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Demosthenes Bouros1*
Argyris Tzouvelekis1
Stavros Anevlas1
Michael Doris1
Stavros Tryfon2
Marios Froudarakis1
Vasiliki Zournatzi1
Asterios Kukuvitis3

1 Department of Pneumonology
University Hospital of Alexandroupolis
Alexandroupolis, Thrace, Greece

2 Department of Pneumonology
General Hospital G. Papankolou
Thessaloniki, Greece

3 Department of Endocrinology
Medical School University of Thrace
and University Hospital of
Alexandroupolis
Alexandroupolis, Greece

Effect of Plasmodium falciparum
Parasitemia on Erythrocyte Zinc
Protoporphyrin

To the Editor:

In regions holoendemic for malaria, both iron deficiency and asymptomatic malaria parasitemia are common (1). Measurements of transferrin receptor and erythrocyte zinc protoporphyrin IX (ZnPPIX) concentrations have been suggested as screening tools to detect iron deficiency (1, 2). Chronic iron-deficient erythropoiesis leads to a relative increase in the insertion of zinc rather than iron into erythrocyte PPX.

Anemia of chronic disease and hyperbilirubinemia can decrease the specificity of an iron deficiency diagnosis based on hematofluorometry-determined increases in the ratio of ZnPPIX to heme. The influence of malaria parasitemia on front-face hematofluorometric ZnPPIX determinations is controversial. Increased ZnPPIX has been statistically associated with malaria, and interference by increased bilirubin from hemolysis has been postulated (3). In a study by Asobayire et al. (4), malaria did not increase ZnPPIX in washed blood, but in that study only 5%–8% of malaria-positive persons had >5000 parasites/μL. In different studies using unwashed blood, increased ZnPPIX concentrations were associated with >5000 parasites/μL (5, 6). These increases in ZnPPIX could either be real, caused by true iron deficiency and/or malaria-related anemia of chronic disease, or false, caused by hyperbilirubinemia, mostly from hemolysis. Bilirubinemia influencing front-face ZnPPIX determinations depends on the instrument (7, 8), use of intact vs lysed specimens (9), or washing of intact cells before use (10). Although jaundice can be present in malarial disease, bilirubinemia is loosely correlated with disease severity (11). Asymptomatic parasitic children are likely to have lower concentrations and incidence of hyperbilirubinemia.

Another potential reason for malarial interference with ZnPPIX determination is hemozoin, the black birefringent intracellular heme crystal. Intra-erythrocytic Plasmodium accumulates more than one half of its heme into hemozoin (12). The heme crystal has a much lower absorbance at 400 nm, suggesting that high malarial parasitemia might lead to increased estimations of erythrocyte protoporphyrin by hematofluorometer (13).

To ascertain whether the intra-erythrocytic parasite influences erythrocyte fluorescence, we prepared a thin blood film smear of synchronized Plasmodium falciparum trophozoite-infected erythrocytes, fixed it with methanol for 1 min, and obtained images on a Zeiss LSM 510 microscope with excitation at 410 nm and emission above 575 nm. These emission and excitation wavelengths correspond to those used on the AVIV front-face hematofluorometer. The individual trophozoite-infected erythrocytes showed much less fluorescence than did uninfected erythrocytes (Fig. 1). The synchronized
ring stages showed no changes in microscopic fluorescence (not shown).

We investigated whether increasing parasitemia interferes with ZnPPIX estimation by front-face hematofluorometry or by determination of free erythrocyte porphyrin (FEP). Using uninfected erythrocytes at 50% hematocrit, we prepared 9 serial dilutions of 200 to 2 x 10⁶ parasites/μL for trophozoite stages and 7 dilutions of 200 to 400 000 parasites/μL for ring stages. The erythrocyte ZnPPIX concentration was measured on a front-face hematofluorometer (AVIV) on 20 μL of parasiticemic erythrocytes washed 3 times with RPMI medium. FEP was extracted and estimated from a calibration curve with excitation and emission wavelengths of 405 and 660 nm, respectively (Perkin-Elmer LS 50B) (14). All reagents were from Sigma.

The mean ZnPPIX by hematofluorometry for control, ring-infected, and trophozoite-infected erythrocytes (n = 10 for all erythrocyte densities) varied by < 5 μmol ZnPPIX/mol of heme. Likewise, the estimated FEP by acid extraction method for both rings and trophozoites did not differ from the estimated values for uninfected erythrocytes. These experiments were designed with plasma-free erythrocytes, and additional studies to correlate bilirubin concentration with parasitemia and ZnPPIX concentrations should be explored.

In conclusion, intracellular Plasmodium parasites can be omitted as an influence on front-face hematofluorometer erythrocyte ZnPPIX determinations, despite the decrease in ZnPPIX microscopic fluorescence seen with individual trophozoite-infected erythrocytes. Because of sequestration of P. falciparum trophozoite-infected erythrocytes, only ring stages reach peak parasitemias near 2 x 10⁷/μL. For Plasmodium vivax, Plasmodium ovale, and Plasmodium malariae, the mean and peak parasitemias are < 20 000/μL and 50 000/μL, respectively (15). Therefore, our experimental results are valid for field malaria studies.

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Girish S. Hiremath1
David J. Sullivan, Jr.2
Abhai K. Tripathi3
Robert E. Black3
Sunil Sazawal1

1 Department of International Health
2 Department of Molecular Microbiology and Immunology
3 Malaria Research Institute
 Johns Hopkins Bloomberg School of Public Health
Baltimore, MD

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* Address correspondence to this author at: Department of Molecular Microbiology and Immunology, Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe St., Baltimore, MD 21205. Fax 410-955-0105; e-mail dsullivan@jhsp.edu.

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Multiplex PCR Assay for the Identification and Differentiation of all Brucella Species and the Vaccine Strains Brucella abortus S19 and RB51 and Brucella melitensis Rev1

To the Editor:

The genus Brucella consists of 6 recognized bacterial species (1) and 2 proposed new species recently isolated from marine mammals (2). Some species have several biovars or biotypes, distinguishable by time-consuming analysis of ∼25