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

THOMAS HANSCHEID, JOSÉ MELO-CRISTINO, AND BERNADINO G. PINTO
Department of Clinical Pathology, Hospital Santa Maria, Lisbon, Portugal

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 (Abott, 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 phagocytized 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 quantified 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
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 hemoglobin-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 that 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 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.
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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Authors’ addresses: Thomas Hänscheid, José Melo Cristino, and Bernadino G. Pinto, Department of Clinical Pathology, Microbiology, Piso 4, Hospital Santa Maria, Av. Prof. Egas Moniz, 1600 Lisbon, Portugal, Fax: +351 21 2881486, e-mail: t.hanscheid@fm.ul.pt.

REFERENCES
