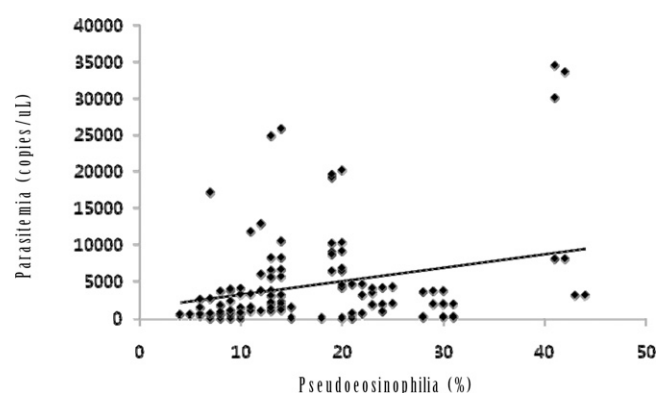


FIGURE 1. Correlation graph of parasitemia (y) against pseudo eosinophilia as measured by the Sysmex XE-2100 analyzer (x). Regression line was $y = 185.4x + 1368$ ($r = 0.270$; $P < 0.001$; $N = 161$).

In conclusion, the Sysmex XE-2100 analyzer is capable of detecting specific abnormalities in the blood of patients with unexpected malaria. Although its overall sensitivity is limited compared with the sensitivities of conventional diagnostic methods such as Giemsa-stained thick blood films, the specificity is high. Therefore, the attention to pseudo eosinophilia and abnormal WBC scattergrams provided by the Sysmex XE-2100 may be useful in the detection of unexpected malaria. Because it is likely that general screening tests like a CBC are always undertaken for patients who present with fever, it can be expected that noticing these abnormalities could prevent delay in the diagnosis of malaria. Clinical hematology laboratories operating the Sysmex XE-2100 analyzer should be aware of these specific parameters, even in the absence of a clinical request. Further studies with XE-2100 analyzer for the detection of unexpected malaria infection are needed in other ethnic groups and patients with non-vivax malaria.

Received September 16, 2009. Accepted for publication December 9, 2009.

Authors' addresses: Jong-Ha Yoo, Young-Kyu Sun, and Young-Ah Kim, Department of Laboratory Medicine, National Health Insurance Corporation, Ilsan Hospital, Goyang, Korea, E-mails: jhyoo92@nhimc.or.kr, labsun@nhimc.or.kr, and yakim@nhimc.or.kr. Jaewoo Song, Kyung-A Lee, and Jong Rak Choi, Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, Korea, E-mails: labdx@yuhs.ac, kal1119@yuhs.ac, and cjr0606@yuhs.ac. Tae Sung Park, Department of Laboratory Medicine, Kyung Hee University School of Medicine, Seoul, Korea, E-mail: 153jesus@khmc.or.kr.

REFERENCES

1. Hänscheid T, Pinto BG, Cristino JM, Grobusch MP, 2000. Malaria diagnosis with the haematology analyser Cell-Dyn 3500:

what does the instrument detect? *Clin Lab Haematol* 22: 259–261.

2. Scott CS, van Zyl D, Ho E, Meyersfeld D, Ruivo L, Mendelow BV, Coetzer TL, 2003. Automated detection of malaria-associated intraleucocytic haemozoin by Cell-Dyn CD4000 depolarization analysis. *Clin Lab Haematol* 25: 77–86.
3. Wever PC, Henskens YM, Kager PA, Dankert J, van Gool T, 2002. Detection of imported malaria with the Cell-Dyn 4000 hematology analyzer. *J Clin Microbiol* 40: 4729–4731.
4. Hänscheid T, Melo-Cristino J, Pinto BG, 2001. Automated detection of malaria pigment in white blood cells for the diagnosis of malaria in Portugal. *Am J Trop Med Hyg* 64: 290–292.
5. Hänscheid T, Pinto BG, Pereira I, Cristino JM, Valadas E, 1999. Avoiding misdiagnosis of malaria: a novel automated method allows specific diagnosis, even in the absence of clinical suspicion. *Emerg Infect Dis* 5: 836–838.
6. Coetzer TL, Scott S, Marshall L, Liebowitz L, Wypkema E, Munster M, Tana M, Nhlangothi P, Lyons C, Mendelow BV, 1999. Automated malaria detection by depolarization of laser light. *Br J Haematol* 104: 499–503.
7. Huh J, Jung J, Yoon H, Chung W, 2005. Pseudo eosinophilia associated with malaria infection determined in the Sysmex XE-2100 hematology analyzer. *Ann Hematol* 84: 400–402.
8. Huh HJ, Oh GY, Huh JW, Chae SL, 2008. Malaria detection with the Sysmex XE-2100 hematology analyzer using pseudo eosinophilia and abnormal WBC scattergram. *Ann Hematol* 87: 755–759.
9. Farcas GA, Zhong KJ, Mazzulli T, Kain KC, 2004. Evaluation of the RealArt Malaria LC real-time PCR assay for malaria diagnosis. *J Clin Microbiol* 42: 636–638.
10. Grobusch MP, Valadas E, Hänscheid T, 2000. Automated malaria diagnosis using pigment detection. *Parasitol Today* 16: 549–551.
11. Hänscheid T, Egan TJ, Grobusch MP, 2007. Haemozoin: from melatonin pigment to drug target, diagnostic tool, and immune modulator. *Lancet Infect Dis* 7: 675–685.
12. Park GB, Cha YJ, 2006. Three cases of pseudo eosinophilia associated with malaria determined in the Sysmex XE-2100 Automated Hematology Analyzer. *Korean J Lab Med* 26: 77–80.
13. Gbadegesin RA, Olumese PE, Adeyemo AA, Amodu OK, 1998. Intraleucocytic malaria pigment and clinical severity of malaria in children. *Trans R Soc Trop Med Hyg* 92: 54–56.
14. White NJ, Tran TH, Nguyen HP, Bethell DB, Nguyen TH, Ly VC, Pham PL, Dinh XS, Phan TL, Pham TD, Day NP, 1996. Clearance kinetics of parasites and pigment-containing leukocytes in severe malaria. *Blood* 88: 4694–4700.
15. Kremsner PG, Mordmiller BG, Metzger WG, 1995. Malaria pigment in leucocytes. *Trans R Soc Trop Med Hyg* 89: 637–638.
16. Grobusch MP, Hänscheid T, Kramer B, Neukammer J, May J, Seybold J, Kun JF, Suttrop N, 2003. Sensitivity of hemozoin detection by automated flow cytometry in non- and semi-immune malaria patients. *Cytometry B Clin Cytom* 55: 46–51.
17. Mendelow BV, Lyons C, Nhlangothi P, Tana M, Munster M, Wypkema E, Liebowitz L, Marshall L, Scott S, Coetzer TL, 1999. Automated malaria detection by depolarization of laser light. *Br J Haematol* 104: 499–503.
18. Padial MM, Subirats M, Puente S, Lago M, Crespo S, Palacios G, Baquero M, 2005. Sensitivity of laser light depolarization analysis for detection of malaria in blood samples. *J Med Microbiol* 54: 449–452.