IgM and IgG against *Plasmodium falciparum* lysate as surrogates of malaria exposure and protection during pregnancy

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**Abstract**

**Background:** Difficulties to disentangle the protective versus exposure role of anti-malarial antibodies hamper the identification of clinically-relevant immune targets. Here, factors affecting maternal IgG and IgMs against *Plasmodium falciparum* antigens, as well as their relationship with parasite infection and clinical outcomes, were assessed in mothers and their children. Antibody responses among 207 Mozambican pregnant women at delivery against MSP¹₁₉, EBA175, AMA1, DBLα and parasite lysate (3D7, R29 and E8B parasite lines), as well as the surface of infected erythrocytes, were assessed by enzyme-linked immunosorbent assay and flow cytometry. The relationship between antibody levels, maternal infection and clinical outcomes was assessed by multivariate regression analysis.

**Results:** Placental infection was associated with an increase in maternal levels of IgGs and IgMs against a broad range of parasite antigens. The multivariate analysis including IgGs and IgMs showed that the newborn weight increased with increasing IgG levels against a parasite lysate, whereas the opposite association was found with IgMs. IgGs are markers of protection against poor pregnancy outcomes and IgMs of parasite exposure.

**Conclusions:** Adjusting the analysis for the simultaneous effect of IgMs and IgGs can contribute to account for heterogeneous exposure to *P. falciparum* when assessing immune responses effective against malaria in pregnancy.

**Keywords:** Malaria, Pregnancy, Heterogeneity, Antibody, Immunity

**Background**

*Plasmodium falciparum* infection during pregnancy, characterized by the accumulation of parasites in the intervillous spaces of the placenta, is a major disease that affects birth outcome in sub-Saharan Africa [1], causing up to 100,000 infant deaths annually [2]. In contrast to non-pregnant adults, pregnant women are at increased risk of malaria infection independently of previous acquisition of immunity [3]. In conditions of high malaria transmission, this susceptibility decreases with subsequent pregnancies [3], suggesting a parity-dependent acquisition of protective immunity. Understanding the targets, quality and quantity of the immune responses that pregnant women mount upon infection and their role, in protection against infection and its adverse effects in pregnancy (i.e., low newborn weight, stillbirth and maternal anaemia) is of critical importance to design effective vaccines specific for pregnant women [4].

Immunoglobulin G (IgG) antibodies against placental infected erythrocytes (IE) and VAR2CSA [5], the major variant surface antigen on the surface of *P. falciparum* infected erythrocytes that binds chondroitin sulfate A (CSA) [6] to enable sequestration in the placenta [7], increase with parity [8]. These antibodies have been...
associated with reduced risk of placental infection [5, 9–
11], low birth weight [12–15] and maternal anaemia [14,
16]. However, several other studies have failed to show
such a protective association [11, 17–22], but instead
suggested that antibodies at delivery reflect exposure to
*P. falciparum* in pregnancy [8, 9, 21–28].

Recent studies show how the power to identify immune
correlates of protection in children [29–31] and pregnant
women [28] under heterogeneous intensities of malaria
transmission can be reduced by inclusion of individu-
als with different degrees of exposure [29]. Heterogene-
ity in exposure to *P. falciparum* [9, 22, 27] can be taken
into account by including in the analysis only women
with proven parasite exposure before delivery (i.e., hav-
ing had a malaria episode during pregnancy) [28]. How-
ever, this requires the morbidity surveillance at antenatal
care units of rural hospitals in African countries that is
not always available. There is a need to develop better
methods to adjust for heterogeneity in parasite exposure
when assessing immune responses that contribute to
protection against malaria infection in pregnancy [30].

The aim of this study was to assess an alternative way
of accounting for the effect of heterogeneous exposure
to *P. falciparum* in the absence of information before
delivery. Specifically, the objective was to assess the rela-
tionship of IgG and IgM against *P. falciparum* antigens
in Mozambican pregnant women at delivery with para-
site infection and adverse clinical impacts in the moth-
ers and newborns. Pregnancy-specific antigens (infected
erthrocytes selected from binding to CSA and placental
isolates), general (non-pregnancy specific) antigens (mer-
ozoite antigens, non-CSA binder *P. falciparum* lines and
isolates) and a parasite lysate (to assess responses against
all whole-parasite antigens) were included. IgG but also
IgM were assessed given their contribution for protection
towards clinical malaria in children during their 1st year of life. The study was conducted in a mother and child cohort living in a malaria endemic area of Mozambique, in the context of a randomized, double-
blind, placebo-controlled trial “[Age of exposure and
immunity to malaria in infants” (AgeMal)] [33].

**Methods**

**Study area**

The study was conducted at the Centro de Investigação
e Saúde da Manhiça (CISM), located in the Manhiça
District, Maputo Province, southern Mozambique. The
area has been described in detail elsewhere [34]. Trans-
mision of *P. falciparum* is perennial and of moderate
intensity with a warm rainy season from November to
April, and a cool dry season during the rest of the year.

**Study design and samples collection**

HIV-negative pregnant women resident in the Manhiça
study area were recruited during the third trimester of
pregnancy at the antenatal clinic of the Maragara Health
Post (MHP), in the south of the study area, from Septem-
ber 2005 to March 2007 [33]. After delivery, neonates
were evaluated for eligibility. Exclusion criteria included
birth weight < 2 kg, twins, congenital malformations,
birth asphyxia or apparent health problems. Three-hun-
dred and 49 eligible newborns were enrolled in the trial.

At delivery, maternal peripheral and cord blood sam-
plies collected into EDTA vacutainers were centrifuged
and plasma stored at −20 °C for future antibody deter-
minations. Thin and thick smears of peripheral and cord
blood were Giemsa-stained and examined for malarial
parasites according to quality-control procedures [33].
Haemoglobin (Hb) levels were determined on full blood
counts performed using a Sysmex KX-21N cell counter
(Sysmex Corporation, Kobe, Japan). Peripheral and cord
blood was also collected onto filter papers for *P. falci-
parum* detection by real-time quantitative PCR (RTqPCR)
in duplicates [35]. Tissue samples obtained from the
maternal side of the placenta were processed for histolog-
ical examination and classified as negative, acute, chronic
or past infections [36]. When the delivery occurred out-
side the maternity post, only the mother blood sample
was collected.

Children were followed up until age 24 months,
with weekly active case detection from birth to age
10.5 months, and monthly home visits from 10.5 to
24 months of age. Children were examined and their par-
asitaemia and haematocrit determined if they presented
fever (axillary temperature ≥ 37.5 °C) or their guardians
referred history of fever in the preceding 24 h. Addition-
ally, passive case detection was carried out at the MHP
and Manhiça District Hospital (MDH) through the mor-
bidity surveillance system to monitor attendances to the
outpatient clinics and admissions to hospital.

Informed consent was sought to enroll pregnant
women and their newborn children in the study upon
birth. The protocol was approved by the National
Mozambican Ethics Review Committee and the Hospital
Clinic of Barcelona Ethics Review Committee. The trial
was conducted according to the ICH Good Clinical Prac-
tice guidelines and reviewed by a Local Safety Monitor
and a Data and Safety Monitoring Board. The trial was
registered in ClinicalTrials.gov (clinical trials identifier
NCT00231452).

**Measurement of antibodies against recombinant antigens**

Recombinant merozoite surface protein 1 (MSP1; C-ter-
ninal 19-kD fragment, 3D7 strain) [37], erythrocyte-
binding antigen 175 (EBA175; region F2, Camp strain)
Measurement of antibodies against whole parasite extract

Whole-parasite lysate was prepared by three freezing/thawing cycles of asynchronous in vitro cultures of 3D7, R29 and E8B laboratory strains at a 5% level of parasitemia and 1% haematocrit, as previously described [21]. Non-infected erythrocyte (NIE) lysate, prepared using the same procedure as whole-parasite lysate, was used as a control of unspecific recognition for each plasma sample. ELISA plates were coated with 50 µL of lysate per well. Wells were blocked with 300 µL of 5% skim milk at 4 °C for 8 h. One hundred microliters of plasma sample were tested in duplicate for IgG and IgM (dilution, 1:1600). Incubation of antibodies and development of the reaction were performed as described above. Malaria-specific antibody recognition was evaluated by subtracting the mean OD value of NIEs from the mean OD value of IEs. The pool of positive plasmas was used to normalize the data from different assays [21] and results were expressed as arbitrary units.

Measurement of antibodies against the surface of Plasmodium falciparum-infected erythrocytes

Cryopreserved O+ erythrocytes infected by two placentals isolates previously confirmed to express VAR2CSA (IEPlac1 and IEPlac2), five pediatric isolates (IECh1–IECh4 and IESev1) from Manhiça [25] and a CSA-binding parasite line adapted to culture (CS2) were thawed and matured to trophozoite stage without in vitro expansion. Erythrocytes at 1% haematocrit were sequentially incubated with test plasma (1:20), rabbit anti-human IgG (1:200) and AlexaFluor donkey anti-rabbit IgG (1:1000) plus 10 µg/mL of ethidium bromide. Reactivity against the surface of IEs was expressed as the difference between the geometric mean fluorescence intensity (MFI) of 1000 IEs and the MFI of NIE obtained in a FACSCalibur flow cytometer (BD, San Jose, USA). A pool of plasma samples from 10 Mozambican pregnant women was used to normalize the data from different assays [21] and results were expressed as arbitrary units.
infection and clinical outcomes in mothers and their children, the association of antibody levels (independent variable) with maternal haematocrit and weight of the newborn (dependent variable) was assessed, including a multivariate analysis with all the antibody responses as independent variables. Negative binomial regression models were used to evaluate the effect of doubling the levels of antibodies on the incidence of multiple malaria episodes in children up to 12 months of age [33]. Analyses were adjusted for maternal age, parity, use of IRS, use of ITN, season, neighbourhood, child anti-malarial intervention [33] and *P. falciparum* infection in the mother. To assess if parity modified the associations, an interaction term was included in the regression models, and ratios and 95% confidence intervals (95% CI) for each antibody responses were estimated after stratifying the regression models. Analyses were performed using Stata 11 (College Station, TX, USA) and significance was defined at \( p < 0.05 \).

**Results**

**Factors related to *P. falciparum* infection in pregnancy**

The analysis of antibody responses included 207 women (Table 1), 42 (20%) with peripheral infection at delivery, 47 (23%) with placental infection (acute infection in 5 [11%], chronic infection in 1 [2%] and past infection in 41 [87%]), and 8 (4%) with cord blood infection. Placental inflammation in 10 (5%) of the women was more frequent in women with peripheral infection than uninfected women. Placental infection decreased with parity (Table 1) and age of the women (35% [25 out of 71] if \( \leq 20 \) years, 16% [9 out of 58] if 21–24 years and 17% [13 out of 78] if \( \geq 25 \) years; \( p = 0.011 \)), and was more frequent among women living in a house that had not received IRS (29% [32 out of 109]) than in those living in a house that received IRS (15% [15 out of 98]; \( p = 0.020 \)). Cord blood infection was more frequent among women with placental inflammation (20% [2 out of 10]) than in those without inflammation (3% [6 out of 197]; \( p = 0.05 \)). The use of ITN was higher among MG women (17% [18 out of 103]) than SG (4% [2 out of 47]) or PG women (5% [3 out of 57]; \( p = 0.020 \)). LBW was more frequent among PG women than SG or MG women (Table 1).

**Factors related to antibody levels at delivery**

Placental infection was associated with higher levels of maternal and cord IgGs against all the antigens measured (except IESEV), as well as with maternal IgMs against MSP1, EBA175, AMA1 and parasite lysate (Additional file 1 Table S1). Peripheral infection (adjusted for placental infection) was associated with increased IgG levels against EBA175, parasite lysate, DBLα and the two placental isolates, as well as cord IgGs against DBLα (Additional file 1 Table S1).

**Table 1** Demographic and clinical factors of mothers at delivery according to their parity

|                  | All (n = 207) | Primigravida (n = 57) | Secundigravida (n = 47) | Multigravida (n = 103) | \( p \) |
|------------------|--------------|-----------------------|------------------------|------------------------|--------|
| Placental infection, n (%) | 47 (23) | 21 (37) | 9 (19) | 17 (17) | 0.013 |
| Peripheral infection, n (%) | 42 (20) | 14 (25) | 7 (15) | 21 (20) | 0.473 |
| Cord infection, n (%) | 8 (4) | 2 (4) | 1 (2) | 5 (5) | 0.895 |
| Placental density | 90.5 (83.9–97.6) | 81.1 (63.9–102.8) | 98.55 (97.7–99.5) | 92.6 (85.6–100.1) | 0.166 |
| Peripheral density | | | | | |
| Microscopy | 3988 (894–17,778) | 6663 (137–323,311) | 3652 | 3058 (507–18,439) | 0.897 |
| PCR | 0.69 (0.25–1.88) | 1.05 (0.15–7.36) | 0.81 (0.02–33.5) | 0.47 (0.12–2.04) | 0.793 |
| Inflammation, n (%) | 10 (5) | 5 (9) | 1 (2) | 4 (4) | 0.270 |
| Age, mean (SD) | | | | | |
| 15–20 years | 71 (34) | 44 (77) | 24 (51) | 3 (3) | <0.001 |
| 21–24 years | 58 (28) | 12 (21) | 20 (43) | 26 (25) | |
| ≥ 25 years | 78 (38) | 1 (2) | 3 (6) | 74 (72) | |
| Neighbourhood (< 2.5 km), n (%) | 41 (20) | 14 (25) | 7 (30) | 20 (19) | 0.494 |
| Use of ITN, n (%) | 23 (11) | 3 (5) | 2 (4) | 18 (17) | 0.002 |
| Household IRS, n (%) | 98 (47) | 24 (42) | 21 (45) | 53 (51) | 0.500 |
| Season (dry), n (%) | 58 (28) | 18 (32) | 16 (34) | 24 (23) | 0.295 |
| Low-birth weight, n (%) | 28 (14) | 15 (26) | 6 (13) | 7 (7) | 0.004 |
| Maternal anaemia, n (%) | 87 (42) | 23 (40) | 20 (43) | 44 (43) | 0.792 |

SD standard deviation, ITN insecticide-treated nets, IRS indoor residual spraying

* 41 past, 5 acute and 1 chronic infection
Maternal IgM against MSP1, EBA175, AMA1 and DBLa were lower in women with placental inflammation compared to women without inflammation (Table 2). IgMAMA1 was higher in *P. falciparum* positive cord blood (10.87, SD 9.40) than in negative cords (6.82, SD 3.75, \( p = 0.023 \)).

The use of ITNs was associated with reduced levels of maternal IgGs against DBLa (Additional file 1 Table S1), while IRS was associated with a reduction in the levels of maternal IgGs against lysate (Additional file 1 Table S1). Antibodies against IEch3 were 1.32-fold higher in neighbourhoods close to the river (<2.5 km) compared to those at more than <2.5 km (95% CI [1.02; 1.69]; \( p = 0.037 \)). There were no significant differences according to season (all \( p \) values > 0.05).

Parity modified the association of maternal IgG and IgM responses as well as cord IgGs with placental infection, as indicated by the statistically significant interaction terms (Fig. 1). The analysis stratified by parity and infection showed that the increase of antibody responses

### Table 2 Antibody responses in women with and without placental infection

| Maternal IgGs | No inflammation (n = 197) | Inflammation (n = 10) | \( p \) | Multivariate* |
|---------------|--------------------------|-----------------------|------|--------------|
| | GM | SD | GM | SD | | Ratio | (95% CI) | \( p \) |
| **MSP1** | 65.41 | 58.42 | 62.84 | 62.76 | 0.891 | 0.88 | (0.49; 1.57) | 0.665 |
| **EBA175** | 44.17 | 33.09 | 42.24 | 26.13 | 0.853 | 0.89 | (0.55; 1.44) | 0.636 |
| **AMA1** | 61.45 | 37.81 | 63.18 | 30.41 | 0.889 | 0.96 | (0.64; 1.42) | 0.824 |
| **DBLa** | 77.56 | 30.62 | 69.28 | 21.26 | 0.375 | 0.83 | (0.65; 1.07) | 0.145 |
| **Lysate** | 31.68 | 40.32 | 43.07 | 26.07 | 0.450 | 1.16 | (0.52; 2.58) | 0.719 |
| **CS2** | 2.16 | 1.62 | 1.83 | 1.07 | 0.492 | 0.93 | (0.79; 1.10) | 0.396 |
| **Women1** | 149.75 | 227.78 | 93.63 | 96.67 | 0.336 | 0.68 | (0.28; 1.62) | 0.386 |
| **Women2** | 412.18 | 580.46 | 216.39 | 228.31 | 0.156 | 0.56 | (0.24; 1.28) | 0.171 |
| **SEV** | 2.72 | 0.88 | 2.39 | 0.90 | 0.228 | 0.97 | (0.93; 1.02) | 0.245 |
| **Child1** | 249.09 | 227.92 | 178.53 | 187.45 | 0.266 | 0.67 | (0.37; 1.22) | 0.193 |
| **Child2** | 641.87 | 547.08 | 544.20 | 448.88 | 0.550 | 0.82 | (0.47; 1.44) | 0.496 |
| **Child3** | 653.83 | 474.55 | 443.80 | 366.64 | 0.103 | 0.67 | (0.42; 1.08) | 0.105 |
| **Child4** | 183.91 | 180.67 | 128.46 | 158.73 | 0.267 | 0.64 | (0.34; 1.23) | 0.186 |
| **Cord IgGs** | | | | | | | | |
| **MSP1** | 75.22 | 45.36 | 40.66 | 32.52 | 0.002 | 0.53 | (0.36; 0.80) | 0.002 |
| **EBA175** | 39.44 | 27.08 | 18.37 | 15.24 | 0.001 | 0.58 | (0.38; 0.76) | 0.001 |
| **AMA1** | 51.87 | 31.25 | 29.61 | 28.32 | 0.006 | 0.58 | (0.38; 0.86) | 0.008 |
| **DBLa** | 76.29 | 38.64 | 39.61 | 35.11 | <0.001 | 0.53 | (0.38; 0.74) | <0.001 |
| **Lysate** | 16.06 | 29.11 | 15.58 | 24.48 | 0.958 | 0.91 | (0.28; 2.93) | 0.87 |
| **CS2** | 2.30 | 1.73 | 2.12 | 1.39 | 0.726 | 0.98 | (0.83; 1.16) | 0.804 |
| **SEV** | 2.61 | 0.81 | 2.35 | 0.74 | 0.304 | 0.98 | (0.94; 1.02) | 0.288 |
| **Cord IgMs** | | | | | | | | |
| **MSP1** | 54.18 | 51.79 | 41.54 | 45.09 | 0.396 | 0.69 | (0.37; 1.28) | 0.237 |
| **EBA175** | 40.16 | 31.79 | 40.62 | 33.98 | 0.065 | 0.92 | (0.55; 1.55) | 0.753 |
| **AMA1** | 60.89 | 40.77 | 67.26 | 27.17 | 0.643 | 1 | (0.66; 1.53) | 0.989 |
| **DBLa** | 60.72 | 27.78 | 65.68 | 28.77 | 0.597 | 0.98 | (0.73; 1.31) | 0.891 |
| **Lysate** | 28.55 | 34.11 | 25.50 | 17.50 | 0.768 | 0.77 | (0.36; 1.64) | 0.495 |
| **CS2** | 2.30 | 1.73 | 2.12 | 1.39 | 0.726 | 0.98 | (0.83; 1.16) | 0.804 |
| **SEV** | 2.61 | 0.81 | 2.35 | 0.74 | 0.304 | 0.98 | (0.94; 1.02) | 0.288 |

*GM geometric mean, SD standard deviation

* Adjusted for parity, age, neighbourhood, ITN, season and IRS*
associated with placental infection was mainly observed in PG women (Fig. 1).

Factors related to pregnancy outcome and incidence of malaria in the children
The newborn weight increased with age and parity of the women, and was lower among women with a placental infection (Table 3). Haemoglobin levels were lower among women who had their pregnancy during the rainy season. Incidence of malaria during the first 1 year of life was higher among children born from a mother with a cord blood infection (Table 3).

The newborn weight decreased with increasing levels of maternal IgM<sub>EBA175</sub> and IgM<sub>DBLα</sub> (reductions of 0.06 kg (95% CI [−0.11; −0.01], p = 0.031 and 0.07 kg (95% CI [−0.14; −0.01], p = 0.032) with twofold increase in antibody levels, respectively; Additional file 1: Table S2). No association was found between antibody levels and maternal anaemia. The risk of malaria in the children during their 1st year of life increased with increasing levels of maternal IgGs (all except IgG<sub>ch1</sub>) and cord IgGs (all except IgG-I<sub>Es</sub>). The multivariate analysis including all antibody levels showed that newborn weight increased with levels of IgG<sub>lysate</sub> whereas decreased with levels of IgM<sub>lysate</sub> (Fig. 2; Additional file 1: Table S3).

Discussion
This study shows that placental infection is associated with increased levels of maternal IgG and IgM against a broad range of parasite antigens and that the newborn weight decreases with increasing levels of IgM specific for EBA175 and DBLα. No association was found between IgG levels against any of the antigens tested and improved pregnancy outcomes when the analysis was performed for specific antibodies. However, higher levels of IgG against a parasite lysate were associated with increasing birth weight when IgG and IgM were included in a multivariate analysis, being IgM against the parasite lysate associated with a reduction in the newborn weight. Overall, these results suggest that adjusting the analysis for the simultaneous effect of IgM and IgG could contribute to adjust for heterogeneity in parasite exposure when assessing immune responses that confer protection against malaria infection in pregnancy, being IgM markers of parasite exposure and IgG markers of protection against poor pregnancy outcomes.

Results of this study confirm parasitological observations already described for malaria in pregnancy. PG and younger women (15–20 year) were at higher risk of placental infection as detected by histology [43]. This increased risk can be attributed to a higher susceptibility to malaria during first pregnancies due to associated immune modulation [25] and/or lack of immunity.
against VAR2CSA expressed by placental parasites [5]. Differential use of preventive tools among women at different parities, as shown by the higher use of ITN among MG women compared to PG, may also contribute to reduce the risk of malaria infection in MG women. In accordance with this reduction of placental infection with increasing parity, low birth weight was more common in PG and the newborn weight was reduced in mothers with placental infection and younger women. Importantly, household IRS was also associated with a reduction in the prevalence of placental infection. In contrast to placental infection, peripheral infection was not found to be associated with parity, possibly because placental submicroscopic infections were not assessed in this study. Results of this study also show that incidence of malaria was higher among children born from women living closer to the river, an association that can be explained by similar risk of exposure to the parasites in mothers and infants living in the same household [21]. Furthermore, this study shows that cord blood infections

| Table 3 | Factors associated with newborn weight, maternal haemoglobin levels and incidence of malaria during the 1st year of life |
|---------|---------------------------------------------------------------------------------------------------------------|
| Parity  |                                                                                                               |
| PG      | 2.8 2.69–2.91 0.001 | 113.1 105.4–120.7 0.878 |
| SG      | 2.9 2.81–2.99 | 113.3 107.9–119.6 1.64 0.61–4.43 0.331 |
| MG      | 3.1 3.05–3.20 | 111.5 107.2–115.8 1.07 0.44–2.59 0.878 |
| Placental infection |                                                                                                               |
| Negative | 3.02 2.95–3.08 0.044 | 113 109.4–116.6 0.473 1 |
| Positive | 2.89 2.77–2.99 | 110.1 102.1–118.1 0.95 0.39–2.27 0.915 |
| Peripheral infection |                                                                                                               |
| Negative | 2.99 2.92–3.05 0.990 | 113.3 109.6–116.9 0.288 1 |
| Positive | 2.99 2.89–3.08 | 108.9 101.1–116.7 1.82 0.79–4.18 0.161 |
| Inflammation |                                                                                                               |
| Negative | 2.98 2.92–3.03 0.106 | 112.6 109.3–116.0 0.488 1 |
| Positive | 3.19 2.92–3.46 | 107.0 90.26–123.7 2.96 0.72–12.02 0.130 |
| Cord infection |                                                                                                               |
| Negative | 2.98 2.92–3.03 0.131 | 112.6 109.3–115.9 0.474 1 |
| Positive | 3.2 2.72–3.68 | 106.4 88.9–123.9 5.90 1.53–22.77 0.010 |
| Age (years) |                                                                                                               |
| 15–20 | 2.78 2.69–2.87 <0.001 | 110.7 104.7–116.8 0.344 1 |
| 21–24 | 3.03 2.95–3.12 | 116.2 110.0–122.3 0.79 0.31–1.98 0.608 |
| ≥ 25 | 3.14 3.05–3.23 | 110.9 105.7–116.0 0.88 0.38–2.05 0.769 |
| Neighbourhood (km) |                                                                                                               |
| > 2.5 | 3.00 2.94–3.06 0.328 | 111.1 114.7–107.5 0.122 1 |
| < 2.5 | 2.93 2.79–3.07 | 117.6 109.9–125.2 1.04 0.42–2.57 0.936 |
| Use of ITN |                                                                                                               |
| Negative | 2.97 2.91–3.03 0.171 | 112.8 109.2–116.5 0.469 1 |
| Positive | 3.09 2.92–3.28 | 109.1 102.4–115.9 1.07 0.34–3.35 0.903 |
| Use of IRS |                                                                                                               |
| Negative | 2.94 2.87–3.02 0.109 | 113.3 108.5–118.1 0.573 1 |
| Positive | 3.04 2.95–3.12 | 111.4 106.8–115.9 0.66 0.32–1.37 0.268 |
| Season |                                                                                                               |
| Rainy | 2.98 2.91–3.05 0.685 | 110.2 106.2–114.3 0.049 1 |
| Dry | 3.01 2.89–3.11 | 117.4 111.8–123.0 0.99 0.44–2.21 0.980 |

PG primigravida, SG secundigravida, MG multigravida, ITN insecticide-treated nets, IRS indoor residual spraying, IRR incidence rate ratio

a p < 0.05 in the multivariate analysis adjusted by age, use of IRS and ITS, season and neighbourhood

b adjusted for treatment, weight and maternal haemoglobin
are associated with an increased risk of malaria during the 1st year of life, suggesting that in utero exposure can have a detrimental effect on the ability of newborns to mount an effective immune response against the parasite.

In this maternal cohort, placental infection is associated with an increase of IgG, as already shown in studies conducted in the same study area [21, 28], but also with increasing IgM. The magnitude of this increase was higher in PG than in MG, probably because parasite densities are higher in first time pregnant women, leading to a higher boosting of antibodies. Antibody responses were associated with a non-significant reduction of haemoglobin levels in the women at delivery, and the multivariate analysis showed that IgM against the lysate were associated with a reduction in the newborn weight. Antibody responses were associated with a non-significant reduction of haemoglobin levels in the women at delivery, and the multivariate analysis showed that IgM against the lysate were associated with a reduction in the newborn weight. Although the large number of comparisons made may have increased the chance of false discoveries, the fact that this associations with antibody responses against parasite lysate are maintained in the multivariate analysis including all the antigens suggest the genuineness of the observation. Moreover, antibody responses were associated with an increase in the incidence of malaria in their children during their 1st year of life. This association may be explained by a similarly high exposure to mosquito bites in pregnant women and their children residing in the same household which would translate into a boosting of antibody responses in pregnant women at delivery and a higher malaria incidence in the children. Overall, these results reinforce the concept of antibodies as markers of parasite exposure and the difficulties of disentangling the protective versus exposure role of antibodies in a situation of heterogeneous exposure. In absence of a continuous morbidity surveillance at antenatal care units to identify malaria cases and exclude from the analysis those women without proven exposure during pregnancy (i.e., having had a malaria episode), these results suggest that adjusting for IgM as markers of exposure to the parasite can control for the heterogeneity of exposure [28] and contribute to identify those IgG that may have a role in protection. Interestingly, data of this study show that IgG against a parasite lysate are more associated to an increase in the newborn weight than IgG against the surface of a CSA-binding parasite line. This association might be explained by an antibody-mediated clearance of circulating parasites early at pregnancy (i.e., before these parasite populations switch to placental-binding, that

![Fig. 2](image-url)
can contribute to prevent low birthweight. Also, immunity against a broad range of \( P. \) falciparum antigens and not only against CS2 may have a role in controlling parasite infections and reducing the adverse consequences of malaria in pregnancy.

### Conclusions

Newborn weight increased with increasing IgG levels against a parasite lysate, whereas the opposite association was found with IgM, suggesting that IgG are markers of protection against poor pregnancy outcomes and IgM of parasite exposure. This study confirms the need for developing methods to control for heterogeneous exposure to the parasite when aiming to identify those immune responses that have a potential role in protection against infection and disease. Further studies are needed to assess novel approaches to disentangle exposure from protection in immunological studies assessing the role of antibodies against the adverse consequences of malaria infection in situations of heterogeneous malaria exposure.

### Additional file

**Additional file 1: Table S1.** Association of antibody responses with maternal infection, use of insecticide treated nets and indoor residual spraying. **Table S2.** Association between antibody levels and weight of newborn, hemoglobin and incidence of malaria in children. **Table S3.** Association of antibody levels with maternal infection, pregnancy outcomes and incidence of malaria in children.

### Abbreviations

- MSP1: merozoite surface protein 1; EBA175: erythrocyte-binding antigen 175; AMA1: apical membrane antigen-1, DBLα: Duffy binding-like alpha domain; ELISA: enzyme-linked immunosorbent assay; IgG: immunoglobulin G; IgM: immunoglobulin M; IE: infected erythrocytes; CSA: chondroitin sulfate A; RTqPCR: real-time quantitative PCR; Hb: haemoglobin; NIE: non-infected erythrocytes; IE: infected erythrocytes; MF1: mean fluorescence intensity; CISM: Centro de Investigación en Salud de la Mancha, MIDH: Mancha District Hospital, MHP: Maragua Health Post; HIV: human immunodeficiency virus; PG: primigravida; SG: secundigravida; MG: multigravida; ITN: insecticide-treated nets; IRS: indoor residual spraying.

### Authors’ contributions

AM, CD, JJA, PLIS designed the study. CG coordinated the field clinical epidemiological study. MNK, AN, RA, AB, MHR, LMQ processed the samples. LMQ, JO performed histological examination placentas. MNK, AJ, PC, CEC performed molecular and immunological laboratory analyses. AM, CD analyzed and interpreted the data. AM wrote the first draft. AN, CG, RA, JO, CM, CD contributed in writing the manuscript. All authors read and approved the final manuscript.

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### Competing interests

The authors declare that there are no competing interests.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate

The protocol was approved by the National Mozambican Ethics Review Committee and the Hospital Clinic of Barcelona Ethics Review Committee. Written informed consent was obtained to enroll mothers and their infants.

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