CASE REPORT

Relapse of *Plasmodium vivax* and Recrudescence of *Plasmodium malariae* Malaria, as Detected by the SYSMEX XE-2100 Fully Automated Blood Cell Analyzer

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All symptoms and signs of uncomplicated malaria are non-specific, as they are shared with other febrile conditions. Two cases of patients with a travel history in Asia are reported who were admitted to the hospital after one week of fever. Laboratory findings for both included anemia, thrombocytopenia and elevated C-reactive protein. In both cases, the DIFF scattergram of the multi-parameter Sysmex XE-2100 (Sysmex Corporation, Kobe, Japan) automated hematology analyzer was abnormal. A subsequent blood smear and the identification of parasites in the film confirmed malaria parasites. The cases described in this report highlight the importance of taking a complete travel history from patients with unexplained febrile illness and suggest that special attention to atypical distribution patterns of the white blood cells (WBC) scattergram is extremely helpful in the diagnosis of malaria.

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Key Words

XE-2100, Automated Hematology Analyzer, *P. malariae*, *P. vivax.*

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INTRODUCTION

Malaria is an increasingly common public health problem in many parts of the world. Today the number of cases is rising worldwide as imported cases of malaria may be present in any country. A febrile illness, even some time after return from the malarious region, should raise suspicion of a possible malarial infection. [1,2] Symptoms of malaria infection are not always dramatic and can easily be dismissed as unimportant or seen as signs of other febrile conditions. Physicians should always consider the possibility of malaria in travelers, because timely diagnosis of malaria infection can be of vital importance. Diagnosis of malaria relies on a complete medical history of symptoms, travel, physical examination, and blood tests including thick and thin Giemsa-stained blood films in order to identify the *Plasmodium* species responsible for the infection. Our data indicate that the modern Sysmex XE-2100 automated hematology analyzer is able to detect certain abnormalities in the blood of patients caused by malaria.

Case 1

A 36-year old woman was admitted to the "Szent László" Hospital, Budapest, on January 27, 2006, because of high fever, chills, muscle and joint pain, vomiting and dry cough that had developed one week before hospital admission.

Earlier she had received anti-fever drugs and ofloxacin treatment without any effect. She had visited Nepal one and a half years earlier and at that time she received Lariam as malaria chemoprophylaxis. In Nepal, she had caught a common cold, but showed no definite signs or history of malaria. The patient was febrile in the hospital. Upon physical examination the liver was palpable, the spleen was slightly enlarged, the blood pressure was 110/70 mmHg, and the pulse rate was 84 beats per minute. The pulmonary and cardiac status were normal. The rest of the examination was unremarkable.

The relevant laboratory investigations included the following: hemoglobin level 110 g/L, white blood cell count 3.5 $\times 10^9$/L and platelet count 59 $\times 10^9$/L, all measured by a Sysmex K4500 18-parameters hematology analyzer. The serum liver enzymes were marginally elevated and the C-reactive protein was 48 mg/L. For 48 hours after hospital admission the patient received only saline infusion and anti-fever drugs. The next day we used the Sysmex XE-2100 multi-parameter hematology analyzer for the complete blood cell analysis. The sample from the patient showed two unexpected distributions of granulocytes in the DIFF scattergram, with an abnormally left-shifted eosinophilic cell population based on side fluorescence and side-scatter signals. The first cluster appearing as a neutrophil cloud was located between the ghost and the neutrophils; the other cluster was above the pseudo-eosinophil cluster. (***Fig. 1A***) For this reason, a blood smear was prepared. The microbiological examination revealed mixed infection caused by *P. vivax* and *P. malariae*. Based on parasite morphology, pigmentation and the shape and size of the infected erythrocytes, different developmental stages of the malaria parasites could be distinguished such as trophozoites, young and mature schizonts and gametocytes. Polymerase chain reaction (PCR) carried out in the Laboratory of Parasitology of the National Center of Epidemiology confirmed the co-infection of *P. vivax* and *P. malariae*. While *P. malariae* seeks only mature red cells, *P. vivax* prefers younger red cells. Loaded with enough parasitic cells, as can be the case with schizonts or gametocytes, these could constitute the left-shifted pseudo-eosinophilic population. The other two abnormal populations are likely to be composed of neutrophils containing phagocytosed hemozoin, a pigment from hemoglobin digestion by the parasite.

One day later the patient had pseudo-eosinophilia (18%) without eosinophils in the blood smear. Hemozoin-containing neutrophils could again be detected on the Sysmex XE-2100 as two atypical populations in the WBC scattergram (***Fig. 1B***). This time, the analyzer classified the hemozoin-containing neutrophils from *P. vivax* as eosinophils, leading to pseudo-eosinophilia. The other pseudo-neutrophil plot from *P. malariae* located between the ghost and the neutrophils was unchanged. The patient was treated with a standard course of chloroquine. 72 hours after the start of the treatment no parasites were detectable and the scattergram became negative as well (***Fig. 1D***). The erythrocytic glucose-6-phosphate-dehydrogenase (G6PD) activity was normal and the malaria treatment was completed with primaquine for 14 days to eradicate the exoerythrocytic forms, especially the hypnozoites responsible for relapses. The patient showed gradual improvement and was discharged from the hospital nine days after admission.

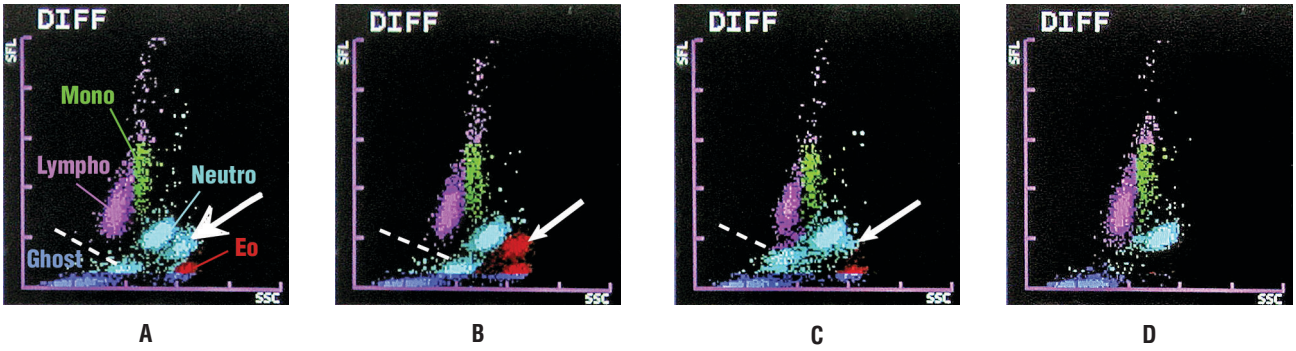

Fig. 1 *Scattergram generated by the Sysmex XE-2100 hematology analyzer*

A. *Sample from the patient on the second day. One group of signals resembling a neutrophil cloud (neutrophils containing hemozoin from P. malariae) (indicated by "----") is located between ghosts and neutrophils, suggesting reduced side scatter, diminished granule content, and low nucleic acid content, the other cluster (neutrophils containing hemozoin from P. vivax) (indicated by "←") is above the pseudo-eosinophil cluster.*
B. *Day 4. Atypical distribution of pseudo-eosinophils (indicated by "←"), which is located closer to the neutrophil cluster corresponding to hemozoin-containing neutrophils, the P malariae-caused pseudo-neutrophil population (indicated by "----") is unchanged.*
C. *Day 5. As a result of treatment, the cluster of pseudo-neutrophils located between lymphocytes and ghosts became larger (indicated by "----"), while the cluster influenced by P. vivax (indicated by "←") decreased in size and moved closer to the regular neutrophils.*
D. *Due to three days of treatment the DIFF scattergram changed to normal.*

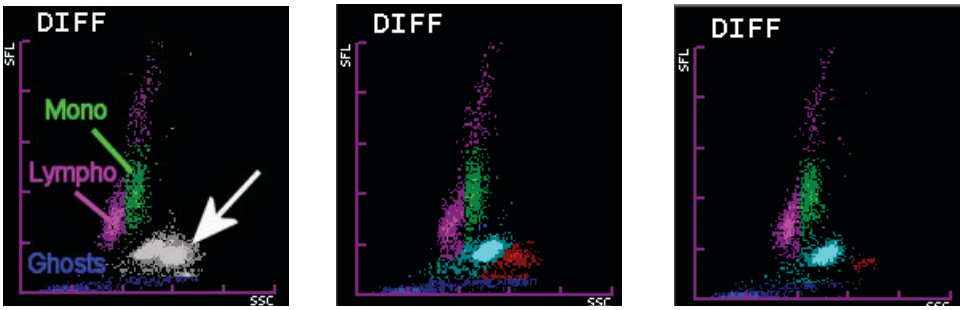

Fig. 2 *Scattergram generated by the Sysmex XE-2100 hematology analyzer.*

A. Initial scattergram with non-classifiable population (indicated by "←")

B. Day 2: abnormal population separates into neutrophil and pseudo-eosinophil population

C. Day 3: successful treatment further reduces the pseudo-eosinophilic population, scattergram approaches normal status.

Case 2

A 21-year-old man originating from the Far East sought care at our hospital emergency department after one week of fever. He did not take malaria chemoprophylaxis. Physical examination revealed mild hepatosplenomegaly. His laboratory findings included mild anemia (Hb: 133 g/L), thrombocytopenia (23 $\times 10^9$/L), marginally elevated lactate dehydrogenase (LDH: 816 U/L) and C-reactive protein (95 mg/L). A smear was prepared. The identification of *P. vivax* parasites in the blood film established the correct diagnosis. The Sysmex XE-2100 hematology analyzer showed the following results: In the DIFF scattergram, the neutrophils could not be classified, with the plot extending from neutrophils toward the area of eosinophils, with enhanced lateral scattered light intensity (***Fig. 2A***). Lymphopenia and "Atypical Lympho" were also found, caused by reactive lymphocytes. The patient was treated with a standard course of chloquine. On the following day atypical eosinophil clusters were observed in the WBC scattergram (***Fig. 2B***), corresponding to schizonts or hemozoin containing neutrophils with mild pseudo-eosinophilia without any eosinophilic cell in the blood smear. Upon three days of proper treatment the DIFF scattergram changed to normal (***Fig. 2C***).

DISCUSSION

It is possible but not very common to develop a relapsing type of malaria months or even years after being infected by plasmodia. Malaria can develop even if prophylactic anti-malarial drugs were taken. Anti-malarial drugs can prevent the development of the symptoms of acute malaria by suppressing the infection in the bloodstream. However, they do not prevent relapses of the infection caused by the strains of the *Plasmodium* parasites which have a persistent liver phase.

In this paper, a very interesting mixed recurrent malaria (*P. vivax* and *P. malariae*) case as well as a case of single *P. vivax* infection are presented. Both *Plasmodium* species can cause recurrent malaria infection.

In the case of *P. vivax* relapse occurs due to the reactivation of hypnozoites stored in the liver. One and a half years ago, the prophylaxis (Lariam) protected the first patient from acute malaria infection. However, it did not prevent the sporozoites from developing into hypnozoites in the hepatocytes. Without Primaquin post-treatment, relapse occurs in approximately half of the infected patients.

In the case of *P. malariae* recrudescence occurs, that is to say, parasitemia falls below detectable levels and then later increases to visible parasitemia. Without appropriate treatment or untreated *P. malariae* causes a long-lasting, chronic infection which in some cases can last a lifetime.

It is difficult to distinguish malaria from other common febrile disorders. It seems to be very useful to carry out a complete blood cell analysis with modern hematological analyzers such as the Sysmex XE-2100. An unexpected WBC scattergram can then suggest preparing a blood smear, which in our cases revealed the malaria infections. Several authors describe the particular potential of an analysis of the depolarization of a laser beam used in flow cytometry in detecting malaria infection using the Cell-Dyn 3500 or 4000 hematology analyzer.[3)]

The automated analysis through laser beam depolarization is performed as a part of the routine full blood count by this multi-parameter hematology analyzer. The abnormal depolarizing patterns are due to the presence of leukocyte-associated malaria hemozoin, a pigment that depolarizes the laser beam.[3)] A recent study using the Sysmex XE-2100 automated hematology analyzer demonstrated unexpected scattergrams in differential WBC plots from patients with malaria infection. Hemozoin is known to be an end product of hemoglobin digestion by the malaria parasite. During malaria schizont rupture, hemozoin is released from infected red cells and is subsequently ingested by host WBC.[4,5)] When this insoluble polymer is released into the host circulation, scavenger neutrophils and monocytes phagocytose the material, which then appears as a black, brown or ambient pigment under light microscopy. The half-life of

neutrophil granulocytes is 6-8 hours and that of a monocyte is several days. Thus, the quantity and distribution of engulfed hemozoin within granulocytes and monocytes may reflect the chronology of a patient's infection. After the apoptosis of granulocytes, the hemozoin polymer is again released into the host circulation. The analyzer classifies some of the hemozoin-containing neutrophils as eosinophils, leading thus to pseudo-eosinophilia. Searching the entries in the electronic literature database of PubMed we found only one report about pseudo-eosinophilia as a result of hemozoin-containing neutrophils using a Sysmex XE-2100 hematology analyzer.[6] Our paper is the first report on mixed infection malaria which results in two hemozoin-containing neutrophil populations, one being above the pseudo-eosinophils with the other located between the lymphocyte and the ghost areas. In our second case, an abnormal scattergram again supports the effect of hemozoin on WBC distribution in the DIFF channel. Therefore, the Sysmex XE-2100 hematology analyzer, while in and of itself not sensitive enough to be a malaria screening tool, may support and contribute to the diagnosis of malaria as well as aid in therapy monitoring. However, complete blood cell count and thin blood smear are always necessary for the patient present with pyrexia, as the abnormalities presented can only suggest the diagnosis of malaria, which might not have been considered initially.

Physicians should always consider the possibility of malaria in travelers, as a timely diagnosis of malaria can be of vital importance.

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