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Research paper

Development and evaluation of a multiplex screening assay for *Plasmodium falciparum* exposure

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ABSTRACT

Transfusion transmitted malaria (TTM) in non-endemic countries is reduced by questioning blood donors and screening of donated blood. Conventional screening is performed by Indirect Fluorescence Antibody Test (IFAT). This method is manual and difficult to standardize. Here we study the diagnostic performance of a multiplex assay for detection of antibodies against *Plasmodium falciparum* in donor blood using IFAT as a comparator. A multiplex assay (MPA) containing the antigens GLURP-R0, GLURP-R2, MSP3, MSP1 hybrid and AMA1 was constructed using xMAP® technology. A discrimination index for exposure to *P. falciparum* malaria was calculated by comparing travelers with clinical malaria (n = 52) and non-exposed blood donors (n = 119). The index was evaluated on blood donors with suspected malaria exposure (n = 249) and compared to the diagnostic performance of IFAT.

At a specificity of 95.8 %, the MPA discrimination index exhibited a diagnostic sensitivity of 90.4 % in travelers hospitalized with malaria. Percent agreement with IFAT was 92.3 %. Screening plasma from blood donors with suspected malaria exposure, we found 4.8 % to be positive by IFAT and 5.2 % by MPA with an agreement of 93.2 %. The calculated index from the MPA exhibits similar diagnostic performance as IFAT for detection of *P. falciparum* malaria. Combining the antibody response against multiple antigens in a discrimination index increased the sensitivity of the MPA and reduced the readout to a single value.

Abbreviations: VFR, Immigrants returning to country of origin to visit friends and family; TTM, Transfusion transmitted malaria; IFAT, Indirect Fluorescence Antibody Test; MPA, Multiplex assay; EIA, Enzyme immune assays; BDNT, Blood donors with no history of travel to endemic countries; SSI, Statens Serum Institut;GLURP-R0, Glutamate-rich protein (non-repetitive amino-terminal region); GLURP-R2, Glutamate-rich protein (carboxy-terminal repeat region); MSP3, Merozoite surface protein 3; MSP1, Merozoite surface protein 1; AMA1, Apical membrane antigen 1; AU, Arbitrary Units; MFI, Median Fluorescence Intensity; TMB, 3,3′,5,5′-Tetramethylbenzidine; CI, Confidence intervals.

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1. Introduction

Malaria is a febrile disease caused by infection with obligate intraerythrocytic protozoan parasite of the genus *Plasmodium*, of which *P. falciparum* is the most lethal (WHO, 2011). In endemic regions, a strong anti-P.falciparum IgG antibody response develops as a function of age and exposure to parasite infections. In non-endemic countries, malaria is mainly diagnosed among travelers returning from endemic areas. Amongst these travelers, immigrants returning from visiting their country of origin (VFR) account for the majority of cases (Keystone et al., 2011). Occasionally, malaria has been reported in patients who have received blood transfusions from infected donors (Kitchen et al., 2005; Mali et al., 2011; Vareil et al., 2011), and although infrequent, transfusion transmitted malaria (TTM) can be severe and sometimes fatal (Stickland et al., 1992). TTM in non-endemic countries is reduced by questioning blood donors concerning exposure and travel history, and screening individuals at risk with immunological or genomic tests (Seed et al., 2005b). In some European countries including Denmark, Indirect Fluorescence Antibody Testing (IFAT) is used to screen donor blood. This manual method depends on maintaining consistency of examination of thin smears of *P. falciparum* asexual blood stages by trained microscopists making inter-laboratory standardization challenging (Seed et al., 2005b; Elghouzzi et al., 2008). Alternative enzyme immune assays (EIA) and ELISAs have been developed and some studies (Kitchen et al., 2004; Seed et al., 2005a; Doderer et al., 2007; Elghouzzi et al., 2008) found comparable diagnostic sensitivity between IFAT and EIA/ELISA whereas others (Silvie et al., 2002) did not. Most EIA/ELISAs utilize one to four antigens for detection of malaria specific antibodies. Bead-based multiplex technology such as the xMAP® system allows simultaneous measurement of antibodies against multiple antigens which should theoretically increase sensitivity. Multiplex assays have been developed for immune epidemiological studies in malaria (Fouda et al., 2006; Ambrosino et al., 2010; Fernandez-Becerra et al., 2010)

The aim of this study is to develop and determine the potential and accuracy of a multiplex assay (MPA) for screening donor blood for *P. falciparum* antibodies compared to IFAT.

2. Materials and methods

2.1. Study population

Archived plasma samples from Ghanaian children aged 0–15 years, Liberian adults, and travelers hospitalized with *P. falciparum* malaria (Table 1) were selected randomly from previously published studies (Gjørup et al., 2007; Dodoo et al., 2008; Olesen et al., 2010). Plasma from blood donors with no history of travel to endemic countries (BDNT) were used as controls (Table 1). Of 460 samples from blood donors classified as donors at risk of malarial infection according to national guidelines and screened for malaria by IFAT at Statens Serum Institut (SSI), 249 were available for testing (Table 1).

2.2. Antigens and conjugation to microspheres

The following recombinant antigens were used for conjugation to microspheres: the non-repetitive amino-terminal region of glutamate-rich protein (GLURP-R0) covering amino acid residues 27–500 and the carboxy-terminal repeat region covering amino acid residues 705–1178 (GLURP-R2) from the F32 laboratory line expressed in *Escherichia coli* (Theisen et al., 1995) the carboxy-terminal conserved region of merozoite surface protein 3 (MSP3) covering amino acid residues 212–382 produced in *E. coli* (Carvalho et al., 2004); a diversity covering hybrid protein of merozoite surface protein 1 (MSP1) containing the N-terminal Block 1 region, the K1 Block 2 synthetic sequence, the RO33 Block 2 sequence and the MAD20 Block 2 synthetic sequence produced in *E. coli* (Cowan et al., 2011); and the ectodomain of apical membrane antigen 1 (AMA1) covering amino acid residues 25 to 544, from the FVO strain was produced in *Pichia pastoris* (Kocken et al., 2002). Specific amounts of the antigens GLURP-R0 (25 μg) , GLURP-R2 (25 μg), MSP3 (25 μg), MSP1 (50 μg) and AMA1 (75 μg) were coupled to bead regions 33, 34, 35, 37, and 39, respectively according to the manufactures instructions (Luminex).

2.3. MPA protocol for antibody determination

Approximately 1250 microspheres from each of the 5 antigen coupled bead regions were mixed, and 100 μL of the resulting mixture was added to wells on a pre-wetted 96-well filter micro titer plate (MSBVS 1210, Millipore) and washed three times with assay buffer E (ABE; 0.1% BSA, 0.05% Tween 20, 0.05% sodium azide in PBS (pH 7.4)). Plasma samples diluted 1:2000 in ABE was added in duplicates at 50 μL/well and incubated with shaking at 1100 rpm for 30 s and 300 rpm for two hours in the dark. After washing three times with ABE, phycoerythrin labeled goat anti-human IgG (Code no. 109-116-098, Jackson ImmunoResearch Laboratories Inc.) diluted 1:200 in ABE was added to the wells and incubated for 1 hour. After washing three times with ABE and resuspension in 100 μL ABE, samples were analyzed on a Luminex 200 (Bio-Rad Laboratories Inc.). Each plate included a calibrator obtained by end-point titration of a pool of plasma from eight hyper immune Liberian adults. The calibration curve was fitted by five point logistic regression modeling using Bioplex manager software version 5 (Bio-Rad Laboratories Inc.). One arbitrary unit (AU) was defined as the reciprocal dilution giving a median fluorescence intensity (MFI) of 10,000. The AU of a test sample was determined by interpolating its MFI value on the calibration curve. A pool of plasma from five hyper immune Liberians and a pool of plasma from eight BDNT served as positive and negative controls. The mean antibody titer and SD for the positive control sample was: GLURP-R0, 19182 ± 1393; MSP3, 21633 ± 838.3; GLURP-R2, 46672 ± 7383; MSP1, 5300 ± 256.7; AMA1, 82339 ± 18000.

2.4. ELISA and indirect fluorescence antibody test

The previously described Afro-immuno assay ELISA protocol (Dodoo et al., 2000; Nebie et al., 2008) was used with minor modifications. Briefly, microtiter plates (Nunc) were coated with recombinant GLURP-R0 and GLURP-R2 at 0.5 μg/mL; MSP3 and AMA1 at 1 μg/mL; and MSP1 at 2 μg/mL. Plates were incubated overnight (4 °C) and blocked with 3% skimmed milk for 1 h. Plasma samples diluted 1:200 were added in duplicate at 100 μL/well and incubated at room temperature for 1 h. Plates were developed with peroxidase-conjugated goat