Malaria and HIV infection in pregnancy are associated with reduced transfer of antimalarial antibodies to the newborn

Running title: Malaria, HIV and transplacental antibodies

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ABSTRACT

Background. Malaria and human immunodeficiency virus (HIV) during pregnancy affect the transplacental transfer of antibodies against several pathogens from mother to fetus, although their effect over antimalarial antibodies remains unclear.

Methods. Total immunoglobulin G (IgG), IgM and IgG subtypes against *Plasmodium falciparum* antigens merozoite surface protein 1-19 ($MSP1_{19}$), erythrocyte binding antigen 175 (EBA175), apical membrane antigen 1 (AMA1) and parasite lysate were measured in 187 mother-cord plasma pairs from Mozambique. Placental antibody transfer was defined as the cord-to-mother ratio (CMR) of antibody levels.

Results. Maternal malaria was associated with reduced CMR of EBA175 IgG (P=0.014) and IgG1 (P=0.029), AMA1 IgG (P=0.002), lysate IgG1 (P=0.001) and MSP1 IgG3 (P=0.01). Maternal HIV was associated with reduced CMR of MSP1 IgG1 (P=0.022) and IgG3 (P=0.023), lysate IgG1 (P=0.027) and IgG3 (P=0.025), AMA1 IgG1 (P=0.001) and EBA175 IgG3 (P=0.001). Decreased CMR was not associated with increased adverse pregnancy outcomes or augmented risk of malaria in the infant during the first year of life.

Conclusions. Placental transfer of antimalarial antibodies is reduced in pregnant women with malaria and HIV infection. However, this decrease does not contribute to increase the risk of malaria-associated morbidity in infancy.

Keywords: malaria, HIV, pregnancy, antibody, placenta, transfer.

INTRODUCTION

Malaria and human immunodeficiency virus (HIV) continue to be major health priorities in endemic countries, especially for pregnant women and their infants. Each year approximately 25 million pregnancies in Sub-Saharan Africa are exposed to the risk of *Plasmodium falciparum* infection, although a high number of these infections are asymptomatic and remain undetected and untreated (1). The effects of HIV on maternal health have been superimposed on those of malaria in Sub-Saharan Africa, where 59% of HIV-infected adults are reproductive age women (2) and where HIV prevalence in pregnancy can exceed 25% (1). The identification of correlates of protection against malaria and how they are affected by HIV infection remains a key research area (3).

During pregnancy, maternal antibodies are transferred to the fetus by an active process mediated by Fc receptors in the placental syncytiotrophoblast (4). This maternal-fetal antibody transfer minimizes deficiencies in antibody production in the fetus and provides a short-term passive immunity (5), conditioning the degree and length of the protection (4) and vaccination success in the newborn (6,7). However, the effect of placental transfer of antimalarial antibodies on infant's immune responses and susceptibility to malaria in their first months of life still remains unclear (8–12).

The efficiency of placental antibody transfer is affected by factors such as maternal antibody levels, density and functionality of receptors, immunoglobulin G (IgG) subclass, avidity, antigen nature, maternal gestational age and parity (13–15). Maternal hypergammaglobulinemia, prematurity, low birth weight (LBW), multigravidity, poor nutritional status and maternal infections, such as HIV infection, have also been associated with decreased transfer (4,15–17), although these factors seem to vary depending on the study population (5). Maternal HIV infection has been consistently associated with reduced placental passage of antibodies against several common viral and bacterial antigens (4,15,18–20). Placental malaria has been

associated with maternal hypergammaglobulinemia (21,22) and reduced transfer of antibodies against pathogens such as measles, tetanus, *Streptococcus pneumonia* or varicella-zoster virus in some studies (4,19,23–25), but not in others (15). However, the information about factors affecting placental transfer of specific antimalarial antibodies defined as the cord-to-mother ratio of antibody levels is scarce and, to our knowledge, no previous work has analyzed the effect of maternal malaria infection (26). Only one study conducted in Kenya addressed the effect of maternal HIV on transplacental transfer and found placental passage of antibodies against the circumsporozoite protein (CSP), but not against other malarial antigens, to be reduced in HIV-infected women when compared to non-infected mothers (26).

Therefore, the aim of this study was to investigate maternal factors affecting the transfer from mother to fetus of antimalarial antibodies and the potential relationship of decreased antibody transfer with adverse pregnancy outcomes and malaria risk in the newborn. We hypothesized that *P. falciparum* and HIV infections in pregnancy might decrease placental transfer of antimalarial antibodies by means of alterations in the architecture of the placenta (27,28), thus contributing to an increased risk of malaria infection in the newborn. To address this, the levels of *P. falciparum*–specific IgG, IgM and IgG subclasses were measured in maternal peripheral and cord plasmas. The effect of HIV infection and malaria diagnosed by histology, microscopy and qPCR, as well as other maternal factors (antibody levels, age, gravidity, preventive treatment and anemia) on antibody placental transfer was assessed.

MATERIALS AND METHODS

Study population

This study was nested in a placebo-controlled trial of intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) for malaria prevention conducted at

the Manhiça Health Research Centre in Manhiça district, southern Mozambique, between 2003 and 2006 (29). Malaria transmission in this semirural area is perennial with some seasonality and *P. falciparum* is the predominant species (30).

Maternal HIV-1 infection was diagnosed with Determine HIV-1/2 rapid test (Abbott Laboratories) and confirmed with Unigold rapid test (Trinity Biotech). At delivery, maternal peripheral and cord blood were collected by venipuncture into ethylenediaminetetraacetic acid vacutainers and thin and thick smears were prepared. Blood was centrifuged and plasma stored at -20°C. Hematocrit level was quantified in a microcapillary tube after centrifugation. Peripheral, cord and placental blood were collected onto filter papers (903TM; Schleicher and Schuell). Biopsies from the maternal side of the placenta were processed for histological examination. Malaria episodes were recorded for infants during the first year of life through a passive case detection system based on the reporting of all malaria cases detected in children attending the outpatient clinic of the Manhiça District Hospital.

The current analysis was conducted in the last 187 women enrolled in the IPTp trial receiving either placebo or SP from whom demographic and clinical data, as well as samples from maternal and cord blood, were available. The study was approved by the Mozambican National Bioethics Committee and the Hospital Clínic of Barcelona Ethics Review Committee.

P. falciparum **detection by microscopy, placental histology and qPCR**

Thin and thick smears were Giemsa-stained and examined for malarial parasites according to quality-control procedures (31). Placental biopsy specimens were processed for histological examination and classified according to previously published criteria (32). DNA was extracted from 50 µL blood drops onto filter paper with an ABIPrism 6700 Automated Nucleic Acid Workstation (Applied Biosystems) and resuspended in 200 µL of water. Five microliters of DNA

117 samples were screened for *P. falciparum* by quantitative polymerase chain reaction (qPCR)
118 targeting the 18S ribosomal RNA gene, as described elsewhere (22).

119

120 **Measurement of antibodies against merozoite antigens and parasite lysate**

121 Maternal and cord plasma samples were tested by enzyme-linked immunosorbent assay
122 (ELISA) for the presence of IgG, IgM and IgG subclasses specific for the recombinant antigens
123 $MSP1_{19}$ (19-kD fragment, 3D7), EBA175 (region F2, Camp) and AMA1 (full ectodomain, 3D7) as
124 previously reported (22,33). High-binding 96-well microplates (Nunc Maxisorp) were coated
125 overnight at 4°C with 200 ng/well of recombinant antigen diluted in carbonate-bicarbonate
126 buffer. After blocking with 2% bovine serum albumin at 4°C for 8 h, 100 µL of plasma diluted
127 1:500 (for IgG and IgM) or 1:200 (for IgG subtypes) were tested in duplicate. Secondary
128 peroxidase-conjugated antibodies were used as follows: goat anti–human IgG 1:30000; IgM
129 1:2000 (Sigma); sheep anti–human IgG1 1:6000, IgG2 1:3000, IgG3 1:6000, and IgG4 1:3000
130 (The Binding Site). Reactions were developed and optical density (OD) values were read at 492
131 nm.

132 Whole-parasite lysate was prepared by three freezing/thawing cycles of asynchronous *in vitro*
133 cultures of 3D7 and HB3 laboratory strains (MRA-102 and MRA-155, MR4, ATCC) at a 5% level
134 of parasitemia and 1% hematocrit. Non-infected erythrocyte (NIE) lysate prepared in the same
135 way was used as a control. Plates were coated with 50 µL/well of parasite extract. Wells were
136 blocked with 300 µL of 5% skim milk at 4°C for 8 h. One hundred microliters of plasma samples
137 were tested in duplicate for IgG (dilution 1:6400) and for IgM and IgG subclasses (1:1600).
138 Incubation of antibodies and development of the reaction were performed as described above.
139 Malaria-specific antibody recognition was evaluated by subtracting the mean OD value of NIEs
140 from the mean OD value of infected erythrocytes (IEs). A pool of plasma samples obtained
141 from 8 Mozambican adults was used to normalize data from different ELISAs (22,33).

Definitions and statistical methods

Pregnant women were classified into those having a first pregnancy (primigravidae), those having a second pregnancy (secundigravidae) and those with at least 2 previous pregnancies (multigravidae). Age was categorized as ≤20, 20-24 or ≥25 years on the basis of maternal age terciles in this population. Maternal anemia was considered if the hematocrit level was <33%. Infection in the pregnant woman was defined if parasites were detected by histology or microscopy in the placenta and/or in the periphery, respectively, or by qPCR in any of the compartments.

Seropositive mothers or newborns for each malaria-specific Ig were defined as those with plasma samples having an OD value above the median OD determined for 10 healthy controls from non-endemic area plus three standard deviations. The efficiency of the placental passage of antibodies was defined as the cord-to-mother ratio (CMR) of ODs in cord and mother peripheral plasma among those women that were seropositive for each specific antigen and Ig. Univariate (Student's t test) and multivariate linear regression models were used to estimate the association of HIV, malaria and other clinical and demographic covariates with antibody levels in cord blood or CMRs after log transformation. Linear regression models were used to estimate associations between CMRs and pregnancy outcomes (gestational age, birthweight and hematocrit) and binomial regression models for malaria incidence in the first year of infant's life. Multivariate models were adjusted for maternal antibody levels, maternal HIV and malaria infection, parity, age, maternal anemia and IPTp group. For all regression models, crude proportions and adjusted effects with their corresponding 95% confidence interval (CI) were computed. Statistical analysis was performed using GraphPad Prism version 6 (GraphPad Software) and Stata Statistical Software version 11.0 (StataCorp).

RESULTS

Characteristics of the study population

The prevalence of *P. falciparum* infection in peripheral blood of the pregnant women included in the study was 9.6% (18/187) by light microscopy, but increased to 29.9% (56/187) by qPCR. Parasites were found in 26 placental sections by histology (13.9%) and 61 (32.6%) infections were detected by qPCR in the placental blood. In total, 116 (62.0%) women presented parasites in one or both compartments by any of the techniques at the time of delivery.

Fifty seven (30.5%) women were HIV-positive. The characteristics of the 187 women at delivery according to their HIV infection status are presented in Table 1. The subset of 187 included in this study and the 1030 women participating in the randomized trial were comparable in terms of IPTp group, parity, age, HIV infection, peripheral and placental malaria infection and hematocrit level. Similarly, no differences were found in parity, age, malaria infection and IPTp intervention between HIV-negative and HIV-positive women. The prevalence of anemia was higher among HIV-infected mothers than among non-infected (Table 1).

Factors associated with levels of antimalarial antibodies in cord blood

Levels of antimalarial antibodies in maternal peripheral and cord plasma samples are shown in Fig. 1. IgG and IgG subtypes levels were comparable between mother and cord pairs (Fig. 1 and Table 2), in contrast to IgM levels that were much lower in cord samples than in mothers. Maternal and cord blood levels of IgG and IgG subclasses were highly correlated, but this was not the case for IgM levels (Suppl. Table 1). The presence of parasites in cord blood was associated with increased seroprevalence of IgG4 against parasite lysate (P=0.036), but not for other IgG subtypes or IgM. Given the very low levels of IgG2 and IgG4 against the three

antigens and parasite lysate, both in mother and cord plasmas (Fig. 1), these subclasses were not further included in the analysis.

Maternal antibody levels and other clinical (maternal HIV, malaria infection, anemia and IPTp group) and demographic (maternal age and gravidity) factors that could influence levels of antimalarial antibodies in cord blood were included in a univariate and multivariate regression analyses. In both analyses, maternal antibody levels were associated with cord levels for all the IgGs (Table 2). The univariate analysis showed that maternal HIV infection was associated with a significant reduction of cord IgG, IgG1, and IgG3 against all the antigens, with the exception of IgG against MSP1 and IgG3 against AMA1 (Fig. 2a). After adjustment for the mentioned variables (Table 2), maternal HIV infection remained associated with a significant decrease of cord IgG1 and IgG3 against MSP1 and lysate, IgG1 against AMA1 and IgG3 against EBA175.

Malaria infection in the mother was associated with increased cord levels of IgG3 against MSP1 in the univariate analysis (Fig. 2b). In the multivariate model, malaria infection was associated with a significant decrease in the levels of IgG against AMA1 and EBA175 and IgG1 and IgG3 against MSP1 (Table 2). No statistically significant association was found between cord antibody levels and gravidity, age, treatment or maternal anemia.

Factors associated with placental transfer of antimalarial antibodies

The CMR was used as a measure of placental transfer of antibodies from mother to fetus. A twofold increase in maternal antibody levels was associated with decreasing CMR of IgG1 and IgG3 against all the antigens, but not against parasite lysate (Table 3). Both in the univariate (Fig. 3a) and multivariate (Table 3) analysis, maternal HIV infection was associated with a significant reduction in the CMR of IgG1 against AMA1, IgG1 and IgG3 against lysate and IgG3

against MSP1 and EBA175. This was also the case for the CMR of IgG1 against AMA1 in the univariate model, but the significance was lost in the multivariate analysis.

Malaria infection in the mother was associated with reduced CMR of IgG against EBA175 and AMA1, IgG1 against all the antigens and IgG3 against MSP1 in the univariate model (Fig. 3b). In the multivariate analysis, reduced CMR of IgG and IgG1 against EBA175, IgG against AMA1 and IgG3 against MSP1 remained significantly associated with malaria infection (Table 3). There were no statistically significant associations between gravidity, age, treatment group or maternal anemia and the CMR of antimalarial antibodies.

Placental transfer of antimalarial antibodies, pregnancy outcomes and malaria incidence during the first year of life

Relationships between adverse pregnancy outcomes (preterm delivery, LBW and anemia in cord blood) or malaria incidence during the first year of life and the CMR were assessed by multivariate or binomial regression analysis, respectively. No significant associations were found with a few exceptions. Only twofold increase in the CMR of IgG against lysate was associated with augmented gestational age (difference in weeks, 0.51 [95% CI, 0.01; 1.00]; P=0.047).

With respect to malaria incidence in the infant, a twofold increase in the CMRs of IgG against lysate and IgG1 against AMA1 was significantly associated with an increased risk of malaria during the first year of life (incidence ratio, 1.48 [95% CI, 1.01; 2.18]; P=0.046; and incidence ratio, 3.11 [95% CI, 1.11; 8.71]; P=0.031, respectively).

DISCUSSION

This study shows for the first time that *P. falciparum* malaria infection in pregnant women at delivery is associated with reduced cord-to-mother ratios of several antimalarial antibodies, mainly IgG and IgG1 against EBA175, IgG1 against parasite lysate and IgG3 against MSP1. In addition, these results demonstrate that HIV infection in pregnant women is associated with a decreased placental transfer of specific antimalarial IgG1 and IgG3 to the newborn. However, this decrease does not contribute to increase the risk of malaria-associated morbidity in infancy. Our results raise the concern about the potential of HIV and malaria to limit the effectiveness of infant immunization strategies based on maternal vaccination during pregnancy, becoming an important barrier for the implementation of this strategy for some vaccine-preventable diseases (34).

This study confirms that IgGs against malaria parasites, but not IgMs, are transferred through the placenta (35,36). The lack of correlation for IgM between maternal and cord samples excludes the possibility of blood contamination and suggest that the low levels of IgM in some cord blood samples could be of fetal origin due to *in utero* exposure (37–39). Although the four IgG subclasses have been shown to be able to cross the placenta (35), the low levels of IgG2 and IgG4 both in mother and cord blood found in our samples cannot confirm a reduced transfer for these isotypes in the case of antimalarial antibodies (37). This is in accordance with previous articles showing that IgG1 and IgG3 are the predominant subclasses produced in response to malaria antigens (40) and present high affinity for Fc receptors, which suggests a preferential transfer (35,37,41–43). Fc receptors, key players of the immune modulation that contribute to the release of inflammatory mediators, have been found upregulated in several inflammatory conditions (44). Future studies analysing the expression of Fc receptors in the context of malaria in pregnancy and HIV will be of great interest to understand the underlying molecular mechanisms.

Malaria infection at delivery, defined as the presence of parasites in peripheral blood or in the placenta either by microscopy, histology or qPCR, was associated with a reduced ratio of antimalarial IgGs in the cord with respect to the peripheral blood of the pregnant women. This is the first study to show such a reduced transfer of antibodies against *P. falciparum* from the mother to the fetus. Placental damages caused by malaria infection (32) may alter Fc receptors and, together with maternal hypergammaglobulinemia (21,22), probably explain the observed reduction in placental transfer. Similarly, HIV infection leads to a reduction in the cord-to-mother ratio of IgGs against AMA1, MSP1 and parasite lysate. This reduction in the antibody transfer is strongest than shown in the only previous report (26) that found a decrease in IgGs against the antigenic determinant $(NANP)_5$ of CSP but not against other antigens. Possible explanations for inefficient transfer of antibodies associated with maternal HIV infection could be the formation of immune complexes impairing transplacental IgG passage, the production of defective IgGs unable to bind to the Fc receptor, direct decrease of receptor levels associated with HIV infection (15), or direct competition by HIV-specific antibodies for a finite number of Fc receptors (18), although further study is required to establish the mechanism.

As a result of this decreased placental transfer of antibodies, we found a reduction in cord levels of several specific antimalarial antibodies in association with maternal malaria or HIV infections that was independent of antibody levels in the mother. Other study in Kenya found reduced levels of antibodies against CSP, LSA1 and RAP1 at birth in newborns from HIV-infected women (12). In contrast, Chizzolini *et al.* found increased parasite-specific IgG1 and IgG3 in cord samples from women with histology-detected placental malaria (39) and Ned *et al.* found no association between antimalarial antibody levels at birth and placental malaria diagnosed by microscopy (12). Variations in the epidemiology and presentation of the diseases in different transmission settings, the diagnostic method used (microscopy and/or histology versus the most sensitive detection of parasites by qPCR (45)) or the statistical analysis performed (i.e., adjusting for levels of antibodies in the pregnant women or use of antibody

levels in the cord as a measure of transplacental transfer instead of the more appropriate CMR value) may account for the differences with our results.

Reduction of placental antibody transfer was not consistently associated with adverse pregnancy outcomes or increased risk of malaria incidence during the first year of life. Although a decrease in the transfer of antimalarial antibodies has been suggested as one of the possible mechanisms explaining the increased predisposition of children born to HIV-mothers to haematological complications when infected with malaria (3,46), our results do not support this hypothesis. An increment in the transfer of IgG against lysate and IgG1 against AMA1 was associated with increased risk of malaria incidence during the first year of life, probably pointing towards these antibodies as markers of risk of infection rather than protection, as previously suggested (10–12,47). It is therefore plausible that other physiological factors, such as the presence of fetal haemoglobin, lactoferrin, secretory IgA or reduced para-aminobenzoic acid, are involved in the relative protection against malaria of infants less than six months of age (10,48).

This study presents several limitations. First, it was not possible to assess the effect of HIV-associated immunosuppression on the transfer of antimalarial antibodies (12,18), as data of CD4+ T cell counts and viral loads were not available for all HIV-infected women. Second, the presence of malaria infection in pregnant women was determined at delivery and does not account for malaria infections occurring earlier during gestation, which could also affect the response and transfer of antimalarial antibodies. The number of women included in this study did not allow further stratification by compartment of infection or diagnostic method, therefore further research will be required to clarify the effect of peripheral infection by itself, as well as the effect of chronic placental malaria, previously associated with reduced antibody transfer (15,19). Finally, the observational approach of the study describes potential associations but causal relationships cannot be inferred.

In summary, this study shows that malaria and HIV infections in pregnancy are independently associated with a decrease in the placental transfer of antibodies against *P. falciparum* asexual blood-stage antigens from mother to fetus. The high prevalence of HIV infection in Mozambique and other parts of Southeast Africa (2), together with declines in malaria transmission (49), may translate into a reduction of antimalarial immunity in pregnant women and affect antibody transfer and immunity development in their infants. The role that antimalarial antibodies transferred from the mother to the fetus have in infant protection should be further investigated in order to evaluate the consequences of the reduction of antibody passage associated with maternal malaria and HIV.

Table 1. Demographic and clinical factors of mothers at delivery according to their HIV status.

	Maternal HIV status		
	Uninfected (n=130), Nº (%)	HIV-infected (n=57), Nº (%)	P[a]
Age (years)			
<20	51 (39.2)	19 (33.3)	
20- <25	40 (30.8)	16 (28.1)	
>=25	39 (30.0)	22 (38.6)	0.506
Parity			
Primigravidae	36 (27.7)	15 (26.3)	
Secundigravidae	25 (19.2)	12 (21.1)	
Multigravidae	69 (53.1)	30 (52.6)	0.953
Anemia			
No	87 (66.9)	23 (40.3)	
Yes	43 (33.1)	34 (59.7)	**<0.001**
Malaria infection			
Negative	76 (58.5)	35 (61.4)	
Positive	54 (41.5)	22 (38.6)	0.706
IPTp group			
Placebo	65 (50.0)	23 (40.3)	
Sulfadoxine-pyrimethamine	65 (50.0)	34 (59.7)	0.224

[a] Chi-square test

Table 2. Association of antimalarial antibody levels in cord blood samples with maternal antibody levels, HIV and malaria infection in the multivariate linear regression model.

	Maternal antibody levels		HIV infection		Malaria infection	
	Effect[a] (95% CI)	P	Effect[b] (95% CI)	P	Effect[b] (95% CI)	P
IgG MSP1	2.14 (1.89; 2.42)	**<0.001**	0.93 (0.77; 1.13)	0.483	0.96 (0.80; 1.15)	0.674
IgG EBA175	2.49 (2.25; 2.74)	**<0.001**	0.85 (0.72; 0.99)	**0.038**	0.83 (0.71; 0.96)	**0.014**
IgG AMA1	2.77 (2.55; 3.01)	**<0.001**	0.91 (0.81; 1.02)	0.118	0.85 (0.76; 0.94)	**0.003**
IgG lysate	1.95 (1.66; 2.29)	**<0.001**	0.83 (0.58; 1.19)	0.322	0.87 (0.62; 1.22)	0.406
IgG1 MSP1	2.14 (2.00; 2.28)	**<0.001**	0.84 (0.74; 0.95)	**0.008**	0.88 (0.78; 1.00)	**0.044**
IgG1 EBA175	2.26 (2.11; 2.42)	**<0.001**	0.89 (0.79; 1.00)	**0.045**	0.90 (0.81; 1.00)	0.055
IgG1 AMA1	2.55 (2.40; 2.71)	**<0.001**	0.88 (0.81; 0.94)	**0.001**	0.94 (0.87; 1.01)	0.073
IgG1 lysate	2.73 (2.43; 3.07)	**<0.001**	0.75 (0.62; 0.90)	**0.003**	0.83 (0.70; 1.00)	**0.048**
IgG3 MSP1	2.13 (1.99; 2.28)	**<0.001**	0.84 (0.74; 0.96)	**0.012**	0.86 (0.76; 0.98)	**0.024**
IgG3 EBA175	1.78 (1.61; 1.97)	**<0.001**	0.68 (0.56; 0.83)	**<0.001**	1.01 (0.84; 1.21)	0.908
IgG3 AMA1	2.39 (2.23; 2.57)	**<0.001**	1.03 (0.90; 1.18)	0.642	0.93 (0.82; 1.06)	0.277
IgG3 lysate	1.89 (1.72; 2.07)	**<0.001**	0.72 (0.57; 0.92)	**0.008**	0.95 (0.76; 1.18)	0.626

Abbreviations: HIV, human immunodeficiency virus; CI, Confidence Interval; IgG, immunoglobulin G; MSP1, merozoite surface protein 1; EBA175, erythrocyte binding antigen 175; AMA1, apical membrane antigen 1.

[a] Defined as the proportional increase in cord antibody levels per doubling the levels in the mother. Adjusted for maternal factors: antibody levels, HIV and malaria infections, parity, age, anemia and IPTp group.

[b] Defined as the ratio of the mean cord IgG levels in the infected women with respect to the non-infected. Adjusted for maternal factors: antibody levels, HIV and malaria infections, parity, age, anemia and IPTp group.

Table 3. Association of cord-to-mother ratio of antimalarial antibodies with maternal HIV and malaria infection in the multivariate linear regression model. Only seropositive women for each malaria-specific antibody were included.

Cord-to-mother ratio	Maternal antibody levels		HIV infection		Malaria infection	
	Effect[a] (95% CI)	P	Effect[b] (95% CI)	P	Effect[b] (95% CI)	P
IgG MSP1 (n=110)	0.91 (0.75; 1.1)	0.326	1.01 (0.79; 1.30)	0.946	1.09 (0.86; 1.37)	0.479
IgG EBA175 (n= 176)	0.92 (0.83; 1.03)	0.165	0.87 (0.73; 1.02)	0.094	0.82 (0.70; 0.96)	**0.014**
IgG AMA1 (n=184)	1.05 (0.96; 1.14)	0.285	0.91 (0.81; 1.02)	0.108	0.84 (0.75; 0.93)	**0.002**
IgG lysate (n=142)	0.87 (0.64; 1.17)	0.358	0.97 (0.67; 1.40)	0.868	0.97 (0.69; 1.36)	0.856
IgG1 MSP1 (n=151)	0.83 (0.76; 0.90)	**<0.001**	0.85 (0.74; 0.98)	**0.022**	0.89 (0.78; 1.01)	0.080
IgG1 EBA175 (n=183)	0.85 (0.79; 0.91)	**<0.001**	0.89 (0.80; 1.00)	0.059	0.88 (0.79; 0.99)	**0.029**
IgG1 AMA1 (n=187)	0.94 (0.88; 1.00)	**0.041**	0.88 (0.81; 0.94)	**0.001**	0.94 (0.87; 1.01)	0.073
IgG1 lysate (n=159)	0.94 (0.80; 1.09)	0.408	0.83 (0.70; 0.98)	**0.027**	0.77 (0.65; 0.90)	**0.001**
IgG3 MSP1 (n=121)	0.89 (0.82; 0.98)	**0.015**	0.82 (0.69; 0.97)	**0.023**	0.80 (0.68; 0.95)	**0.010**
IgG3 EBA175 (n=139)	0.76 (0.68; 0.84)	**<0.001**	0.73 (0.61; 0.88)	**0.001**	0.90 (0.75; 1.09)	0.288
IgG3 AMA1 (n=170)	0.90 (0.83; 0.97)	**0.009**	1.02 (0.89; 1.17)	0.735	0.93 (0.82; 1.06)	0.255
IgG3 lysate (n=163)	0.91 (0.80; 1.03)	0.140	0.79 (0.65; 0.97)	**0.025**	0.92 (0.76; 1.11)	0.402

Abbreviations: HIV, human immunodeficiency virus; CI, Confidence Interval; IgG, immunoglobulin G; MSP1, merozoite surface protein 1; EBA175, erythrocyte binding antigen 175; AMA1, apical membrane antigen 1.

[a] Defined as the proportional increase in cord antibody levels per doubling the levels in the mother. Adjusted for maternal factors: antibody levels, HIV and malaria infections, parity, age, anemia and IPTp group.

[b] Defined as the ratio of the mean cord IgG levels in the infected women with respect to the non-infected. Adjusted for maternal factors: antibody levels, HIV and malaria infections, parity, age, anemia and IPTp group.

Fig. 1. Levels of antimalarial antibodies in maternal (grey triangles) and cord (black triangles) samples represented as ELISA optical densities (OD). Black lines correspond to the geometric mean of the population.

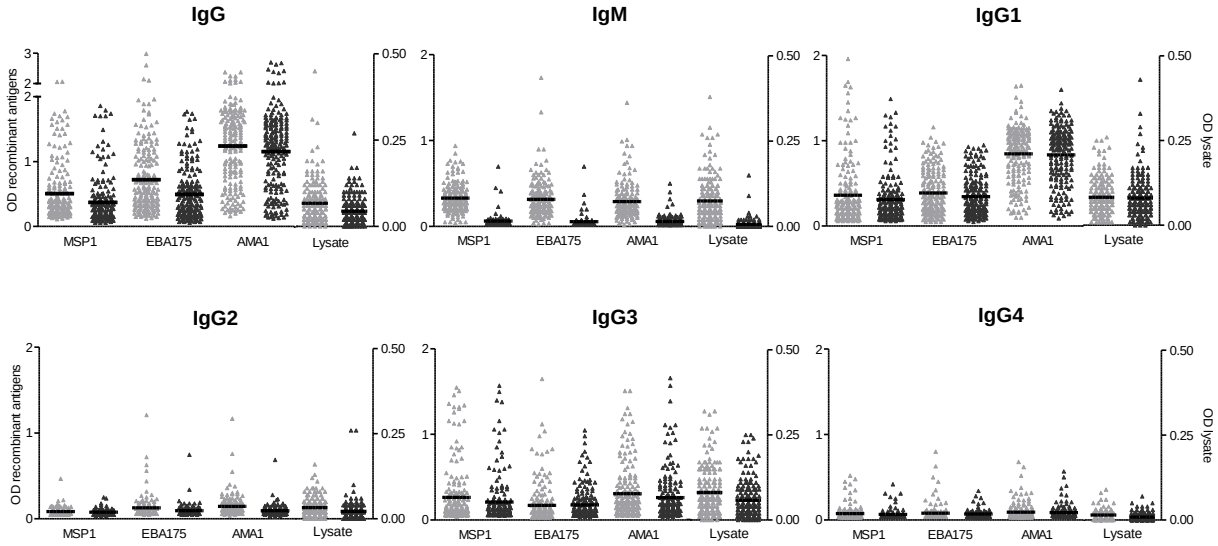
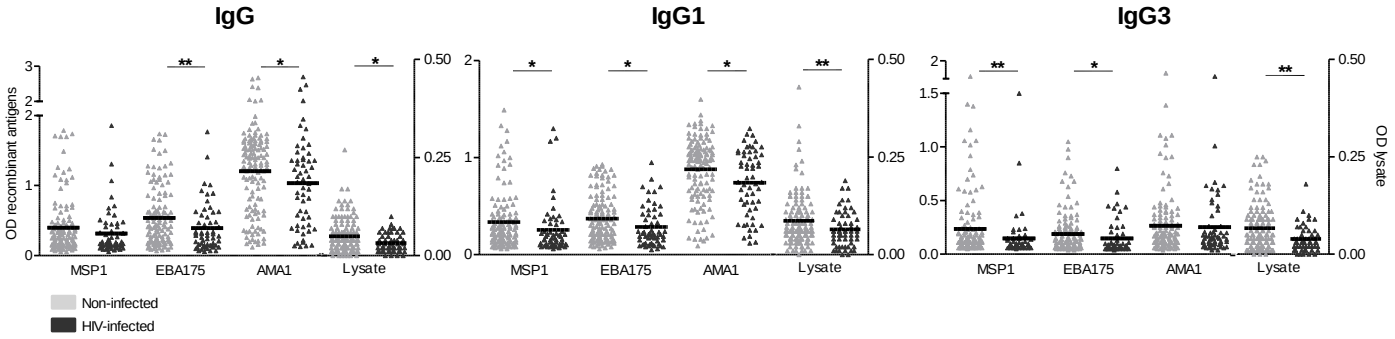


Fig. 2. Cord levels of antimalarial antibodies by maternal infection status: (a) HIV and (b) malaria infection in the univariate analysis represented as ELISA optical densities (OD). Horizontal black lines correspond to the geometric mean of the population. P-values from Student's t test are represented as * (P<0.05), ** (P<0.01) or * (P<0.001).**

(a)



(b)

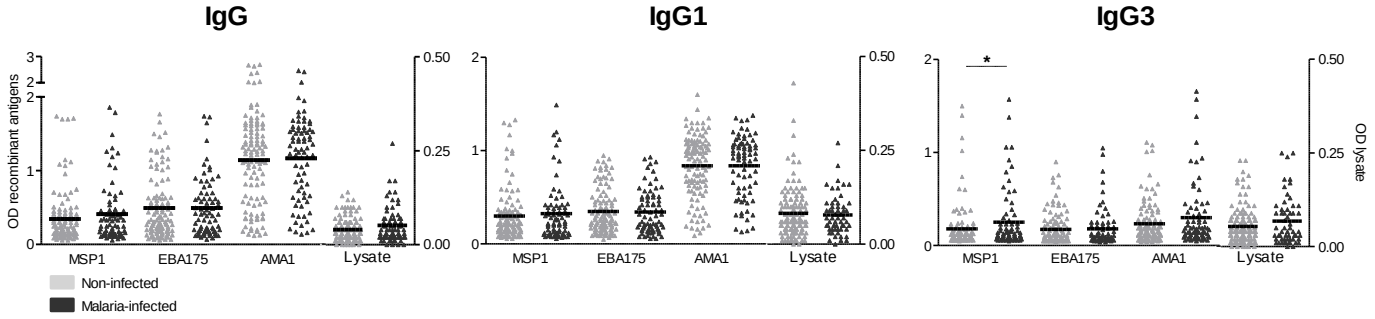
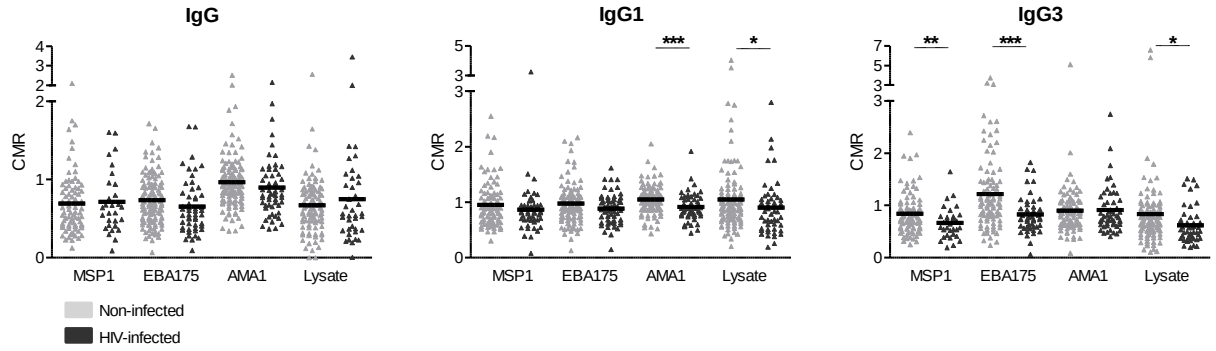
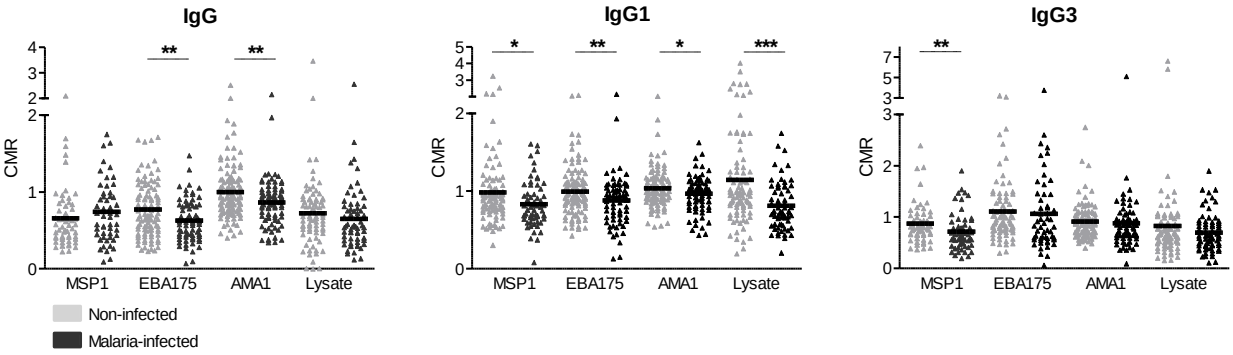


Fig. 3. Cord-to-mother ratio (CMR) of antimalarial antibodies by maternal infection status: (a) HIV and (b) malaria infection in the univariate analysis. Horizontal black lines correspond to the geometric mean of the population. P-values from Student's t test are represented as * (P<0.05), ** (P<0.01) or * (P<0.001).**

(a)



(b)



Supplementary Table 1. Correlations between maternal and cord antibody levels evaluated by Spearman's rank correlation test.

Antibody	Spearman's rho	P
IgG MSP1	0.598	**<0.001**
IgG EBA175	0.803	**<0.001**
IgG AMA1	0.792	**<0.001**
IgG lysate	0.728	**<0.001**
IgM MSP1	0.074	0.316
IgM EBA175	0.039	0.593
IgM AMA1	0.136	0.063
IgM lysate	-0.004	0.967
IgG1 MSP1	0.836	**<0.001**
IgG1 EBA175	0.865	**<0.001**
IgG1 AMA1	0.82	**<0.001**
IgG1 lysate	0.762	**<0.001**
IgG2 MSP1	0.469	**<0.001**
IgG2 EBA175	0.556	**<0.001**
IgG2 AMA1	0.590	**<0.001**
IgG2 lysate	0.412	**<0.001**
IgG3 MSP1	0.748	**<0.001**
IgG3 EBA175	0.578	**<0.001**
IgG3 AMA1	0.842	**<0.001**
IgG3 lysate	0.808	**<0.001**
IgG4 MSP1	0.616	**<0.001**
IgG4 EBA175	0.496	**<0.001**
IgG4 AMA1	0.837	**<0.001**
IgG4 lysate	0.294	**<0.001**

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

MEETING(S) WHERE THE INFORMATION HAS PREVIOUSLY BEEN PRESENTED

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