The Correlation of Abnormal Information in Sysmex Hematology Analyzers XE-2100 and XS-1000*i* with Diagnosis of Plasmodium Infection

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Purpose: To study the correlation of the interpretive messages (IP message or "flag") from the automated hematology analyzers XE-2100 and XS-1000i (Sysmex Corporation, Kobe, Japan, hereinafter called "XE-2100" and "XS-1000i") and plasmodium infection based on abnormal scattergram in routine hematology analysis.

Method: Blood films were examined microscopically for Plasmodium parasites in erythrocytes , when increasing eosinophils (EO) were not detected on the smear even though the "Eosinophilia" IP message and/or EO abnormal scattergram was shown by XE-2100 and XS-1000i.

Results: In 9 out of 1501 cases with "Eosinophilia" or "EO abnormal scattergram" on the XE-2100, microscopic EO results were normal. In 6 out of 9 cases that displayed the "Eosinophilia" flag an alteration in the scattergram could be detected, where the space between the EO and the neutrophils (NEU) population had narrowed. In the other 3 cases from the XE-2100, the analyzer showed "WBC Abn Scattergram", and EO and NEU results were not obtained. Upon re-examination of these 3 cases on the XS-1000i, "Eosinophilia" and the narrow space between EO and NEU on the scattergram were displayed. In a retrospective study, 289 cases from XS-1000i analysis showed an "Eosinophilia" message and/or EO abnormal scattergram. Only 3 out of the 289 cases were inconsistent with microscopic results, and plasmodium trophozoites, schizonts or gametocytes were found in the erythrocytes of those specimens.

Conclusion: If EO results obtained by XE-2100 and XS-1000i in routine hematology analysis are inconsistent with microscopic examination even though "Eosinophilia" and/or EO abnormal scattergram was shown by XE-2100 and XS-1000i, the blood smear should still be examined microscopically for the presence of malaria parasites in the erythrocytes.

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Key Words Hematology Analyzer, Malaria Plasmodium, Eosinophil (EO)

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INTRODUCTION

Automated hematology analyzers are widely used in routine blood testing, making significant improvements in turn-around-time and accuracy. In addition to accurate counting of normal cells, the analyzers are able to supply suggestive information, through continuous development of new technologies, about the presence of abnormal cells although it might not always be able to identify the specific type of abnormal cells. However, this information is a helpful suggestion for laboratory technicians to focus on specific cells in the blood smear thus making microscopy more efficient.

We carried out RBC microscopic examination based on the automated hematology analyzers XE-2100 and XS-1000*i* (Sysmex Corporation, Kobe, Japan, hereinafter called "XE-2100" and "XS-1000*i*") displaying a "Eosinophilia" flag and/or an "EO abnormal distribution". As a result, it was confirmed that 9 patients had been infected with malaria plasmodium.

MATERIALS AND METHODS

1. Materials

1) Hematology analyzers and reagents

XE-2100 and XS-1000*i* are fully automated 5-part-differential hematology analyzers. A special feature of the new-generation analyzer XS-1000*i* is that it can use ordinary blood sampling tubes as well as micro-tubes with only 20μL blood. In the instruments, leukocytes are counted and identified using side scattered light (SSC, reflecting the complexity of cell contents) and side fluorescence intensity (SFL, reflecting the nuclear acid content). The lysing reagent for leukocytes contains macromolecular organic acid. This acid binds specifically to the eosinophil (EO) granules thus increasing the complexity of the EO cell contents. Consequently the EO cells can be distinguished from other neutrophils[1]. The scattergram is shown in ***Fig. 1***.

All reagents, calibrators and quality control materials

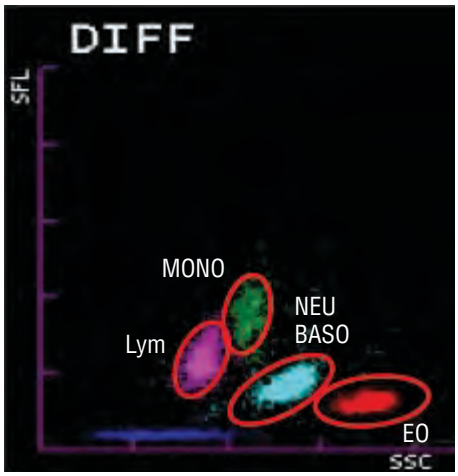

Fig. 1 *DIFF scattergram of Sysmex automated hematology analyzer*

were genuine products purchased from Sysmex Corporation.

2) Microscope and blood film staining reagents
Microscope: Olympus BX50 (Olympus Corporation, Tokyo, Japan)
Wright-Giemsa-stained dye: self-made according to standard clinical laboratory procedure[2)].

2. Patient information and specimens

1501 cases analyzed in our hospital on the XE-2100 from Aug. 2006 to Oct. 2007 and 289 cases on the XS-1000*i* produced an "Eosinophilia" IP message flag and/or an EO abnormal distribution. RBC microscopic examination was carried out in these 1790 cases.
The patient information of the 9 confirmed cases of plasmodium infection is as follows.
There were 6 males and 3 females, aged from 20 to 40 years, from Fujian or Anhui province. They were seen by internal medicine physicians in our hospital because of fever, fatigue and headache. Only one female patient was seen by a hematologist in our hospital because of reduction of three blood parameters. Platelet, hemoglobin and leukocyte were 45×10^9/L, 105g/L and 3.4×10^9/L respectively.

3. Methods

1) Measurement by hematology analyzers
2mL of peripheral blood was collected for examination on XE-2100 and XS-1000*i* or 60µL for micro-tube mode examination by XS-1000*i*. The specimens were collected into a tube containing 1.5mg/mL of EDTA-K2. The assays were performed within 0.5 to 2 hours after sample collection.
The analyzers had been calibrated with the Sysmex SCS-1000 calibrator (Sysmex Corporation), every six months. Daily internal quality control was carried out using three levels of e-CHECK (Sysmex Corporation) quality control material in order to confirm the instrument was in good condition.

2) Microscopic examination
From every specimen with an "Eosinophilia" flag and/or EO abnormal distribution, a blood smear was prepared. After Wright-Giemsa stain, the leukocytes were classified under the oil immersion lens of the microscope. If the manual EO count was inconsistent with the result from the instrument, microscopic RBC morphology examination was performed to search for malaria parasites.

RESULTS

1. 9 out of 1501 cases with an "Eosinophilia" flag and/or an EO abnormal distribution on the XE-2100 did not show any eosinophils irregularity upon microscopic review and thus the IP message (flag) could not be confirmed. In these cases, two different types of abnormal scattergrams could be seen. Type 1 was common in the 6 cases with an "Eosinophilia" flag and displayed a smaller space between the EO and neutrophil (NEU) area in the DIFF scattergram (***Fig. 2***). Type 2 was seen in 3 cases with "WBC Abn Scattergram" flag. The EO and NEU population could not be distinguished (***Fig. 3***) in the type 2 cases. Microscopic examination revealed a normal EO count in both types. The 3 cases which did not produce results for EO and NEU with the XE-2100 were re-examined with XS-1000*i*. The results were similar, producing a type 1 scattergram. In a retrospective study on results obtained by XS-1000*i*, only 3 of 289 cases with an "Eosinophilia" flag and/or an EO abnormal distribution showed inconsistency with microscopic results.

2. All 9 plasmodium-containing samples identified by microscopy showed eosinophils within the normal range. Different stages of plasmodium were detected in the erythrocytes: trophozoites, schizonts and gametocytes (***Fig. 4-6***). The results of the hematology examination are shown in ***Table 1***.

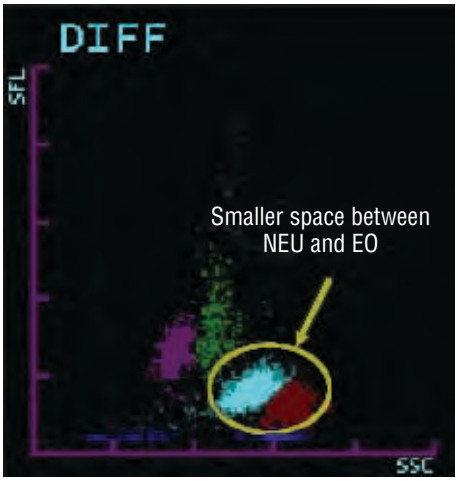

Fig. 2 *DIFF scattergram with "Eosinophilia" (Type 1)*

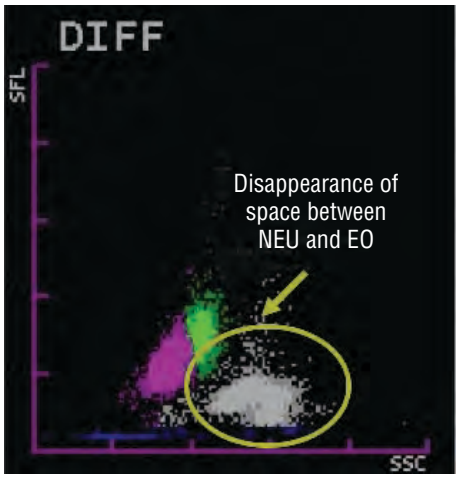

Fig. 3 *DIFF scattergram with "WBC Abn Scattergram" (Type 2)*

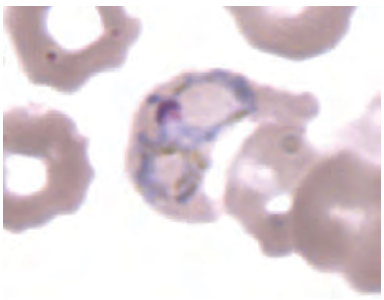

Fig. 4 *Piasmodium trophozoites*

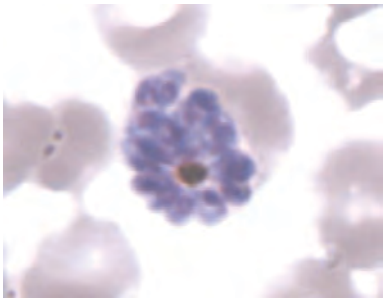

Fig. 5 *Plasmodium schizont*

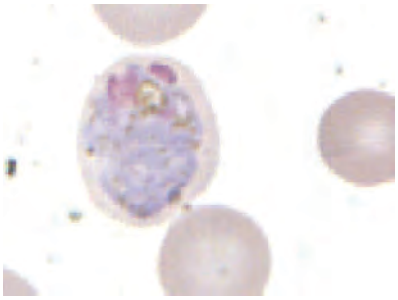

Fig. 6 *Plasmodium gametocyte*

Table 1 *The results of 9 malaria-containing specimens*

Case No	Sex	Age	Results from hematology analyzers			Results from microscopy		
			NEU (%)	EO (%)	IP message	NEU (%)	EO (%)	Morphology of Plasmodium
1	M	38	74.0	10.7	Eosinophilia	90	0	Trophozoite
2	F	20	66.6	23.5	Eosinophilia	88	0	Trophozoite Gametocyte
3	M	20	23.2	20.0	Eosinophilia	50	0	Trophozoite Gametocyte
4	M	34	-- 58.2*	-- 13.2*	WBC Abn Scg Eosinophilia*	65	0	Trophozoite Gametocyte
5	M	30	-- 56.1*	-- 14.5*	WBC Abn Scg Eosinophilia*	60	1	Gametocyte
6	M	40	85.9	5.2	Eosinophilia	89	0	Trophozoite
7	F	38	78.9	9.1	Eosinophilia	81	1	Trophozoite Gametocyte
8	F	29	43.1	22.5	Eosinophilia	58	0	Gametocyte
9	M	22	-- 40.2*	-- 35.6*	WBC Abn Scg Eosinophilia*	57	1	Gametocyte

*: Results from XS-1000*i*; the others from XE-2100.

DISCUSSION

We investigated cases with an "Eosinophilia" IP message (flag) or an "EO abnormal scattergram" from the automated hematology analyzers XE-2100 and XS-1000*i*, and re-assessed them by microscopic examination. In 9 of 1501 samples (0.6%) from XE-2100 and 3 of 289 samples (1%) from XS-1000*i* a plasmodium infection was detected. Leukocyte classification in XE-2100 and XS-1000*i* is based on side scattered light and fluorescence intensity, which reflect the complexity of cell contents and the nuclear acid content respectively. Pseudo-eosinophilia appears to be caused by the presence of components which have a side scattered light and fluorescent intensity similar to an EO. Although it is unknown what these components are, we would like to suggest the following hypothesis. The Malaria pigment (hemozoin) is a degradation product of hemoglobin and is a kind of double refraction crystal released from plasmodium-infected cells. It is ingested by the host leukocytes after the plasmodium schizont ruptures. Thus the neutrophil cluster (NEU) containing malaria pigment will appear in the EO area of the scattergram because of the change of side scattered light intensity[3-6].

In our study, a certain type of patients was suspected to have eosinophilia as indicated by an IP message (flag). However, this pseudo-eosinophilia appeared in a different area of the scattergram compared with the usual increased EO signal, and showed the characteristic of a decreased distance between the EO and NEU population (***Fig. 2***). Another type of patients showed "WBC Abn Scg" with the disappearance of the space between EO and NEU (***Fig. 3***).

Through the above experience of detecting plasmodium infection through a detailed check of inconsistent result between instrument IP message and microscopy, we first have to recognize that we should not easily conclude the instrument IP message was an error. Even if it is a low incidence of 0.6% to 1% in all specimens, we have to search for the real reasons to avoid some cases being overlooked, particularly when there is no clinicians' request for a special inspection.

Secondly, we should be fully aware of the instrument's working principle, attach great importance to the flag information and understand its meaning. In order to determine other possible evidence based reasons for instrument flags, we must have the knowledge to accurately integrate alarm signals and abnormal scattergram information of the instrument with microscopic confirmation. Based on the technology used by the analyzer and the abnormal appearance of the scattergrams, we concluded that the inconsistency between the analyzer result and the microscopic examination was attributed to the influence of some substance in the normal leukocyte that intensified the side scatter light signal. As a result, NEU side-light intensity might shift to right (higher) in the scattergram without a true increase in the eosinophils concentrations in the blood film.

In addition, we have also noted that the IP message "Eosinophilia" was encountered in 6 patients with smaller spacing between EO and NEU (***Fig. 2***), and 3 samples showed "WBC Abn Scg" without EO and NEU classification results (***Fig. 3***). The 3 samples with "WBC Abn Scg" were re-examined on the XS-1000*i*, and "Eosinophilia" and smaller spacing was shown in XS-1000*i*. In the same instrument, some cases of malaria infected samples displayed "Eosinophilia" with smaller spacing between EO and NEU, and other cases displayed no-classification. We consider it might be caused by the amount of hemozoin in the patients. Some specimens showed different results between the instruments: no-classification in XE-2100 and "Eosinophilia" message in XS-1000*i*, albeit identical working principles. This might be due to sensitivity differences of the two instruments to side-light intensity. It reminds us that we should pay more attention to the condition of the instruments.

In conclusion, if "Eosinophilia" or EO abnormal scattergram is shown in routine hematology analysis by XE-2100 and XS-1000*i* although there is no abnormal EO population upon validation by microscopy, it might suggest the existence of malaria plasmodium in the patient.

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