Two cases of *Plasmodium falciparum* malaria analyzed on Sysmex Xn-9000 and Mindray Bc-6800 Plus

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ABSTRACT

Malaria is an infection caused by the protozoan *Plasmodium*, transmitted to humans by Anopheles mosquitos. The diagnosis involves a combination of clinical observations, clinical history, and microscopic blood examination. In this study, we assessed *Plasmodium falciparum* infection in two patients of african origin with malaria diagnosis, using two different microscopic examination and hematological analyzers. The Sysmex XN-9000 is able of revealing, by the white cell differential (WDF) scattergram panel, an abnormal cluster indicative of the accumulation of plasmodium infected red blood cells, associated with a specific alarm signal on the Q-FLAG panel. The Mindray BC-6800 Plus also showed an anomalous cell cluster, linked to parasitized red blood cells in the differentiation channel (DIFF). Also in this case the cluster was associated with a specific alarm of 'Infected red blood cells' ('InR'), identifying the number and percentage of infected red blood cells. Microscopic analysis confirmed the presence of ring forms of the parasite in both cases. Both XN-9000 and BC-6800 Plus, which integrate cytofluorimetric and cytomorphological analysis, demonstrated the potential for rapid and accurate diagnosis of malaria infection.

Key words: *malaria, Plasmodium falciparum, hematology analyzer*

CASE REPORT 1

A 40-year-old woman of Nigerian nationality, living in Italy for 13 years, arrived at the emergency department (ED) of the Tor Vergata University Hospital, showing the following symptoms: elevated temperature (40°C), hypotension, tachycardia and general malaise. No allergies to drugs or foods, or use of substances of abuse were reported. Due to a significant language barrier, only a few other pieces of information were obtained at this stage. Interestingly, the patient had returned recently from a trip to Nigeria; she also said she had not taken any kind of malaria chemoprophylaxis. The patient was thus thoroughly investigated at the ED.

The patient showed normal renal function, electrolyte concentration, and only slight alteration in liver function, characterized by a total bilirubin concentration of 1.53 mg/dL (r.v. ≤1.20) and a value of C-reactive protein (CRP) of 114.60 mg/dL (r.f.<5.00).

Complete blood count (CBC) test reported: red blood cells (RBC) 4.24 $x10^{12}$/L (r.i. 3.50-5.20), hemoglobin (Hb) 92.0 g/L (r.i. 12.0-16.0), platelets 75 $x10^{9}$/L (r.i 150-450), white blood cells (WBC) 2.91 $x10^{9}$/L (r.i. 4.30-10.80).

The CBC was performed using a Sysmex hematology analyzer, XN-9000 (XN, Sysmex, Kobe, Japan). This analyzer employs flow cytometry with an optical laser method for the identification and quantification of hematological cells according to Forward-Scatter (FSC), Side-Scatter (SSC) and Fluorescent Intensity (SFL), which reflect cell size, cell granularity/internal complexity and cell DNA/RNA content, respectively. In this case, in a specific area of the white cell differential (WDF) scattergram, a supplementary deep purple colored abnormal cell cluster (ghost) was visible (Figure 1, panel

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A, yellow arrow). This cell population is characterized by high SSC and very low SFL signals. The high SSC signal of a parasitic red blood cell cluster (pRBC) could probably be related to the presence of hemozoin crystals produced by mature parasites during Hb degradation (1).

The presence of this cluster, the pRBC flag generated in the output channel, was recently renamed the iRBC ('inclusion red blood cells') (Figure 1, panel B). The flag detects the abnormal shape or positioning of the cell populations in the WDF channel. This is achieved using a proprietary algorithm for cell cluster analysis ("SAFLAS", Sysmex Adaptive Flagging Algorithm). Using this software, when specific abnormalities are detected in the WDF (and WNR) scattergrams, the XN-9000 automatically generates the pRBC flag.

In this instrument, the probability of the presence of abnormal cells is indicated by the Q-FLAGs panel, where specific flags span on a scale from 0 to 300 arbitrary units. The pRBC flag is highlighted in red when the value is >100, 300 in this case (Figure 1, panel B, upper right panel). The flag value strictly depends on the number of events counted by the XN-9000 in the ghost area (Figure 1, panel A, purple population). The progressive reduction and disappearance of the pRBC cluster is an indicator of the resolution of the disease.

Furthermore, the evaluation of the XN-9000 WDF scattergram relating to lymphocyte activation (increase in high fluorescence events in the acute phase of the disease and subsequent reduction due to disease remission), as well as platelet count (usually reduced during the acute phase of the disease) and the Hb value are other indicators helpful in monitoring the course of the disease. With the pRBC cluster, in addition to reporting the suspicion of malaria, the tool excludes all anomalous events in that cluster from the WBC and leukocyte count. Therefore, the reported results can be considered unbiased by interference, and were consistent with a diagnosis of malaria.

The pRBC flag is an extremely useful screening tool to identify clinically suspected and unsuspected cases of malaria, but microscopy and peripheral smear are necessary for more accurate diagnosis.

The morphological examination was performed using Sysmex DI-60 (Digital Imaging), which allowed us to observe the infected RBC with double dotted rings (Figure 1, panel C) and the presence of anisopoikilocytosis, a condition characterized by varying shapes and sizes of red blood cells (RBCs) that supports malaria diagnosis. Furthermore, the diagnosis was also confirmed by the positive identification of *Plasmodium falciparum* using Paramax-3 test. The patient was admitted in the hospital's infectious disease ward and treated with standard antimalarial therapy, consisting of Eurartesim 40/320 mg (4 tablets/day) based on body weight and with antipyretic therapy using paracetamol and Novagin for two days.

In the following days, the patient showed marked remission of the initial symptoms. Successively, due to the improvement in thrombocytopenia and the reduction in inflammation indices, the patient was discharged from the hospital. The patient was instructed to continue antimalaria therapy for complete remission.

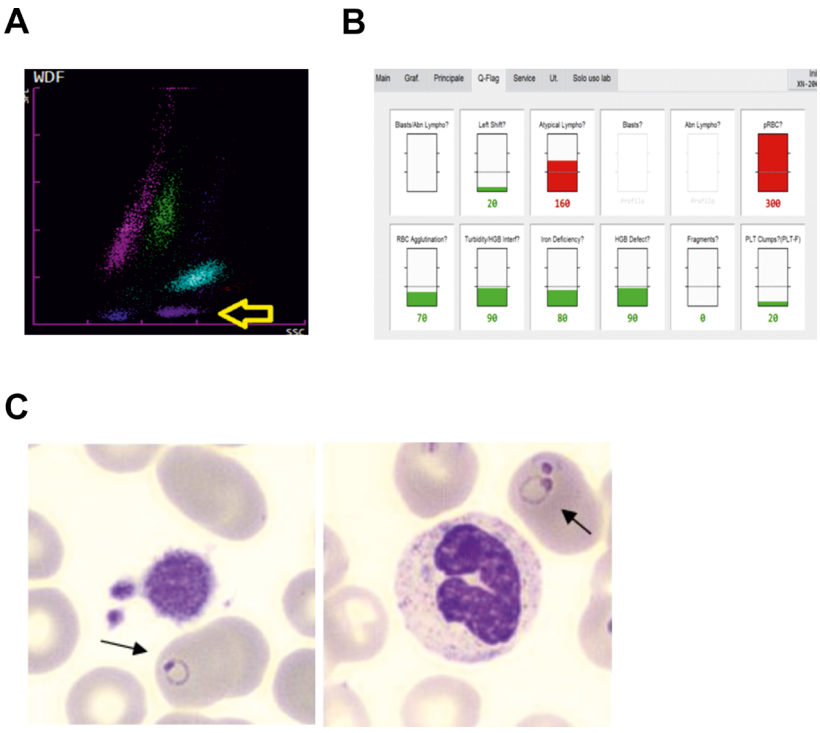

Figure 1

A. Anomalous cluster (ghost) in the lower part of the y axis (yellow arrow), almost close to the axis, below the neutrophil cluster which represents an accumulation of red blood cells infected by Plasmodium.
B. "Ghost" cluster on the WDF scattergram revealed by the alarm signal on the Q. FLAGs (pRBC =300).
C. Microscopical revision of peripheral blood smear: the arrows indicated the presence of infected red blood cells (e.i. double dotted rings typical of P. falciparum).

CASE REPORT 2

A 53-year-old male patient, from Senegal, arrived in the ED of the hospital with elevated temperature (40 °C) associated with headache, weakness, and no other accompanying symptoms. The patient was not treated with any kind of pharmacological therapy. During anamnesis, he reported a history of gastritis and blindness in the left eye after childhood trauma. This patient also referred of a recent trip to Senegal, without having completed any kind of malaria chemoprophylaxis. The CBC was performed using the Mindray BC-6800 Plus hemocytometer (Mindray, Shenzhen, China), reporting RBC 4.29 x10^{12}/L (4.40-6.00), Hb 123 g/L (120-160), platelets 142 x10^{9}/L, WBC 5.92 x10^{9}/L, CRP 282 mg/L, mild hyper transaminasemia, and indices of cholestasis and renal function within reference limits. The BC-6800 Plus is the first hemocytometer to use a combination of fluorescence flow cytometry and selective lysis systems to identify possibly parasitized RBC in cases of malaria, using qualitative and quantitative alarms.

The BC-6800 Plus hemocytometer performs a differential count of WBC (DIFF channel, Figure 2, panel A, B), where normal red cells are lysed, and white cells are differentiated into different subpopulations according to size (forward scatter, FS), internal complexity (side scatter, SS), and nucleic acid content (fluorescence, FL). Therefore, a 3-dimensional (3D) cube is generated (Figure 2, panel A); it differentiates the WBC into lymphocytes, monocytes, neutrophils (with basophils) and eosinophils. The immature cells, if present, are shown with a higher fluorescence.

Furthermore, in the DIFF channel, the infected RBC resistant to instrumental cell lysis and containing nucleic acid bind to the M-6 FD fluorescent dye, form a cluster that is positioned close to the neutrophil cluster, made visible by rotating the 3D cube. Moreover, the BC-6800 Plus analyzer has the possibility to inform the operator with a panel showing specific alarms, that can also reveal the presence of malaria parasites in red cells. In this specific case, the cut-off for the alarm of infected RBC was placed at 40 units from the manufacturer (Figure 2, panel C).

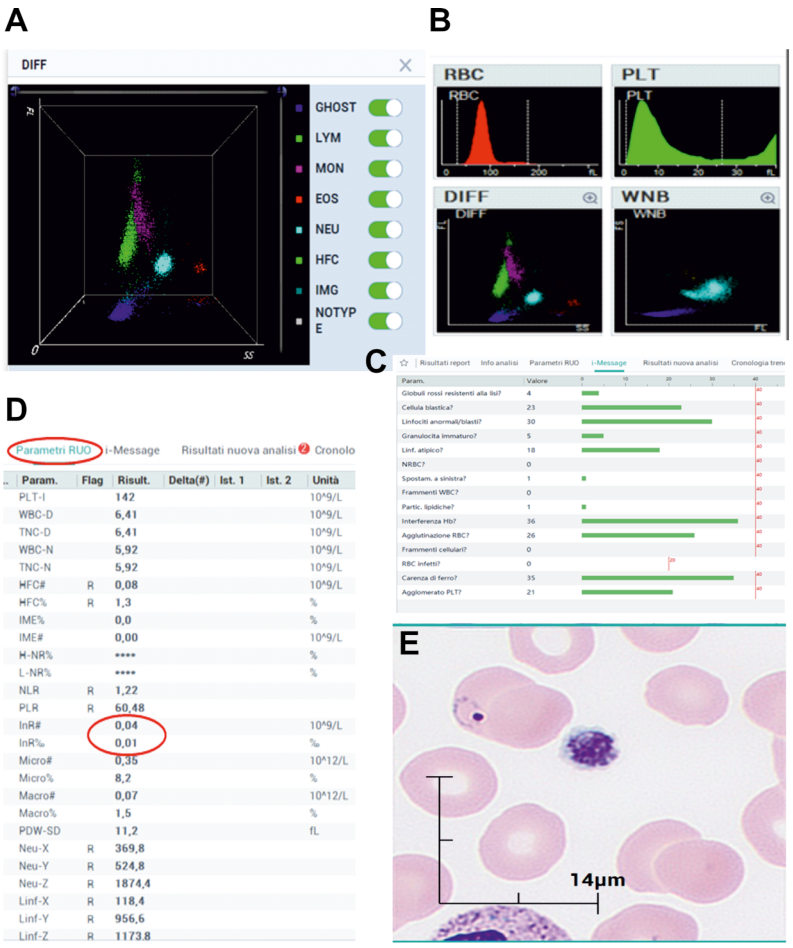

Figure 1

A. On the left are shown RUO parameters where BC-6800 Plus reports the "Infected RBC" as morphological alarm, and the " InR (# 0.04 109/L, ‰ 0,01)"; On the right different bidimensional cell's cluster are showed;
B. Different cluster of malaria-infected Red Blood Cells in threedimensional analysis scattergrams (SF Cube);
C. Microscopical revision of peripheral blood smears showing "ring form" characteristics of Plasmodium falciparum with MC-80 analyzer (Mindray, Medical System).
D. On the left are shown RUO parameters where BC-6800 Plus reports the " InR" (# 0.04 109/L, ‰ 0,01)" as morphological alarm
E. Microscopical revision of peripheral blood smears showing "ring form" characteristics of Plasmodium falciparum with MC-80 analyzer (Mindray, Shenzhen, China)

Furthermore, for the Research Use Only parameters (RUO), the instrument shows the 'InR#' and the 'InR‰' parameters outside the normal values (Figure 2, panel D). These parameters are used to calculate the morphological alarm of 'Infected RBC'. In fact, in this case 'InR#' (= 0.04 × 10^9/L) represents the number of malaria-infected RBC (x10^9/L) and 'InR‰' (= 0.01 ‰) indicates the number of infected RBC x1000 RBC. The increase in infected RBC correlates proportionally with these parameters (2). For this reason, in accordance with the data detected in the patient, the cut-off value has been positioned at 5 units instead of 40. The diagnosis of malaria was confirmed by a peripheral blood smear (Figure 2, panel E) in which the typical ring form of *Plasmodium falciparum* was clearly visible, thus confirming the alarm of the RUO parameters 'InR' and accounting for the change of cutoff to 5. Again, *Plasmodium falciparum* positivity has been further confirmed by the Paramax-3 rapid test for malaria. The patient was treated with standard antimalarial therapy, consisting of Eurartesim 40/320 mg (4 tablets/day) for three consecutive days. Successively, due to the absence of alterations in blood tests, the treatment for malaria was concluded and the patient was discharged.

DISCUSSION

Malaria is a major health problem throughout the world with a high impact, especially in the African regions, and can be considered one of the main causes of morbility and mortality in this area (3). The infection is caused by five species of protozoan *Plasmodium: P. vivax, P. falciparum, P. malariae, P. ovale,* and *P. knowlesi.* All these strains infect humans using the Anopheles mosquito as a vector. In particular, *Plasmodium falciparum* is the most prevalent malaria parasite. This parasite goes through different stages: sporozoite, merozoite, and trophozoite. Generally, for the diagnosis of malaria, the most detected form is the "ring-form" trophozoite. It is made up of a central vacuole, external cytoplasm, and a nucleus located on the opposite side, such as 'a ring' with a stone (4). Failure to recognize and treat this species within 24 hours can lead to severe clinical complications, potentially with fatal outcomes (5).

The main clinical symptoms include fever and chills, headache, muscle pain, nausea and vomiting, abdominal pain, and diarrhea. A comprehensive approach is necessary for the clinical diagnosis of malaria, involving clinical observations, case history, and blood microscopic examination. The gold standard for malaria diagnosis is thick blood smear and thin blood smear, but blood cell counters and, in case of strong suspicion, rapid antigenic test play a crucial role in the current diagnosis of the disease (6-7).

The diagnosis of malaria is considered an emergency diagnosis. Therefore, techniques that allow for a rapid and accurate diagnosis must be employed. The work by Capizzi et al. indicates hematologic analyzers as potentially useful tools to detect, by blood count of affected patients, abnormalities that lead to timely diagnosis of malaria (8). Malaria infection by *P. falciparum*, if not identified and treated within 24 hours, can lead to worsening of the patient's clinical condition, eventually proving fatal. In this study, we have presented two cases involving African patients who were characterized by very high fever and general illness which were evaluated in the ED of the Rome Tor Vergata University Hospital. The diagnosis of malaria in the presence of P*lasmodium Falciparum* infection was made by using both microscopic examination and hematological instrumentation with two different instruments. We evaluated the efficacy of Sysmex hematology analyzer, XN-9000, and Mindray BC-6800 Plus, in detecting malaria even in the absence of specific diagnostic tests, with the aim of establishing parameters useful to an accurate therapeutic intervention, or a second level analysis in the absence of a specific malaria molecular test. The presented results aim to demonstrate the possibility of detecting malaria using these routine clinical laboratory instruments if properly set up. Thrombocytopenia and morphological alarms detected by the RBC counter could be indicative of the presence of malaria parasites. On the XN-9000, Q-FLAGs detect the pRBC alarm, which can be indicative of parasite infection, as this flag can detect false WBC counts caused by intraerythrocytic parasites (1). Furthermore, the BC-6800 Plus hemocytometer is the first instrument in its class that can provide a dedicated (RUO) flag for the parameters of InR (#, ‰), which identify the number and percentage of parasitized RBC in the sample (9). The latest generation hemocytometers may be valuable for malaria screening in non-endemic countries; however sensitivity and specificity strictly depend on the instrument properties, extent of parasitemia, and maturation stage of the parasite. False positive could be related to preterm infant samples with very high resistance to lysis of RBC or to samples with the presence of interference, such as very high concentrations of fibrin filaments or an excessive number of RBC with other inclusions (Jolly bodies). For emergency diagnosis, it would be interesting to study further implementation by combining hemoscopy examination with a diagnostic algorithm. Furthermore, rapid molecular biology tests, which provide results in an hour or less, could potentially replace the rapid antigen test for Plasmodium (10). In the future, this red blood count examination could become a valid alternative to specific methods due to its speed, simplicity, low cost, and utility as a routine analysis for all patients with mild symptoms. In conclusion, examination of the peripheral blood smear allows the confirmation and identification of cellular anomalies detected in the blood count. This use could facilitate the preparation of a comprehensive laboratory report that serves as a valuable tool for clinicians, helping them with diagnosis and the request for further information and appropriate second-level laboratory tests.

CONFLICT OF INTEREST

None

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