Flagging performance of Sysmex XN-10 haematology analyser for malaria detection

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ABSTRACT

Aim The aim was to assess the flagging performance of Sysmex XN-10 haematology analyser for malaria detection through the parasitic red blood cell ('pRBC') alarm.

Methods We retrospectively studied 584 blood samples performed on the Sysmex XN-10 analyser that were tested for malaria. Sensitivity, specificity, positive and negative predictive values, and prevalence were established for the pRBC alarm.

Results Sensitivity, specificity, and positive and negative predictive values for the pRBC flag were 7.8%, 100%, 100% and 87.7%, respectively. The prevalence of pRBC flag of 0.026% in the overall population was significantly different from the prevalence of 1.027% in the population tested for malaria.

Conclusions Considering the excellent specificity and the low prevalence of the flag in the overall population, we suggest, in case of the presence of pRBC flag, the implementation of a rapid review of the blood smear looking for *Plasmodium*, mostly if the patient had fever and had not been tested for malaria.

INTRODUCTION

Microscopy or rapid diagnostic tests remain the main tools for malaria diagnosis according to WHO 2018 guidelines, while new methods, including isothermal DNA amplification, are now available in non-endemic countries.[1] Prompt malaria diagnosis is a key component of the prevention of disease severity, leading to poor outcome with a special emphasis on patients presenting with imported malaria in areas where this diagnosis is uncommon.

Automated complete blood count (CBC) is usually indicated for patients admitted with fever. Sysmex XN-10 was indeed reported to detect mature stages of *Plasmodium* through the flow cytometry White blood cells Differential Fluorescence (WDF) scattergram.[2 3] Automated CBCs performed on Sysmex XN-10 haematology analyser (Sysmex Corporation, Kobe, Japan) may therefore represent a tool to suspect malaria diagnosis. A 'parasitic red blood cell' ('pRBC') flag was recently developed by Sysmex Corporation for XN-10 (IPU V.21.12) in the presence of additional fluorescence in WDF scattergram (figure 1). Intraerythrocytic parasites can indeed result in additional fluorescence due to incomplete lysis of red blood cell (RBC) by the lysing reagent. To investigate the performance of the pRBC flag for malaria positive patients' detection in a non-endemic area, we retrospectively reviewed CBCs of patients screened for malaria using conventional methods which took place over a 10-month period.

This multicentre study was set up in the University Hospital of Lyon (Hospices Civils de Lyon, France). All patients from various departments of admission who had a malaria screening and a concomitant automated CBC were included during a 10-month period (from December 2017 to September 2018) without any exclusion criteria. CBCs of these patients were retrospectively screened for the pRBC flag. According to the national guidelines, malaria diagnosis was performed using a microscopic examination and a rapid diagnostic test (Vikia; Biomerieux, France). Microscopic examination (Leica, Germany) included a thin smear (Diff-Quick; RAL Diagnostics, France), and a thick smear (Giemsa, Merck, Germany) was performed by two independent operators. Automated CBC analysis was performed on the Sysmex XN-10 haematology analyser IPU V.21.12 (Sysmex Corporation). This analyser uses fluorescence and flow cytometry technology with a semiconductor laser to categorise white blood cells. The pRBC flag was recently developed by Sysmex Corporation for XN-10 (IPU V.21.12) and renamed 'inclusion red blood cell' (IPU V.22) to correct white blood cell counts on the WDF scattergram in case of additional fluorescence interference. No additional sample was collected from patients for the purpose of this study. Sensitivity and specificity, as well as positive predictive value (PPV) and negative predictive value (NPV), of the pRBC flag were calculated to evaluate its performance for *Plasmodium* detection. The prevalence of the pRBC flag was also determined in the overall population on a 2-month period. Moreover, the level of agreement of conventional methods and pRBC flagging was calculated with Cohen's kappa statistic.[4]

RESULTS

A total of 584 CBCs from samples tested for malaria were examined. Seventy-seven samples were positive for malaria, including 59 positive for *Plasmodium falciparum (Pf)*, 6 for *Plasmodium ovale (Po)*, 5 for *Plasmodium malariae (Pm)*, 3 for *Pf* and *Pm* coinfections, and 4 for *Pf* and *Po* coinfections (table 1). Several stages of parasite were detected on microscopic examination: ring-stage trophozoites, schizonts and gametocytes. Parasite density ranged from less than 0.01% to 13.5%. Among malaria positive patients, 72.7% (56/77) had thrombocytopenia. 'Abnormal lympho/blast'

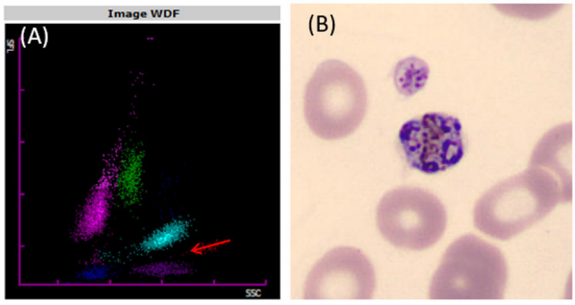

Figure 1 Detection of malaria parasite. (A) Sysmex XN-10 haematology analyser shows an abnormal White blood cells Differential Fluorescence (WDF) scattergram with a supplementary purple cluster in the presence of parasitic red blood cell flag (red arrow). (B) Peripheral blood smear stained with May-Grünwald-Giemsa: mature form of *Plasmodium malariae* (schizont). SFL, side fluorescence light; SSC, side scatter light.

and 'Atypical lymphocyte' alarms were observed in 14 malaria patients (18.2%) and 26 patients (33.8%), respectively.

Among the population who was screened for malaria, pRBC flag had a sensitivity of 7.8% (6/77) and 20.8% (16/77) if the defined thresholds are 100 and 10, respectively. Regarding the mature stages (schizonts and gametocytes) mostly found in non-*Pf* species, pRBC flag had a sensitivity of 46.1% (6/13) and 100% (13/13) for defined threshold of 100 and 10, respectively (table 1). pRBC flag was not recovered for samples that were only positive for ring-stage trophozoites on the blood smear and this, despite a high parasite density. pRBC flag had a specificity of 100% (507/507) in patients who were tested for malaria, a PPV of 100% (6/6) and a NPV of 87.7% (507/578) if the defined threshold is 100.

The prevalence of pRBC flag of 0.026% (29/110800) in the overall population on a 2-month period was significantly different from the prevalence of 1.027% (6/584) in the population who was tested for malaria on a 10-month period.

Cohen's kappa of 0.619 with an expected agreement of 0.679 and an observed agreement of 0.878 indicates a moderate level of agreement.

Of note, 19 out of the 29 false-positive samples (65.5%) were obtained from patients under 1 year of age. Microscopic examination of these samples reports five cases of inclusions, such as jolly bodies in RBCs, eight cases of fibrin filaments or platelet aggregates and one case of interference on fluorescence due to the presence of immature nucleated RBCs.

Table 1 *Plasmodium* species and stages and Sysmex XN analyser data

	Pf (n=59)	*Pm* (n=5)	*Po* (n=6)	*Pf and Pm* (n=3)	*Pf and Po* (n=4)
Trophozoites	58	5	6	3	4
Schizonts	0	2	1	2	0
Gametocytes	3	4	2	0	1
'Abnormal lympho/blasts' flag	10	1	1	1	1
'Atypical lymphocytes' flag	18	2	3	1	2
'pRBC' flag (limit of ≥100)	0	5	1	0	0
pRBC flag (limit of ≥10)	5	5	3	2	1

Pf, *Plasmodium falciparum*; *Pm*, *Plasmodium malariae*; *Po*, *Plasmodium ovale*; pRBC, parasitic red blood cell.

DISCUSSION

We studied CBCs of 77 malaria-positive patients mostly infected with *Pf*. Thrombocytopenia, known to be related to malaria infection,[5] was observed in 72.7% of patients. A specific flag developed on haematology analyser could help to detect *Plasmodium* in patients who were not yet tested using conventional tests. We showed evidence that the pRBC flag from Sysmex XN-10 revealed a poor sensitivity of 7.8% for malaria parasites, notably for *Pf* samples (0/59). However, as previously demonstrated on the XN-10 analyser,[2 3] the sensitivity was increased to nearly 50% if samples are positive for mature forms of *Plasmodium* that are mostly recovered during *non-Pf* infections. The sensitivity was also improved using a lower defined threshold of 10. The specificity was proved to be excellent in patients who were tested for malaria (100%). False-positive samples in the overall population were extremely rare and mostly recovered for young children under the age of 1 year and this, with or without erythrocytes or platelets abnormalities. The prevalence of pRBC flag in the overall population was low compared with the population who was tested for malaria. Whereas pRBC flag is mostly contributive for the detection of *non-Pf* infections, the use of pRBC flag to detect new cases of malaria is clinically relevant. First, the diagnosis of malaria is urgently needed to start a prompt antimalarial treatment, and any tool that will help to complete malaria diagnosis using conventional methods is very welcome. Second, non-*Pf* infections are expected to increase in the future as the fight against malaria is mostly directed to *Pf*, leading to increasing relative risk of non-falciparum infection.

Considering the excellent specificity and the low prevalence of the flag in the overall population, we suggest, in case of the presence of pRBC flag, the implementation of a rapid review of the blood smear looking for *Plasmodium*, mostly if the patient had fever and had not been tested for malaria.

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