AUTOMATED DETECTION OF MALARIA PIGMENT IN WHITE BLOOD CELLS FOR THE DIAGNOSIS OF MALARIA IN PORTUGAL

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Abstract. A novel automated method (Cell-Dyn® 3500) allows malaria diagnosis by detecting malaria pigment in white blood cells during routine full blood counts. In Portugal, 174 samples from 148 patients who presented to the emergency department were analyzed. Compared with microscopy the sensitivity was 95% and the specificity was 88%. In 5 cases, false-positive Cell-Dyn® 3500 results were from patients who had a recent history of treated malaria, indicating that the method may remain positive during convalescence. Six patients were diagnosed due to the changes observed with the automated method only, because clinicians had not requested malaria smears. This instrument appears to provide a promising method for the diagnosis of malaria, especially where automated full blood counts are routine in the work-up of febrile patients.

INTRODUCTION

The inherent disadvantage of all malaria diagnostic methods is that they have to be requested explicitly by an alert clinician. In a recent report from Canada, 59% of malaria cases were missed on first presentation, and 16% required 3 or more physician contacts before malaria smears were ordered. Other routine laboratory tests, e.g., automated full blood counts (FBC) showed only unspecific changes present in malaria, but which can be present in many other diseases.

A recent study from South Africa investigated a new automated FBC-analyzer, the Cell-Dyn® 3500 (Abbott, Santa Clara, CA). The instrument generates white blood cell (WBC) differentials, based on the different scatter characteristics of laser-light at 4 angles. The separation of eosinophils from neutrophils is achieved by utilizing the depolarizing properties of the eosinophilic granules. In a lobularity/granularity plot (lobularity = x-axis, measure of 90°-scatter; granularity = y-axis, measure of 90°-depolarized scatter) eosinophils are depicted as a green-dotted population above a threshold line, which separates them from the other WBC populations. Hemazoin-containing WBC are detected by the instrument due to the depolarizing properties of the pigment. The appearance of monocytes (purple-coded events, PCEs) in the eosinophil area (green-coded events) is a sign of phagocytosed hemazoin and consequently of malaria.

The aim of this study was to assess the performance of this novel method and to determine its usefulness for the diagnosis of imported malaria in a routine laboratory setting.

MATERIALS AND METHODS

The study was conducted in the laboratory of the emergency department of a large teaching hospital in Lisbon, Portugal, during 9 months in 1999. Samples from patients who presented to the emergency department and for whom attending clinicians had requested full blood counts and malaria smears were included in the study. Whole blood was obtained by venepuncture and anticoagulated with EDTA. Samples were processed as part of the normal routine with the CD3500 analyzer for FBC. The CD3500 plots were regarded as positive for malaria if 2 or more purple-coded events (pigment-containing monocytes) were detected in the eosinophil area of the lobularity/granularity plot, as seen on

the color monitor of the instrument. The number of these events was recorded. Thick and thin blood films were stained with Giemsa and examined by microscopy as described previously. The parasite number was quantitated per 200 WBC in thick blood films or per 1,000 red blood cells in thin blood films. Samples were regarded as negative if examination of at least 200 microscopic fields of a thick blood film under 1,000× magnification showed no parasites. If a patient had more than one sample analyzed, the sample on first presentation was considered for calculating sensitivity and specificity. During a 2-month portion of the study the FBC-results from all samples without a request for malaria smears were reexamined for the presence of purple-coded events (PCEs) at 24 hour intervals. Any sample with 2 or more PCEs was examined by microscopy for the presence of parasites.

RESULTS

The results are summarized in Table 1. Forty-eight patients had Plasmodium falciparum, 6 had Plasmodium vivax, one had Plasmodium ovale, and 2 had Plasmodium malariae infection. No mixed infections were observed. The median parasitemia was 0.05% (25th percentile: 0.01%; 75th percentile: 0.8%). The CD3500 failed to detect 3 microscopically-positive cases with a parasitemia of 0.0001%, 0.02%, and 0.06% respectively. In 12 patients (17 samples) with a negative microscopy result, the CD3500 was positive. The instrument showed a sensitivity of 95% and a specificity of 88%. Five of the 12 patients with false-positive CD3500 results (10 samples) had a history of treated malaria in the preceding 3 weeks. In the other 7 patients (7 samples) no reason for the positive CD3500 plots could be found. No correlation was observed between the parasitemia and the number of PCEs. During the 2 month period when all FBC results without a request for malaria smears were reexamined, 10 patients were identified, whose samples showed ≥ 2 PCEs in the CD3500 histogram. In 6 of these patients, microscopy confirmed a diagnosis of malaria and review of the clinical notes revealed a lack of clinical suspicion. In the other 4 patients microscopy for malaria was negative.

DISCUSSION

Detection of hemazoin has been the basis for several microscopic methods to diagnose malaria. The CD3500 is
the first automated method based on this principle that allows detection of malaria during routine analysis for full-blood-counts.

The higher sensitivity (95%) in our study when compared to the one from South Africa (72%) could be explained by differences in the populations studied; one important aspect may be that in Portugal all malaria cases are imported via travelers to malaria-endemic areas. It has been suggested that the kinetics of pigment-containing WBC may vary between different populations and could be related to severity of infection and host immunity.

Of non-immune adults with severe malaria in Vietnam, a hypoendemic area, 92% had pigment-containing WBC on admission upon counting 100 neutrophils and 30 monocytes. In Gabon, a hyperendemic area, counting 100 neutrophils and 100 monocytes on admission, different percentages were found for children with severe malaria (100% had pigment-containing monocytes, 95% had pigment-containing neutrophils), children with mild malaria (87% and 32%, respectively) and adults with mild malaria (58% and 16%, respectively). In the same study the absence of a correlation between pigment-containing monocytes and parasitemia was noted. In agreement with this, we did not observe a correlation between the number of purple-coded events and parasitemia. However, the CD3500 failed to detect 2 cases with a degree of parasitemia (0.06% and 0.02%) that usually does not pose diagnostic difficulties. This finding suggests that the sensitivity of the instrument depends ultimately on the various factors that influence the kinetics of hemozoin-containing WBC in each individual.

The specificity (88%) observed was lower than in the South African study (96%). In our study ≥ 2 rather than ≥ 1 PCEs was used as cut-off point. Rarely, a single PCE was noted just above the calculated separation line between the eosinophil and other WBC populations. Due to their minimal difference in the degree of granularity (y-axis = 90° depolarized light scatter) as compared with the normal monocyte population this was regarded as a spurious event. In samples positive for malaria, usually ≥ 2 events were noted that showed a much higher degree of granularity. However, when applying ≥ 1 PCE as the criterion for positivity, only an additional 5 samples would have been regarded as positive. In 4 samples the single PCE lay just above the separation line and disappeared on reanalysis; all samples were negative by microscopy. Only one case of malaria would have been identified (parasitemia 0.06%); however, the PCE was situated well inside the eosinophil area.

The main reason for false positive CD3500 results may be the persistence of hemozoin-containing monocytes after parasite clearance, since 5 of the 12 patients had a history of recently-treated malaria. Pigment-containing WBC were still present in more than 70% of patients after parasite clearance in one study and the clearance time of pigment-containing monocytes has been reported to be up to 2–3 weeks. Therefore, a positive CD3500 plot does not necessarily indicate acute malaria, but may be found in a successfully-cured patient. The discordant results between both methods in 5 of 26 follow-up samples provides further support for this explanation. Patients tested 4–7 days after initiation of treatment were already negative on microscopy but still had positive CD3500 results. It may be difficult to interpret the clinical relevance of a positive CD3500 result in countries with endemic malaria where frequent infections and subclinical parasitemia are common. On the other hand, the cause for the false-positive CD3500 results in 7 patients could not be established. Nonetheless, when all FBC results were reexamined at the color monitor of the CD3500 during a 2-month period (average of 382/day), only a total of 4 patients with false-positive results was found. Their samples showed few PCEs (2, 2, 3, and 3, respectively) which disappeared when the samples were reanalyzed.

In imported malaria, insufficient experience with malaria microscopy is a concern. This is especially important when considering the large number of laboratory staff who work during on-call hours when no immediate expert help may be available. Interestingly, we observed that almost half of all malaria smears were performed out-of-hours or other than from 9:00 AM to 5:00 PM, Monday through Friday when 61% of patients with malaria were diagnosed. In this context the instrument might alert the on-call microscopist to the possibility of a positive smear result.

It should be noted that the instrument and its software were not specifically designed to diagnose malaria, nor does the instrument flag its presence or give automated counts for pigment-containing WBC. The CD3500 analyzes up to 10,000 WBC. However, only a partial number of WBC are represented on the monitor’s plot. Consequently, the number of PCEs is not equal to the number of pigment-containing monocytes in the peripheral blood. Furthermore, in many samples positive for malaria the eosinophil population was abnormally scattered. This suggests that the instrument also detects pigment-containing neutrophils but classifies them incorrectly as eosinophils, which is not surprising, considering that the instrument’s algorithm to differentiate the eosinophils from neutrophils is based precisely on the detection of intracellular depolarizing particles. The detection of intra-erythrocytic parasites with pigment may also be possible, although in the normal operating mode all erythrocytes are rendered invisible to the laser light after dehemoglobinization with a hypotonic salt solution. We observed one case of Plasmodium vivax malaria in a patient who had erythrocytes with increased osmotic resistance. Further to the usually observed purple-coded events, red-coded events were noted in the eosinophil area that represent erythrocytes.

Our findings suggest that this novel method is a useful addition to conventional microscopy. Most importantly, it may permit the automated diagnosis of cases where no clinical request was made other than an FBC. However, further studies are necessary to validate this new method in areas and populations where the kinetics of pigment-containing
WBC may be different. It would also be desirable if the software allowed a correct enumeration of hemazoin-containing WBC. Unfortunately, the price for purchase and maintenance of this sophisticated instrument is beyond the means of many countries where malaria is endemic, and is thus a significant limitation.

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