Antibodies to variant surface antigens of Plasmodium falciparum infected erythrocytes associated with protection from treatment failure and development of anaemia in pregnancy

Gaoqian Feng[1], **Elizabeth Aitken**[1], **Francisca Yosaatmadja**[1], **Linda Kalilani**[2], **Steven R Meshnick**[3], **Anthony Jaworowski**[4,5], **Julie A Simpson**[6], and **Stephen J Rogerson**[1,*]

[1] Department of Medicine (RMH/WH), University of Melbourne, Parkville VIC 3052 Australia

[2] College of Medicine, University of Malawi, Blantyre Malawi

[3] Departments of Epidemiology and Microbiology and Immunology, University of North Carolina, Chapel Hill NC USA

[4] Centre for Virology, Burnet Institute, Melbourne VIC 3004, Australia

[5] Dept. of Medicine, Monash University, Clayton VIC

[6] Centre for Molecular, Environmental, Genetic & Analytic Epidemiology, School of Population Health, University of Melbourne, Parkville VIC 3052 Australia

Abstract

Background—In pregnancy associated malaria (PAM), *Plasmodium falciparum* infected erythrocytes (IEs) express variant surface antigens (VSA-PAM) that evade existing immunity and mediate placental sequestration. Antibodies to VSA-PAM develop with gravidity and block placental adhesion or opsonise IEs for phagocytic clearance, protecting women from anemia and low birth weight

Methods and findings—Using sera from 141 parasitemic pregnant Malawian women enrolled in a randomized trial of antimalarials and VSA-PAM-expressing CS2 IEs, we quantitated levels of IgG to VSA-PAM by flow cytometry and opsonizing antibodies by measuring uptake of IEs by THP1 promonocytes. After controlling for gravidity and antimalarial treatment, IgG against VSA-PAM was associated with decreased anemia at delivery (OR=0.66, 95% confidence interval [CI] 0.46, 0.93; P=0.018) and weakly associated with decreased parasitological failure (OR=0.78; 95% CI, 0.60, 1.03; P=0.075), especially re-infection (OR=0.73; CI, 0.53,1.01; P=0.057). Opsonizing antibodies to CS2 IE were associated with less maternal anemia. (OR=0.31, 95% CI, 0.13, 0.74; P=0.008) and treatment failure (OR=0.48; 95% CI, 0.25, 0.90; P=0.023), primarily due to recrudescent infection (OR=0.49; 95% CI, 0.21, 1.12; P=0.089).

Conclusion—Both IgG antibody to VSA-PAM and opsonizing antibody, a functional measure of immunity correlate with parasite clearance and less anemia in pregnancy malaria.

INTRODUCTION

Globally, 247 million people are infected with malaria every year[1], which causes 881,000 deaths annually. Pregnant women have an increased risk of *Plasmodium falciparum* infection which is maximal in the first and second pregnancy [2]. Maternal malaria infection occurs

*Corresponding author: A/Prof Stephen J Rogerson, University of Melbourne Department of Medicine,, Post Office Royal Melbourne Hospital, Parkville VIC 3050, Australia, T +61 3 8344 3259; F +61 3 9347 1863; sroger@unimelb.edu.au.

partly because infected erythrocytes (IEs) accumulate in the placenta [3]. Studies suggest that the *var2csa* variant of *P. falciparum* membrane protein 1 (PfEMP1) is the key protein which mediates this accumulation [4].

Women acquire immunity to pregnancy associated malaria (PAM) by generating antibodies against PAM variant surface antigens (VSA-PAM) in a gravidity dependent manner [5–8]. The level of PAM-specific antibodies remains low before their first or even second pregnancy and increases significantly with increased gravidity. These antibodies have been associated with protection from maternal malaria and its consequences in subgroups of pregnant women [5, 9,10]. This protection may result from blocking binding of IEs to chondroitin sulfate A (CSA) on syncytiotrophoblasts in the placenta [5,8,11], or from promoting clearance by opsonic phagocytosis of IE in the peripheral blood and the placenta [12–14]. Levels of opsonizing antibodies are correlated with levels of PAM specific IgG [12], but their relationship to clinical outcomes is unknown.

Host immunity against malaria is believed to be an important factor in malaria treatment success [15], and studies in children or non-immune adults have demonstrated associations between specific measures of immunity to malaria, most commonly levels or titres of IgG to defined antigens measured by ELISA, and treatment outcome [16–21]. Such studies are lacking in pregnant women.

Prevention of malaria in pregnancy in Africa still relies on sulphadoxine-pyrimethamine (SP), but parasite resistance leads to treatment failures in children [22]. Beneficial effects of SP are seen in pregnant women, even where there are moderate levels of pediatric treatment failure [23]. We hypothesized that immunity to VSA-PAM, and in particular levels of antibodies that opsonise IE for phagocytic clearance, could be important components of the acquired maternal immune response involved in clearing infection and protecting pregnant women from treatment failure and adverse pregnancy outcomes.

In the present study we compared a recently developed assay for VSA-PAM specific opsonic activity with flow cytometry measurements of total IgG to VSA-PAM to measure antibody in sera collected from parasitemic Malawian women in mid pregnancy. Antibody levels with each assay were examined as predictors of clinical outcomes including treatment success, maternal anemia at delivery and birth weight.

METHODS

Study population

141 serum samples were collected during a randomized clinical trial of antimalarials for treatment of parasitemia in pregnancy, conducted at Mpemba and Madziabango Health Centers in Blantyre District, Malawi from September, 2003 to September, 2004 [24]. Women 14–26 weeks pregnant, with parasitemia on peripheral blood film, were eligible to participate whether or not they had symptoms. Participants were randomly assigned to SP (3 tablets; 500 mg sulfadoxine and 25 mg pyrimethamine per tablet); SP plus azithromycin (1 g/day for 2 days) or SP plus artesunate (200 mg/day for 3 days) treatment groups. All participants received 2 doses of drug treatment irrespective of whether or not they experienced recurrence of parasitemia. Participants' general demographic information and malaria infection history were collected together with blood samples at time of enrolment. All the participants were followed up until delivery. At delivery, infant birth weight and mothers' and infants' hemoglobin concentrations were recorded. Anemia was defined as maternal hemoglobin lower than 11 g/ dl and low birth weight was defined as infant's birth weight lower than 2500 g. Parasitological treatment failure was defined as a further episode of parasitemia from the 7th day after treatment till the end of study, and Heteroduplex Tracking Assays were performed to distinguish

recrudescence (isolation of genetically identical parasites at a subsequent time point) from re-infection (isolation of novel parasite types not seen on enrolment), based on polymorphisms in the merozoite surface protein 1 (msp-1) gene [25].

Culture of Plasmodium falciparum

The laboratory adapted *P. falciparum* line CS2 which expresses *var2csa* and binds to CSA was cultured in RPMI-HEPES with 0.5% Albumax and 0.18% $NaHCO_3$. A second *P. falciparum* line, E8B-ICAM, which binds to ICAM-1 was cultured in RPMI-HEPES with 0.25% Albumax, 5% heat-inactivated human serum and 0.18% $NaHCO_3$. All parasites were synchronized by gelatin selection weekly [26].

Culture of THP1 cells

THP1 cells were maintained in RPMI-1640 with 0.002 mol/L L-glutamine 1.5 g/L sodium bicarbonate, 0.01 mol/L HEPES, 5×10^{-5} mol/L 2-mercaptoethanol, and 10% fetal bovine serum. Cell density was monitored closely between 1×10^5 and 1×10^6 cell/ml. Cells were passaged every 6 days, when cell density approached 1×10^6 cells/ml.

Flow cytometry

In vitro cultured IEs were used at 4% to 8% parasitemia to quantify total level of IgG against VSA expressed on the surface of erythrocytes infected with CS2 or E8B-ICAM parasite lines. After washing with 0.1% newborn calf serum (NCS) in phosphate buffered saline (PBS), IEs were resuspended at 0.1% haematocrit in 0.1% NCS in PBS and incubated with individual serum samples at 1:20 (v/v) for 30 minutes. Then IEs were washed three times with 0.1% NCS in PBS and incubated with Polyclonal Rabbit Anti-Human IgG (Dako A0424) at 1:100 dilution for 30 minutes, followed by three washes and incubation with Alexa Fluor 488 donkey anti-rabbit IgG (Molecular Probes A-21206) at 1:500 dilution plus 10 μg/ml ethidium bromide (EtBr) for 30 minutes. Using a FACScalibur with CELLQuest software, two thousand IEs were counted, and geometric mean of fluorescence intensity was recorded. Pooled sera from Malawian hyperimmune multigravid women were used as a positive control, while the negative control was pooled sera from malaria-naive Melbourne donors. Both positive and negative controls were included in each assay. Adjusted mean fluorescence intensity (MFI) was calculated by subtracting the MFI in channel FL1 of the EtBr negative cell population from that of the EtBr positive cell population. Finally we used the relative level of IgG compared to the positive control (relative MFI) as the indicator of total IgG level.

Opsonic phagocytosis

We adapted previously published methods to examine phagocytic uptake of opsonised IEs [13,27]. THP1 cells were plated into 96-well plates at 5×10^4 cells/well in THP1 culture medium plus 1×10^{-7} mol/L phorbol myristate acetate (PMA) for 12–24 hours to allow differentiation to adherent macrophage-like cells. This was replaced with culture medium without PMA on the second day and cells were incubated for a further 2–3 days before use.

Trophozoite-stage CS2 IEs at ≥ 8% parasitemia were purified by percoll gradient centrifugation to 90–95% parasitemia, followed by 3 washes in fetal calf serum (FCS) coated tubes and resuspended in cold PBS. 2.25 μl of heat-inactivated patient sera were incubated with 8×10^6 IEs for 1 hour at room temperature for opsonization. Unbound serum components were removed by 3 washes with PBS and 1×10^6 IEs were added to wells containing THP1 cells in quadruplicate and incubated for 2 hours to allow phagocytosis. After incubation, 100 μl cold PBS was added to each well to stop phagocytosis, followed by incubation for 3 min in 100 μl 0.2% NaCl to lyse non-phagocytosed IEs. The wells were washed 4 times with warm THP1 culture media to remove lysed IEs. THP1 cells and phagocytosed IEs were lysed by adding

100 μl 0.2 mol/L Tris-HCl, 6 mol/L urea for 30 minutes to release hemoglobin. After lysing the cells, 100μl 1 mg/ml 2,7-diaminofluorene (Sigma D17106–1G) with 0.3% hydrogen peroxide was added into each well, and hemoglobin release was measured spectrophotometrically at 620 nm. Standard curves were constructed in triplicate using unopsonized IEs from the same culture. Eleven 2-fold serial dilutions were made from a starting concentration of 2.5×10^5 unopsonized cells. The standard curve was used to estimate the number of ingested erythrocytes from the amount of hemoglobin released which was then converted to a phagocytic index of ingested erythrocytes per 100 macrophages. The same pooled serum from multigravid Malawian pregnant women described above was used as a positive control. Relative Phagocytosis Index (relative PI) was calculated for each individual serum sample as the percentage of phagocytic index obtained using the positive control pooled serum.

Statistical analysis

Data were entered into Microsoft EXCEL and analyzed using Stata 9.2 (StataCorp, College Station, Texas, USA). Total level of IgG (relative MFI) and total level of opsonic antibodies (relative PI) were log to the base 2 transformed. The associations between relative MFI or relative PI and gravidity were estimated using linear regression. To investigate the association between immunity against PAM and malaria treatment outcome, maternal anemia and infants' birth weight, relative MFI against CS2 and E8B-ICAM and relative PI at enrollment were defined as exposure variables. Recrudescence, re-infection, parasitological failure, maternal anemia at delivery and infant low birth weight were defined as outcome variables. Maternal gravidity which was classified as first pregnancy and second or subsequent pregnancies was considered as a confounding factor, as was participant's treatment group. Multiple logistic regression was performed to investigate the association between exposure and outcomes variables with adjustment for gravidity and treatment group.

Ethics

Ethics approval was obtained from the College of Medicine Research Ethics Committee, University of Malawi and from the Melbourne Health Human Research Ethics Committee.

RESULTS

Summary of study population

Serum samples were tested from 141 pregnant women with a mean age of 21.2 years (SD = 4.7). Characteristics of the study population are listed in Table 1. 47 participants were assigned to SP mono-therapy group; 47 participants were assigned to the SP plus azithromycin group; 47 participants were assigned to the SP plus artesunate group.

Relative MFI against CS2 IE increased with gravidity. Linear regression showed that the relative MFI against CS2 IEs was higher in secundigravidae (ratio of geometric means=2.01; 95% CI: 1.17, 3.43 P=0.012) and multigravidae (ratio of geometric means =2.62; 95% CI: 1.82, 3.78; P<0.001) compared with primigravidae. In contrast, the relative MFI against E8B-ICAM did not differ between the gravidity groups (Figure 1A, 1B).

Relative PI was similar between primigravid and secundigravid women (ratio of geometric means = 1.05; 95% CI: 0.84, 1.32; P=0.680), however, it was significantly higher in multigravid women compared to primigravid women (ratio of geometric means = 1.64; 95% CI: 1.39, 1.91; P<0.001) (Figure 1C). There was a moderate correlation between relative PI and total IgG against CS2 IEs (R = 0.56, P<0.001).

Immunity against PAM: Association with malaria treatment outcome

Both relative MFI against CS2 IEs and relative PI were associated with measures of treatment outcome. The associations between relative MFI against CS2 IEs or relative PI and parasitological failure are shown in Table 2, Table 3 and Figure 2. Every two fold increase in relative MFI against CS2 IEs, which is the indicator of total level of IgG against VSA-PAM, was associated with a 22% reduction in the odds of parasitological failure after malaria treatment (OR=0.78; 95% CI: 0.60, 1.03; P=0.075). A similar, but stronger association was observed between relative PI and parasitological failure (OR=0.48; 95% CI: 0.25, 0.90, P=0.023).

When parasitological failures were classified as recrudescence or re-infections [25], relative MFI against CS2 IEs was associated with both recrudescence and re-infection at a marginally significant level (Table 2). Every two fold increase in relative MFI against CS2 IEs was associated with a 25% decrease in the odds of recrudescence (OR=0.75; 95% CI: 0.56, 1.01; P=0.057) and a 23% decrease in the odds of re-infection (OR=0.77; 95% CI: 0.57, 1.03; P=0.083). After adjustment for gravidity and treatment group, the relationship with recrudescence was no longer significant, but there remained a marginally significant relationship with risk of re-infection (OR=0.73, 95% CI: 0.53, 1.01; p=0.057). Relative PI was more strongly associated with the risk of recrudescence (Table 3). For each 2 fold increase in relative PI there was a 59% decrease in the odds of recrudescence (OR=0.41; 95% CI, 0.20, 0.85; P=0.016). After adjustment for gravidity and treatment, the magnitude of the effect attenuated slightly (OR=0.49, 95% CI: 0.21, 1.12, p=0.089).

Immunity against PAM: Association with maternal anemia and infants' low birth weight

Host immunity against PAM was associated with risk of maternal anemia at delivery. Both relative MFI against CS2 IEs and relative PI were significantly associated with a decrease in the risk of maternal anemia. As shown in table 2 and figure 3, a 2 fold increase in relative MFI against CS2 IEs was associated with 36% decrease in the odds of maternal anemia (OR=0.64; 95% CI: 0.46, 0.88; P=0.007). Relative PI was more strongly associated with a decreased odds of anemia (figure 3). Logistic regression showed that a 2 fold increase in relative PI was associated with 70% reduction in the odds of maternal anemia (OR=0.30; 95% CI: 0.13, 0.68; P=0.004). These associations remained highly significant after adjusting for gravidity and treatment group (table 3).

Levels of IgG to E8B-ICAM were not associated with parasitological failure (OR=0.99 95%: CI 0.75, 1.30; P=0.935) or maternal anemia (OR=0.94; 95% CI 0.67, 1.32; P=0.714). Relative MFI and relative PI against CS2 IEs and relative MFI against E8B-ICAM IEs were not associated with infant birth weight, or the risk of low birth weight (<2500 g) delivery.

DISCUSSION

We measured levels of total IgG to VSA-PAM (relative MFI against CS2 IEs) and opsonic antibodies against VSA-PAM (relative PI) in sera from parasitemic pregnant women, and related antibody measures to treatment and pregnancy outcomes. As in previous studies, we found that antibody-mediated immunity against VSA-PAM was generated in a gravidity dependent manner [5,7,8,12,28,29], whereas antibody to VSA of the parasite line E8B-ICAM was not.

Both opsonizing antibodies and total IgG to VSA-PAM were associated with treatment outcome and with a lower prevalence of maternal anemia at delivery, but the relationship between opsonizing antibody levels and treatment outcome or maternal anemia was stronger than that between total IgG to VSA-PAM and outcome, suggesting that measurement of the

opsonizing function of antibodies may be a more specific assay of the protective effect of IgG in patient sera. These data provide the first evidence that antibodies against VSA-PAM expressed on the surface of CSA-binding IEs may be important determinants of malaria treatment outcome in pregnant women; moreover, they may be useful predictors of anemia as a consequence of such infection.

Outcome of malaria treatment varies with age [15], and relatively more pregnant women than children clear infection when treated with a partially effective drug [30]. Primigravid women, who are at highest risk of malaria in pregnancy, are more likely to fail drug treatment than multigravidae [31]. Our data suggest that this may be attributable, at least in part, to the lower levels of antibody to VSA-PAM found in primigravidae [8,12].

Antibodies of the cytophilic subclasses IgG1 and IgG3 can opsonise infectious agents for phagocytosis, and IgG1 and IgG3 responses to VSA-PAM develop in a gender and parity dependent manner [28,29]. The Fc domains of cytophilic antibodies bind to Fc receptors expressed on the surface of macrophages followed by phagocytic clearance. Decreased antibody to VSA-PAM may partly explain susceptibility of HIV infected pregnant women to malaria[32], and Keen et al recently reported that HIV infection significantly decreases opsonic activity and levels of IgG1 and IgG3 directed against VSA-PAM in pregnant Kenyan women. [12] Together with our observations that opsonizing antibodies are important predictors of treatment outcome and are associated with protection from anemia, this suggests that opsonic activity is a biologically important function of PAM-specific antibodies in addition to their ability to prevent placental sequestration.

Antibodies against maternal malaria could block IEs adhering to CSA preventing placental sequestration [5,9,10], or they may opsonise IEs and promote phagocytic clearance [12,13]. We chose not to test sera for adhesion-blocking antibodies in this study, because in a separate cohort we found weaker correlations between assays of these antibodies and either HIV serostatus or pregnancy outcomes [33]. It would nevertheless be of interest to compare different assays, including assays directly measuring antibody to VAR2CSA protein, the dominant VSA-PAM (reviewed in [6,34]), in longitudinal studies such as this.

The optimal format for assays of opsonizing activity has yet to be resolved. Keen et al assayed opsonizing antibody using thioglycollate elicited mouse macrophages or human monocyte derived macrophages and direct counting of ingested cells by microscopy [12]. We used PMA-primed THP1 human promonocytic cells and adapted a published protocol [13] to give a spectrophotometric measure of ingested erythrocytes using a 96 well plate format [27,35], allowing increased throughput, necessary for clinical studies. Primary human macrophages are most relevant to the in vivo situation, but inter-host variability in phagocytic activity [36] makes them less useful for large sample sets, because of the need to use cells from multiple donors. We are examining protocol modifications that might allow sample sets of 100–200 sera to be tested using the same cells. In each case, it is unlikely that other host serum factors influence assay outcome, as opsonization is followed by extensive washing before opsonized IEs are added to macrophages.

Malaria in pregnancy may cause anemia, which is a risk factor for maternal mortality and morbidity, as well as for low birth weight of the offspring [37]. In Malawi, the risk of anemia at delivery among malaria infected pregnant women decreases with increased gravidity [38]. Maternal anemia is caused by a variety of factors [6,34] but, as our data show, opsonizing antibody at time of study entry was associated with significantly lower risk of anemia at delivery. Every 10 units increase in relative PI against CS2 IEs resulted in approximately 50% decrease in the prevalence of anemia at delivery. A similar, but weaker, relationship was seen between relative MFI against CS2 IEs and anemia. We previously demonstrated a strong

epidemiological association between malaria and mild or moderate degrees of anemia in pregnancy in Malawi [38]. Opsonizing antibodies may facilitate clearance of malaria infection, and remove its effects on red cell destruction and suppression of erythropoiesis [39].

Our data suggest that opsonizing antibodies may also protect pregnant women from recurrence of parasitemia after intermittent preventive treatment, which is recommended by WHO as an important prevention approach against PAM. Moreover, this increased immunity decreases the risk of maternal anemia. Our sample size precluded us from restricting analysis to women of a specific gravidity, as others have done [5,9,10], and we did not find any relationship between either antibody measure and birth weight.

Because our study had a relatively small sample size we could not investigate if the associations between antibody response and the outcome measures were modified by gravidity or treatment. The levels of antibodies to VSA-PAM (and especially of opsonizing antibodies) remained significantly associated with treatment outcome and protection from anemia after controlling for these variables except for relative MFI in association with parasitological failure (Tables 2 and 3). When treatment failures were divided into recrudescences and re-infections, total IgG to VSA-PAM was particularly associated with a decrease in new infections, whereas opsonizing antibody was associated with protection from recrudescence, although this was of borderline significance (OR 0.49, CI 0.21–1.12, p=0.089). Further studies are required to confirm these findings, which suggest that opsonizing antibodies may play a particularly important role in elimination of infection in pregnant women.

HIV is known as an important factor in malaria infection [40,41], however, only 78 (55%) participants were tested for HIV infection. Among this subgroup, HIV infection was not correlate with either measurement of immunity against PAM (data not shown). Thus we did not adjust our results for HIV infection.

In conclusion, opsonic phagocytosis was particularly strongly associated with decreased risk of further episodes of parasitemia, or of maternal anemia at delivery, suggesting that opsonizing antibodies form a key component of immunity to malaria in pregnant women. These potential protective effects suggest that active immunization with vaccines against VSA-PAM that elicit opsonizing antibodies may improve effectiveness of preventive treatment and pregnancy outcomes. Development of simple assays of antibody to VSA-PAM may allow identification of women who are at particular risk of complications of pregnancy malaria.

Acknowledgments

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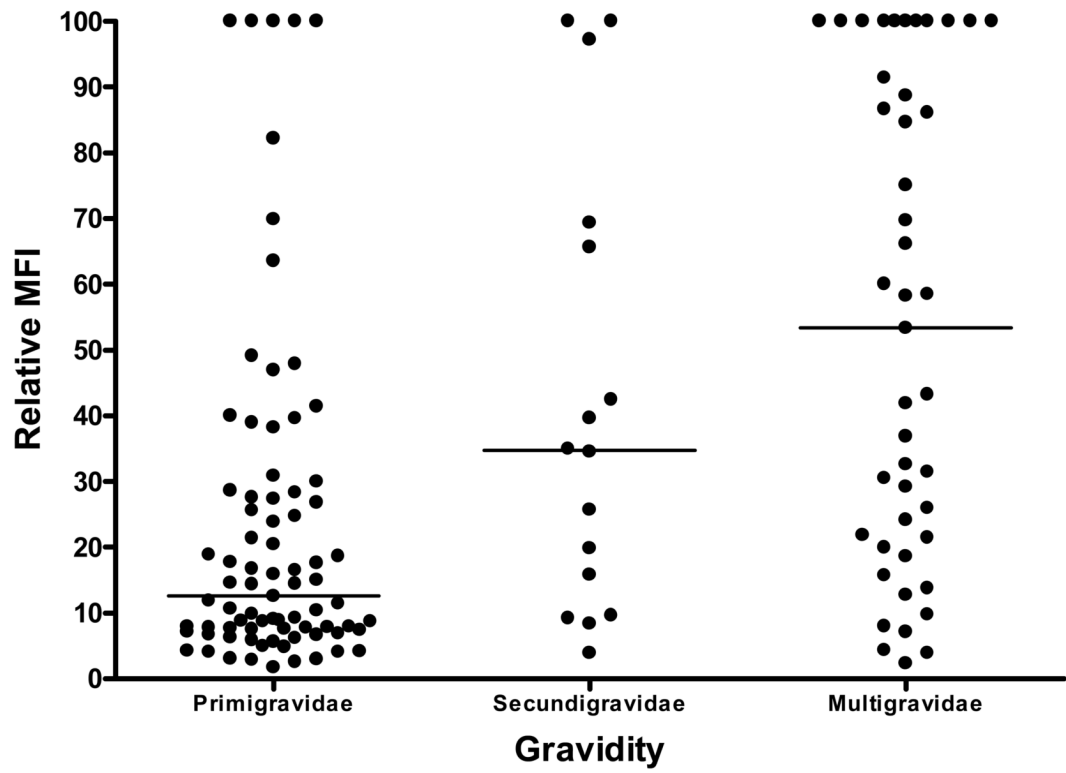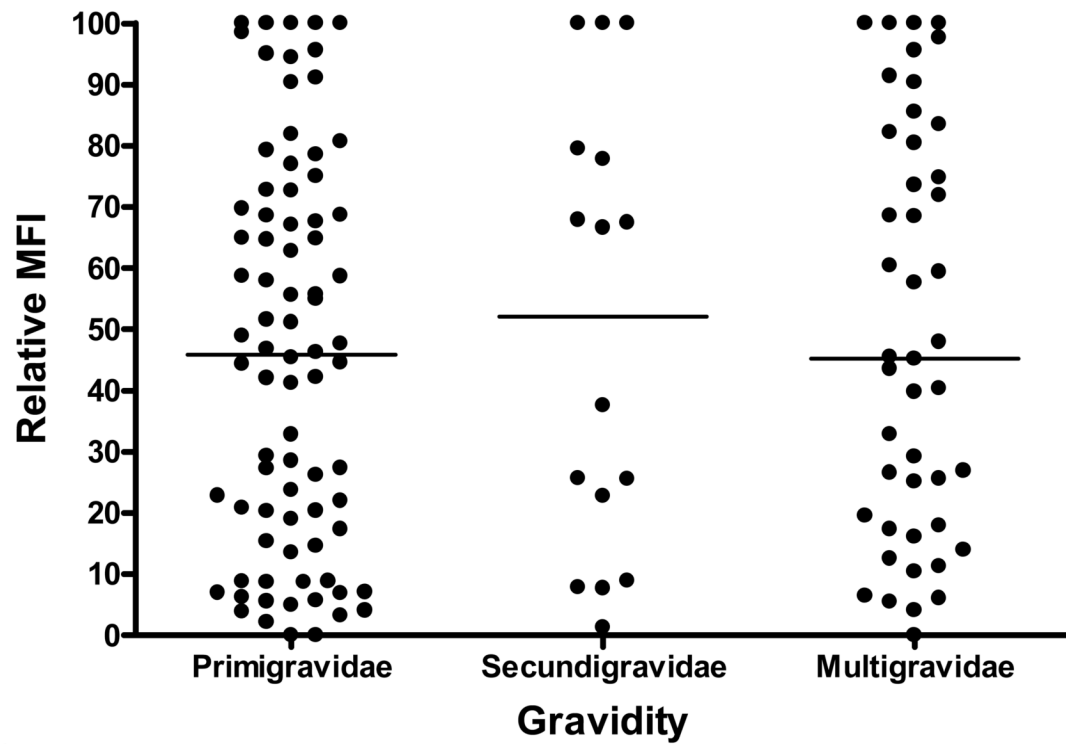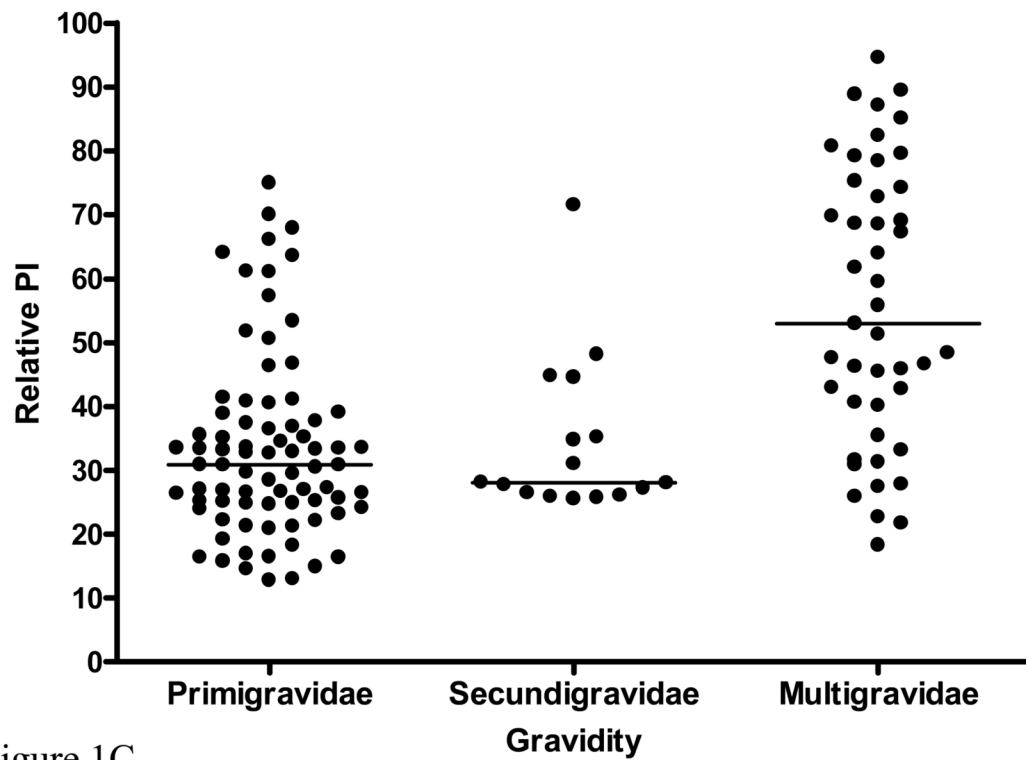
Figure 1A

Figure 1B

Figure 1C

Figure 1.
Relationship between antibody to VSA and gravidity. Dot plot show individual values and line indicate the median of relative MFI in each group. (A) Total level of IgG (relative MFI) against CS2 IEs at enrollment in each gravidity group. (B) Total level of IgG (relative MFI) against E8B-ICAM IEs at enrollment in each gravidity group. (C) Level of opsonic antibodies (relative PI) in each gravidity group.
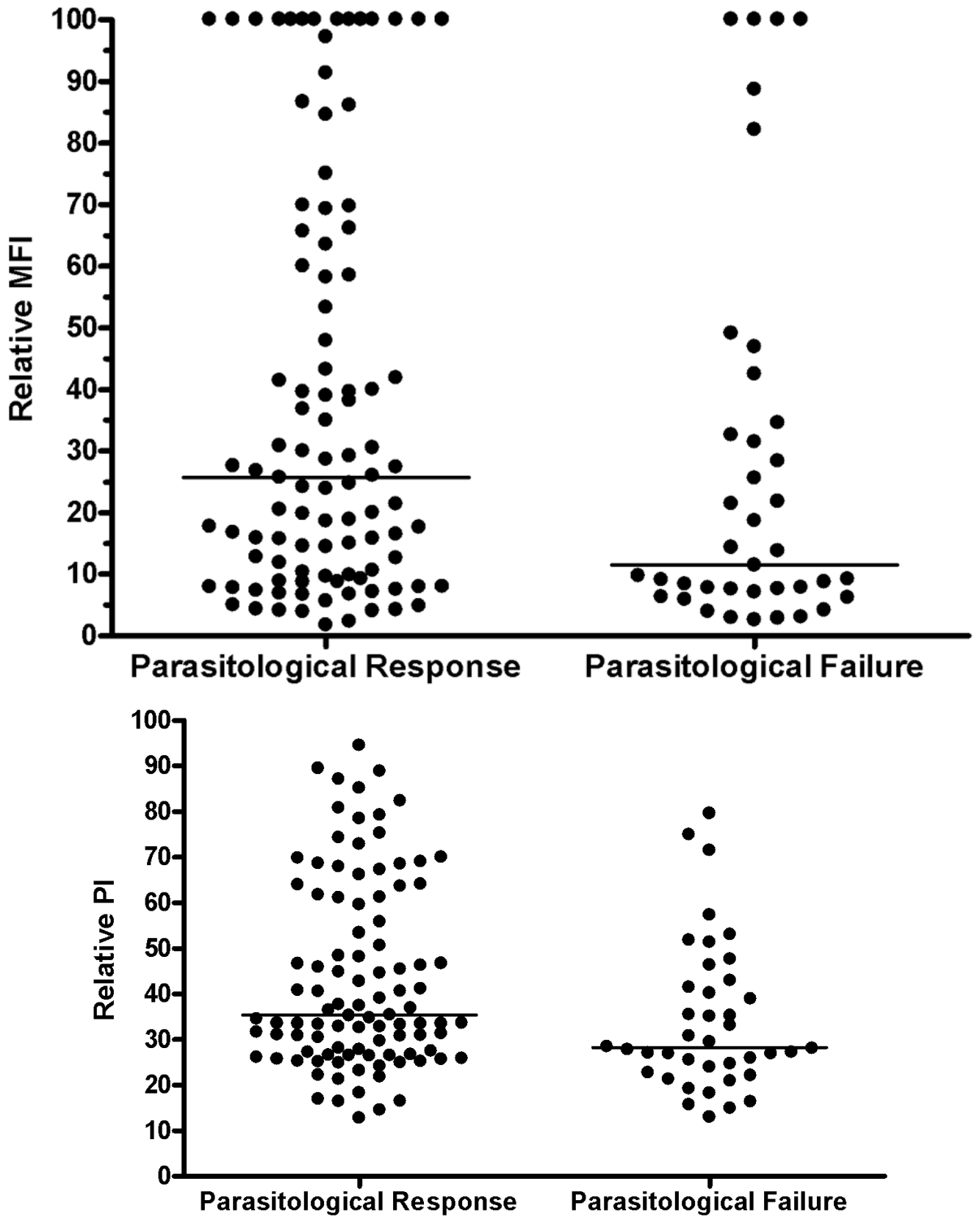
Figure 2.
Level of opsonic antibodies (relative PI) and total level of IgG (relative MFI) against CS2 IEs in patients with parasitological failure vs. patients who cleared infection and remained parasite free (Parasitological response). Dot plots show individual values; line indicates the median of relative MFI and relative PI in each group.
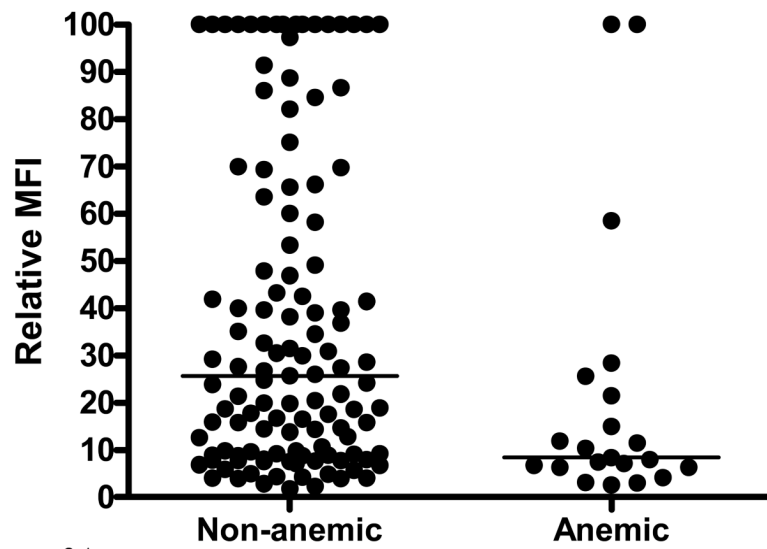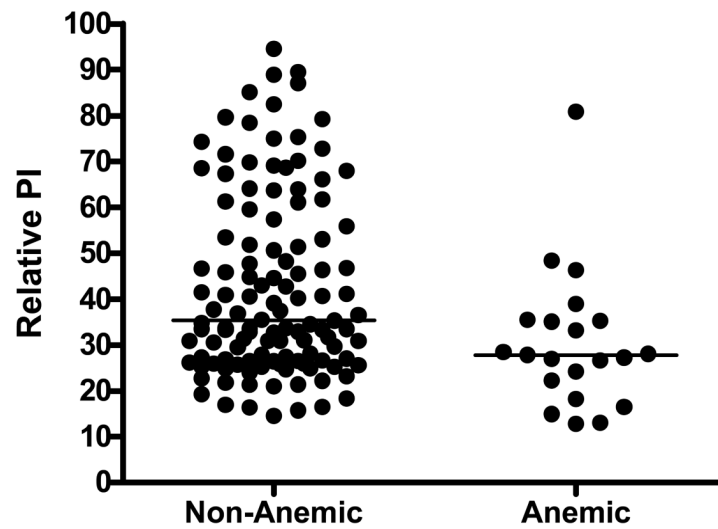
Figure 3A

Figure 3B

Figure 3.
Level of opsonic antibodies (relative PI) and total level of IgG (relative MFI) against CS2 IEs in patients with anemia vs. patients without anemia at delivery. Dot plots show individual values; line indicates the median of relative MFI and relative PI in each group.

Table 1

Summary of study population by gravidity and treatment groups

	Primigravidae	Secundigravidae	Multigravidae	SP only	SP plus Azithromycin	SP plus Artesunate
Parasitological failure[^]	26/80 (33.8%)	5/16 (31.3%)	9/45 (17.8%)	17/47 (36.2%)	12/47 (25.5%)	11/47 (23.4%)
Recrudescence[^]	17/80 (21.3%)	3/16 (18.8%)	3/45 (4.4%)	14/47 (29.8%)	4/47 (8.5%)	5/47 (8.5%)
Re-infection[^]	14/80 (18.8%)	2/16 (12.5%)	7/45 (15.6%)	5/47 (12.8%)	9/47 (19.1%)	9/47 (19.1%)
Age (years)[*]	18.3 ± 1.78[*]	22.1 ± 3.0	26.1 ± 4.7	21.5 ± 4.9	21.2 ± 4.8	20.9 ± 4.7
Maternal anemia	15/80 (18.8%)	1/16 (6.3%)	5/45 (11.1%)	8/47 (17.0%)	8/47 (17.0%)	5/47 (10.6%)
Birth weight (g)[*]	2705 ± 528	3088 ± 289	2998 ± 468	2869 ± 625	2785 ± 537	2836 ± 482

[*] Mean ± SD

[^] Participants may have recrudescence after one treatment and re-infection after the other. But they were only counted once in parasitological failure

Table 2

Summary of the association between relative MFI against CS2 IEs and parasitological failure, recrudescence, reinfection or maternal anemia

	Parasitological Failure[*]			Recrudescence			Reinfection			Maternal anemia[**]		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Relative MFI (unadjusted)	0.76	0.59, 0.97	0.029	0.75	0.56, 1.01	0.057	0.77	0.57, 1.03	0.083	0.64	0.46, 0.88	0.007
Relative MFI[^]	0.78	0.60, 1.03	0.075	0.84	0.59, 1.18	0.313	0.73	0.53, 1.01	0.057	0.66	0.46, 0.93	0.018
Primigravidae[^]	1	-	-	1	-	-	1	-	-	1	-	-
Multigravidae	0.87	0.37, 2.02	0.738	0.48	0.16, 1.47	0.200	1.28	0.46, 3.56	0.637	0.73	0.24, 2.20	0.578
SP only[^]	1	-	-	1	-	-	1	-	-	1	-	-
SP plus azithromycin	0.66	0.27, 1.62	0.363	0.23	0.07, 0.78	0.018	2.22	0.67, 7.38	0.192	1.17	0.38, 3.59	0.781
SP plus artesunate	0.51	0.20, 1.31	0.164	0.29	0.09, 0.90	0.032	1.96	0.58, 6.64	0.278	0.64	0.19, 2.20	0.480

OR - Odds Ratio;

[*] Parasitological failure: any further episode of parasitemia from D7 to delivery;

[**] Maternal anemia: hemoglobin <10 g/dl at delivery;

[^] - Mutually adjusted for relative MFI, gravidity and treatment

Table 3

Associations between relative PI and parasitological failure, recrudescence, reinfection or maternal anemia

	Parasitological failure*			Recrudescence			Reinfection			Maternal anemia**		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Relative PI (unadjusted)	0.46	0.26, 0.83	0.009	0.41	0.20, 0.85	0.016	0.61	0.31, 1.21	0.159	0.30	0.13, 0.68	0.004
Relative PI^	0.48	0.25, 0.90	0.023	0.49	0.21, 1.12	0.089	0.58	0.28, 1.21	0.146	0.31	0.13, 0.74	0.008
Primigravidae^	1	-	-	1	-	-	1	-	-	1	-	-
Multigravidae	0.90	0.39, 2.07	0.797	0.58	0.19, 1.81	0.351	1.06	0.39, 2.86	0.913	0.77	0.25, 2.34	0.648
SP only^	1	-	-	1	-	-	1	-	-	1	-	-
SP plus azithromycin	0.66	0.27, 1.65	0.376	0.24	0.07, 0.81	0.021	2.17	0.66, 7.15	0.204	1.21	0.39, 3.76	0.739
SP plus artesunate	0.54	0.22, 1.37	0.197	0.27	0.09, 0.85	0.026	2.08	0.63, 6.81	0.228	0.59	0.17, 2.04	0.406

OR - Odds Ratio;

*
Parasitological failure: any further episode of parasitemia from D7 to delivery;

**
Maternal anemia: hemoglobin <10 g/dl at delivery;

^
- Mutually adjusted for relative PI, gravidity and treatment