Novel biomarkers in the pathogenesis of placental malaria in sub-Saharan Africa

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5. ABO PHENOTYPES AND MALARIA-RELATED OUTCOMES IN MOTHERS AND BABIES IN GHANA

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b. Barcelona Centre for International Health Research (CRESIB), Department of Pathology, Hospital Clínic-Universitat de Barcelona, Barcelona, Spain

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Abstract

Background
ABO blood group phenotypes modulate malaria risk in non-pregnant individuals but few studies have assessed their role in pregnancy-associated malaria.

Methods
A cross-sectional study of placental malaria was previously carried out in Kumasi, Ghana. The primary objective of the current analysis was to assess the association of ABO phenotypes and malaria-related outcomes. ABO grouping was defined on antiserum agglutination and placental malaria was classified according to characteristic histopathological changes. The secondary objective was to describe antiphospholipid profiles in relation to ABO blood groups.

Results
The prevalence of placental malaria was 33% (n=97). Among primiparae, blood group A was associated with more placental infections than the other blood groups combined (OR 6.18; 95% CI 1.10 – 34.70; p=0.038). Among multiparae there was no association between placental malaria and blood group. After adjustment there was a trend for reduced placental weight (p=0.062) and greater feto-placental weight ratio (p=0.070) with blood group O. There was no association between blood groups and anti-phospholipid profiles.

Conclusions
These data contrast with a number of previous studies identifying blood group O as a risk factor for malaria among primiparae. However, they are compatible with others reported from non-pregnant individuals which identified blood group A as a risk factor for malaria infection. Further work to clarify the glycobiology of placental malaria is suggested.
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Keywords

abo blood-group system
anti-phospholipid antibody
β glycoprotein I
cardiolipin
phosphatidyl-serine
malaria
placenta
Background

In regions of the world which are highly endemic for *P. falciparum* malaria, red cell polymorphisms conferring resistance to malaria are widespread (1). It has been observed that in such regions, a higher proportion of the population has blood group O than in non-malarious regions (2). Although results are inconsistent, the data from several studies of non-pregnant subjects suggest that blood group O may have a pivotal role in providing protective immunity against severe forms of the disease while blood group A is a risk factor for severe malaria and acts as a co-receptor for *P. falciparum* rosetting (3).

ABO blood groups are carbohydrate histo-blood antigens that are expressed in many tissues and which have important modulating roles in infection and some types of cancer. The antigens are formed by terminal glycosylation of glycoproteins and glycolipid chains present on cell surfaces. Glycosylation is integral to cell-cell interactions including adherence, which is implicated in disease severity in malarial pathophysiology (2).

Few studies have examined the role of ABO blood groups in relation to malaria infection during pregnancy, which is characterised by dense parasite sequestration in the placental vascular beds and adverse neonatal sequelae including low birthweight and preterm delivery, particularly in primiparae (4). We have previously reported the first associations between ABO blood group phenotypes, placental malaria and birth outcomes in The Gambia (5) and Malawi (6). In contrast to studies from non-pregnant subjects, in both these studies blood group O was associated with increased prevalence of placental parasitaemia in primiparae and reduced prevalence in multiparae. We concluded that the ABO blood group system was a component of the well-described parity-related susceptibility to *P. falciparum* placental infection.

We have also reported an association between parity and specific moieties of anti-phospholipid antibodies (aPL) in malarial pregnancy, which was related to parasite clearance from the placenta in multiparae though not in primiparae (7). It is not known if aPL concentrations are associated with ABO blood groups but they be related components of the parity-specific immunity to placental malaria.

The aim of the present study was to assess the association of ABO phenotype and malaria-related outcomes in pregnant urban Ghanaian women living under conditions of holo-endemic malaria transmission. A further objective was to assess the association between ABO blood groups and aPL antibodies.

Methods

Study Design

A cross-sectional survey was carried out between April and June 2003 at the labour unit at the Komfo Anokye Teaching Hospital, Kumasi, Ghana. Kumasi is the second-largest city in Ghana, with a population of 1.2 million (8). Located in the Ashanti region of central Ghana, the climate is semi-humid tropical, with peak rainfall between April and June and an intense perennial malaria transmission, with the predominant parasite being *P. falciparum*. 

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The target population was gravid women delivering at the hospital during the study period and their babies. The current study was a component of an analysis of transplacental transfer of maternal measles antibody (MMA) in a malarial environment, on which the sample size calculation was based (9).

**Enrolment of Subjects**
Gravid women who delivered vaginally and consented to be enrolled were consecutively recruited at delivery in the labour ward. Those with hypertension (essential or pregnancy induced), twin delivery, or who had received a blood transfusion less than 24 h before delivery were excluded. At enrolment, basic demographic data and antenatal care were documented on a pre-prepared questionnaire. Information was obtained from each patient's antenatal health card, if available; participants who did not have such a card were questioned directly. Women were weighed on an electronic balance to the nearest 0.1kg. Mid-upper arm circumference (MUAC) was measured at the mid-point of the left arm using a paper tape measure.

Only babies delivered alive after the 24th week of gestation (assessed using a modified Ballard Score (10)), and whose mothers gave consent, were recruited. Shortly after delivery, each baby was weighed on an electronic balance, and crown-heel length measured on an infantometer. The readings of each parameter were obtained and values recorded to the nearest 0.05kg and 0.5cm respectively. Each baby was examined and classified as appropriate, small or large for gestational age. Ponderal index was calculated from weight/length3 (Rohrer’s index).

**Collection of Specimens**
Maternal blood (5 mL) was obtained from a peripheral vein within 4 h of delivery. Cord blood (8 mL) was collected from a large vein on the fetal side of the placenta immediately after delivery. Sera were stored at -70°C within 8 h of collection, until assayed. Placental tissue samples (1cm3) were obtained from an off-centre position and stored in 20 mL of 10% formaldehyde in phosphate buffer until processed for histological examination. The placenta was weighed on an electronic balance to the nearest 0.05kg, after removing blood clots and cutting the cord close to its insertion (2-3cms).

**Malaria Diagnosis**
Paraffin-embedded sections (5µm thick) of the placental biopsy specimens were stained with hematoxylin-eosin and examined under light microscopy (×40) and under polarized light at the Pathology Department, University of Barcelona. Placental malaria infection was defined by the presence of parasites, malaria pigment and by the histological features of malaria. Classification was into non-infected, acute infection, chronic infection and past infection as previously described (11, 12). In subsequent analyses, active infection included both acute and chronic infection. Thick and thin Giemsa-stained films were prepared with blood obtained from the cord and examined under light microscopy (x100) for malarial parasites at the laboratory in Komfo Anokye Teaching Hospital, and the number and species of parasites were measured against 200 white cells in the standard manner. A negative count was recorded if no parasites were seen in 100 fields from each blood film.
Haematology
Maternal and cord haemoglobin concentrations were measured with the HemoCue® (Angelholm, Sweden). ABO blood groups were typed by agglutination using commercial antisera (Biotech Laboratories Ltd., Ipswich, Suffolk, UK).

HIV Testing
Anonymous HIV testing was carried out on the maternal samples using a similar, but non-quantitative ELISA technique (Genscreen® HIV 1 / 2 version 2, Bio – Rad, France). Subjects who were HIV-positive were withdrawn from the study to avoid confounding of the primary analyses which were of the association between placental malaria, malarial outcomes and ABO blood grouping.

aPL assays
The aPLs, anti-phosphatidylserine (aPS) and anti-cardiolipin (aCL), were obtained from Sigma (Sydney, Australia) and anti-β2 glycoprotein I (aβ2GPI) was purified from normal human plasma as described previously. Antibody screening was conducted using our published methods (7). Concentrations were expressed as multiples of the median (MoM). Total serum immunoglobulin concentrations were measured by laser nephelometery using an Array Protein System (Beckman Coulter, High Wycombe, UK).

Statistical analysis
Data entry was carried out in Excel (Microsoft) and analysis was carried out in STATA (version 9) (Statacorp). Dichotomous variables were assessed with chi-square or Fisher exact tests, with p values less than 0.05 considered statistically significant. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated using logistic regression. Differences between means and standard errors (SE) were assessed by linear regression where data were normally distributed, or the Mann-Whitney/Wilcoxon Test where they were not. Multiple linear regression was used to analyse factors associated with malarial outcomes. Factors included were: maternal blood group (O versus non-O; A versus non-A), primparity and active placental infection. Interaction terms were used to adjust for effect modification in stratified analyses.

Ethical considerations
The study was approved by the ethical committees of the Liverpool School of Tropical Medicine and Kumasi University prior to commencing the fieldwork. All laboratory data, with the exception of haemoglobin measurements made at the bedside were anonymous. Codes linking results to individual patients were erased prior to data entry. Informed consent was taken for each mother in the study, and anonymous HIV testing was specifically discussed.

Results

Patient cohort
During the study, 12 women refused consent or left the hospital before consent could be obtained. In addition, 4 mother–infant pairs were excluded because of maternal hypertension, 2 pairs because of congenital abnormalities and 2 pairs because of sampling failure. It is estimated that approximately 12% of the deliveries occurring during the day were not recruited for the study, because of logistical difficulties. This
did not reflect any deliberate selection bias. Complete data sets were not available for all deliveries. We excluded the data from 3 HIV-positive women in order to avoid possible confounding from another maternal infection impacting on placental malaria susceptibility and on fetal growth through the immunosuppressive effects of the virus. Of 123 HIV-negative women recruited for the study, blood group and histological data were available for 97.

Mean maternal age in this sample was 26.4 years (SE +/-0.66) and 38 (39%) were primiparae (multiparity refers to all parities greater than one). Mean neonatal gestational age was 38.4 weeks (+/- 0.37), and 20 (21%) neonates were born preterm (<37 weeks gestation). Mean birth weight was 3031 g (+/- 46) and 6 (6.2%) neonates had low birthweight (<2500 g). Most women (97%) had documented attendance for at least one antenatal clinic.

Evidence of malaria infection was present in 32 placentas (33%) and there were no cases of cord parasitaemia. Active infection occurred in 17 (53%) and past infection in 15 (47%). There were 19 (50%) primiparae with placental malaria (all categories) compared with 13 (22%) multiparae (OR 3.54; 95% CI 1.46 – 8.57; p=0.005). Over half of the subjects (52%) reported using antimalarial medication during pregnancy.

The male/female ratio was 1.02.

**ABO blood group phenotypes and malaria infection**

Table 1 summarises the data on ABO phenotype, placental malaria and parity. There was no association between blood groups and malaria infection (all infection) within the sample as a whole ($\chi^2$ 2.36; p=0.501).

Among primiparae, blood group A was associated with more placental infections than the other blood groups combined (OR 6.18; 95% CI 1.10 – 34.70; p=0.038). There was no association between blood group AB (OR uncalculated; n=1), blood group B (OR 0.21; 95% CI 0.02 – 2.07; p=0.181) or blood group O (OR 0.65; 95% CI 0.18 – 2.37; p=0.512) and placental malaria.

Among multiparae there was no association between placental malaria and blood group A (OR 0.38; 95% CI 0.07 – 1.91; p=0.239), blood group AB (OR 1.19; 0.11 – 12.54; p=0.882), blood group B (OR 0.75; 95% CI 0.14 – 3.98) or blood group O (OR 2.27; 95% CI 0.64 – 8.03; p=0.202).

**Impact of parity, placental malaria and ABO blood group phenotype on maternal and neonatal outcomes**

Recognised malarial parameters are summarised in relation to parity, malaria infection and maternal ABO blood group phenotypes in table 2. After adjusting for the effects of active placental malaria infection and parity, there was a trend for reduced placental weight (p=0.062) and greater feto-placental weight ratio (p=0.070) with blood group O. There was no association between blood group A and any of the malarial parameters under investigation (data not shown), adjusting for the effects of active placental malaria infection and effect modification by parity.
Impact of parity, placental malaria and ABO blood group phenotype on maternal anti-phospholipid antibody profiles

Table 3 summarises aPL concentrations in relation to parity, malaria infections and maternal ABO blood group phenotypes. After adjusting for the effects of active placental malaria infection and parity there were no associations between any maternal blood group and maternal aPL concentrations.

Discussion

In the current study blood group A was associated with increased prevalence of placental malaria in primiparae only but had no measurable effect on malarial outcomes. Blood group O was not associated with placental malaria infection but was associated with a trend for smaller placentas and higher feto-placental weight ratio, after controlling for active placental malaria infection and parity. There was no association of blood group phenotype and parity-specific aPL profiles which we previously described in placental malaria (5, 6).

In our original study from The Gambia, the OR for active placental parasitaemia among primiparae with blood group O was 3.0 (95% CI 1.2 – 7.3) but was 0.8 (95% CI 0.3 – 1.7) among multiparae (5). Placental parasitaemia occurred at least twice as frequently in primiparae but only among blood group O women. This effect of parity, one of the cardinal features of placental malaria, was not observed in non-O phenotypes for any of the placental histological types of infection. Similarly to the current study, blood group O was associated with higher mean feto-placental weight ratios in multiparae compared with non-O phenotypes. This can be interpreted as evidence of an association between blood group O and the well-described parity-specific protective immunity in placental malaria.

In the largest study yet published on this topic (n=647), primiparae from Malawi with blood group O were at increased risk of active placental malaria compared to those with non-O phenotypes (OR 2.18, 95% CI 1.05 – 4.55) (6), while among multiparae the O-phenotype was associated with less frequent active placental infection compared with non-O phenotypes (OR 0.59, 95% CI 0.36 – 0.98), together with higher newborn ponderal indices. The consistency of the findings across the two studies from The Gambia and Malawi suggested that the ABO interaction with placental malaria affects placental and fetal growth.

In Eastern Sudan, an area of unstable malaria transmission, women with blood group O were reported at higher risk of past placental malaria infection but had higher haemoglobin concentrations than women with non-O phenotypes, regardless of parity (13). Other birth outcomes were not different between groups. In a later study conducted in the same area, no association between blood group phenotypes and placental malaria was found (14).

In Gabon, the association between ABO blood groups and active placental malaria was investigated and compared directly with the findings from the other published studies described above (15). The OR for placental malaria comparing mothers from Gabon with blood group O to non-O blood groups was 0.3 (95% CI 0.05 – 1.8) for primigravidae and 0.7 (95% CI 0.3 – 1.8) for multigravidae. However this study used placental blood smears for diagnosis rather than placental histology, as was used in
the previous studies. This precluded detection of past malaria infection and the overall rate of detected infection was much lower at 7.1%. In addition there was no parity difference in the prevalence of placental parasitaemia, which is unusual for African studies conducted under perennial malaria transmission. It is likely this study underestimated the number of active infections in which chronically parasitised red cells were adherent to the syncytiotrophoblast. The results from Gabon should therefore be interpreted cautiously as the statistical power was low and the confidence intervals of the main analyses were wide.

A meta-analysis was performed from the 3 studies which had published adequately detailed data and which were conducted in hyper-/holo-endemic settings with stable transmission (The Gambia (5), Malawi (6) and Gabon (15), using a random effects model. The meta-analysis suggested that blood group O offered some protection against placental malaria in multiparae (combined OR 0.65, 95% CI 0.44 – 0.96) (15). In primiparae, the opposite effect was noted but the findings were not statistically significant. These estimates could not be adjusted for other possible confounding variables such as maternal age or gestational duration. There was also wide variation in prevalence of placental malaria, primiparity and blood group O across these studies. In addition diagnosis of placental malaria was not standardised. Meta-analyses of a small number of small studies using various methodologies and from heterogenous settings may be further weakened by the ‘file-drawer’ phenomenon, whereby studies which do not show an effect in the expected direction are not published for one reason or another. It is important that other well-controlled data relating to the same variables, such as those from the current study, are also published in order to minimise this bias.

In the only other published study we could find, blood group O was reportedly more common among women in south-eastern Nigeria with placental parasitaemia than among women with no placental parasites although placental histology was not examined and no effect size was reported (16).

Our current study was small and lacked power to examine malarial outcomes precisely. It is in agreement with three other studies (13-15) which reported no association between blood group O and placental infection, in contrast to the findings from our original studies in The Gambia (5) and Malawi (6), and replicated in Nigeria (16). The findings are consistent with observations in non-pregnant individuals in whom blood group O has been associated with reduced risk of severe clinical malaria, and blood group A with increased risk (3).

The binding of parasitised red cells to non-infected red cells in peripheral blood classically results in rosette formation, which has been associated with severity of clinical malaria in children and with the blood group A phenotype (17). Rosettes are not observed in placental malaria but impaired rosette formation may be only one mechanism by which individuals with the blood group O phenotype and other red cell polymorphisms demonstrate greater innate resistance to malaria.

Assuming that the exposure rate of P. falciparum infection is comparable across parities, then enhanced parity-specific immunity among multiparae with blood group O phenotype and improved indices of feto-placental growth must relate to improved parasite clearance from the placenta. A key feature of pregnancy-associated malaria is
parasite sequestration in the placenta mediated by cytoadherence of infected red cells expressing the unique variant surface antigen VAR2CSA to the placental syncytiotrophoblast, which expresses the glycosaminoglycan chondroitin sulphate A (CSA) (4). Infected red cells can also bind to the proteoglycan thrombomodulin, present on endothelial cells and placental syncytiotrophoblast, via CSA side chains (18). Soluble adhesion molecules and endothelial markers (including von Willebrand factor and E-selectin) are associated with ABO phenotypes, especially thrombomodulin, which is found in lower concentrations in blood from group O individuals (19).

Autoantibodies including aPL are associated with infections and the pathogenesis of certain pregnancy complications. In a previous study we observed β2GPI-independent IgM antibodies to cardiolipin (p = 0.018) and phosphatidylserine (p= 0.009) in multiparae, which were most concentrated in past placental malaria infection. Trends for improved clinical parameters in infected women with levels of aCL beyond the 99th multiple of the median for a healthy, non-malarious population were also observed (7). In the current study we found no association between ABO blood group and aPL suggesting that the phenotypes are not closely-related components in the mechanism of parity-specific immunity to placental malaria.

It is possible that the interactions of ABO blood group phenotypes and placental malaria could relate to other mechanisms affecting P. falciparum infection such as the biting-rate by Anopheles gambiae (20) or antigen-sharing between the parasite and ABO phenotypes leading to modulated immune responses (21). ABH antigens have been described in the O-glycans of glycophorin A (GPA), an important determinant of successful parasite penetration into the red cell, mediated through GPA-sialic acid (22). ABO phenotypes differ in sialic acid content and composition, with group O showing the highest membrane content, but a lower percentage of sialoglycoproteins, which may indicate that blood group O-bearing red cells are easier for the parasites to invade but harder to manipulate into cytoadherence (23). We have shown previously that placental expression of α2,6-linked sialic acid is upregulated in placental malaria and could form part of the immune response to parasitisation (24). The lectin histochemistry of the human placenta has not been reported in relation to maternal ABO phenotypes.

It has been estimated that 40% of women living in Sub-Saharan Africa are exposed to malaria infection during pregnancy (25). Existing public health interventions can only achieve a partial reduction in the health hazards associated with placental malaria. In this context, greater understanding of host susceptibility to placental infection with P. falciparum is the basis for improved control. ABO blood groups and related cell surface glycans which are already known to have a particular relevance in reproductive biology could also be important in malarial pathophysiology via cellular adhesion and other interactions. Such findings may help to define particular groups of women and babies at higher risk from malaria in pregnancy, and inform future host-parasite studies in immunology and pathogenesis.
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Abbreviations

aβ2GPI: anti-glycoprotein I
daCL: anti-cardiolipin
daPS: anti-phosphatidylserine
daPL: anti-phospholipids
CI: confidence interval
HIV: human immunodeficiency virus
CSA: chondroitin sulphate A
GPA: glycophorin A
MoM: multiples of the median
OR: odds ratio
SE: standard error

Competing interests

None declared

Authors’ contributions

SO conceived and designed the original study, collected the samples and demographic data, analysed the results and wrote the first draft of the manuscript. MPL conceived the current study, suggested the analysis plan and revised the manuscript. JO supervised the placental histology and revised the manuscript. BB supervised the original study and edited the final manuscript.

Acknowledgements

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References

Table 1: ABO phenotype by placental malaria category and parity group

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<th>Placental malaria</th>
<th>Phenotype n (%)</th>
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<tr>
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<td>Type</td>
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<tr>
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<td>Past</td>
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<td>46 (78)</td>
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<td></td>
<td>Total</td>
<td>59 (100)</td>
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<table>
<thead>
<tr>
<th>Parity</th>
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<th>Blood group</th>
<th>n</th>
<th>n (%)</th>
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<tr>
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<td>Active</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Primiparae</td>
<td></td>
<td>O</td>
<td>6</td>
<td>93 (5.6) 2750 (112) 2.26 (0.06) 442 (24) 6.27 (0.20) 0.62</td>
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<td></td>
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<td>Non-O</td>
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<td>114 (8.1) 2850 (119) 2.55 (0.06) 538 (66) 5.53 (0.57) 1.18</td>
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<tr>
<td></td>
<td>Past or no infection</td>
<td>O</td>
<td>16</td>
<td>110 (3.1) 2900 (145) 2.46 (0.08) 481 (25) 6.12 (0.31) 1.17</td>
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<td></td>
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<td>Non-O</td>
<td>12</td>
<td>109 (6.0) 2700 (137) 2.37 (0.06) 463 (23) 5.98 (0.40) 0.83</td>
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<tr>
<td></td>
<td>Active</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Multiparae</td>
<td></td>
<td>O</td>
<td>4</td>
<td>96 (4.6) 2800 (108) 2.29 (0.15) 525 (52) 5.48 (0.52) 1.00</td>
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<td>23</td>
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<td>Non-O</td>
<td>29</td>
<td>118 (2.7) 3190 (81) 2.54 (0.06) 544 (21) 6.83 (0.22) 1.03</td>
</tr>
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*Expressed as geometric mean
§ p=0.062
§§ p=0.070

Table 2: Pregnancy outcomes by parity, malaria infection and maternal blood group phenotypes

<table>
<thead>
<tr>
<th>Parity</th>
<th>Placental malaria</th>
<th>Blood group</th>
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<th>Malarial parameters: Mean (SE)</th>
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<td>Maternal Hb (g/L)</td>
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<tr>
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<td>O</td>
<td>6</td>
<td>93 (5.6)</td>
</tr>
<tr>
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<td></td>
<td>Non-O</td>
<td>4</td>
<td>114 (8.1)</td>
</tr>
<tr>
<td></td>
<td>Past or no infection</td>
<td>O</td>
<td>16</td>
<td>110 (3.1)</td>
</tr>
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<td></td>
<td></td>
<td>Non-O</td>
<td>12</td>
<td>109 (6.0)</td>
</tr>
<tr>
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<td>Active</td>
<td>O</td>
<td>4</td>
<td>96 (4.6)</td>
</tr>
<tr>
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<td></td>
<td>Non-O</td>
<td>3</td>
<td>126 (26.0)</td>
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<tr>
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<td>Past or no infection</td>
<td>O</td>
<td>23</td>
<td>117 (3.8)</td>
</tr>
<tr>
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<td></td>
<td>Non-O</td>
<td>29</td>
<td>118 (2.7)</td>
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Table 3: Anti-phospholipid antibody profiles by parity, malaria infection and maternal blood group phenotypes

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<tr>
<th>Parity</th>
<th>Placental malaria</th>
<th>Blood group</th>
<th>n</th>
<th>Median (interquartile range) aPL concentration, expressed as MoM</th>
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<td></td>
<td>aCL IgG</td>
</tr>
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<td>Active</td>
<td>O</td>
<td>6</td>
<td>1.7 (1.0-1.8)</td>
</tr>
<tr>
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<td></td>
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<td>4</td>
<td>1.35 (1.3-1.95)</td>
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<td>16</td>
<td>1.6 (1.0-2.4)</td>
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<td>12</td>
<td>1.5 (1.1-1.6)</td>
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<tr>
<td>Multiparae</td>
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<td>4</td>
<td>1.95 (1.55-2.25)</td>
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<tr>
<td></td>
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<td>23</td>
<td>1.15 (1.0-1.7)</td>
</tr>
<tr>
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<td></td>
<td>Non-O</td>
<td>29</td>
<td>1.3 (0.8-1.7)</td>
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</tbody>
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