Quantification of the Dynamics of Antibody Response to Malaria to Inform Sero-Surveillance in Pregnant Women

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Keywords: Malaria, Pregnancy, Antibodies, PfAMA1, PfEBA175, PfMSP2, PfMSP3, PvAMA1, PfVAR2CSA, Longitudinal data

Posted Date: September 14th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-885654/v1>

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Version of Record: A version of this preprint was published at Malaria Journal on March 5th, 2022. See the published version at <https://doi.org/10.1186/s12936-022-04111-y>.

Abstract

Background: Malaria remains a major public health threat and in low malaria transmission areas new tools are needed to detect infections for prompt treatment and to progress elimination efforts. Pregnant women are particularly vulnerable to malaria infections and access routine antenatal care, presenting a unique sentinel population to apply novel sero-surveillance tools to measure malaria transmission. The aim of this study was to quantify the dynamic antibody responses to multiple antigens during pregnancy to identify a single or multiple antibody response of exposure to malaria in pregnancy.

Methods: Antibody-mediated immunity responses to six parasite antigens (five commonly studied merozoite antigens and the variant surface antigen 2-chondroitin sulphate A (VAR2CSA), a pregnancy-specific erythrocytic antigen) were measured over the gestation period until delivery (median of 7 measurements/woman) in 250 pregnant women who attended antenatal clinics located at the Thai-Myanmar border. A multivariate mixture linear mixed model was used to cluster the pregnant women into groups that have similar longitudinal antibody responses to all six antigens over the gestational period using a Bayesian approach. The variable-specific entropy was calculated to identify the antibody responses that have the highest influence on the classification of the women into clusters, and subsequent agreement with grouping of women based on exposure to malaria during pregnancy.

Results: Of the 250 pregnant women, 135 had a *Plasmodium* infection detected by light microscopy during pregnancy, defined as cases. The antibody responses to all six antigens accurately identified the women who did not have a malaria infection detected during pregnancy (93%, 107/115 controls). Antibody responses to *P. falciparum* merozoite surface protein 3 (PfMSP3) and *P. vivax* apical membrane antigen 1 (PvAMA1) were the least dynamic. Antibody responses to the antigens *P. falciparum* apical membrane antigen 1 (PfAMA1) and PfVAR2CSA were able to identify the majority of the cases more accurately (63%, 85/135).

Conclusion: These findings suggest that the combination of antibodies, PfAMA1 and PfVAR2CSA, may be useful for sero-surveillance of malaria infections in pregnant women, particularly in low malaria transmission settings, leading to the early detection and treatment of malaria infections in pregnant women.

Background

Malaria is a major infectious disease causing around 229 million clinical cases and 409,000 deaths globally in 2019 (1). Pregnant women are particularly vulnerable to malaria infection, as well as presenting with more severe symptomatic infections (2). Each year, around 125 million pregnant women, living in malaria endemic countries, are at risk of malaria infection (3, 4). Malaria in pregnancy poses substantial risks to the pregnant woman and their baby, increasing the risk of maternal anaemia, hypertensive disorders, miscarriage, stillbirth and neonatal death and as such there are several prevention and treatment strategies provided to women attending antenatal care to reduce the burden of malaria in pregnancy (5, 6). Pregnant women routinely attending antenatal care are also considered an easy-access population which can serve as sentinel surveillance populations to estimate malaria transmission (7).

The development of novel serological surveillance (sero-surveillance) tools for use in sentinel populations of pregnant women is a potential powerful technique for detecting recent and ongoing malaria infections, and monitoring malaria transmission (8, 9). This is particularly pertinent in low malaria transmission settings such as Southeast Asia, where parasite density is often low and standard surveillance methods are insensitive to submicroscopic and asymptomatic infections (10). Antibodies targeting blood-stage antigens, predominantly relatively conserved antigens expressed on the merozoites, have been the focus of sero-surveillance studies in non-pregnant populations (9, 11). In pregnant women, serological studies have also investigated antibody responses to the pregnancy-specific *P. falciparum* antigen (PfVAR2CSA), which is expressed on the surface of infected erythrocytes (IEs), and mediates sequestration of *P. falciparum* in the placenta via binding to placental chondroitin sulphate A (CSA) receptors (12, 13). Antibodies specific for PfVAR2CSA can reduce the accumulation of the IEs in the placenta (14). High antibody levels against PfVAR2CSA can be acquired with successive pregnancies (15), reducing the susceptibility to falciparum malaria in multigravida women by clearing the parasites sequestered in the placenta (16).

However, while numerous studies have investigated PfVAR2CSA antibodies in pregnant women as markers of infection (17), few studies have incorporated non-pregnancy specific antibodies (18, 19), and none have considered the combined effects of the antibodies.

The aim of this study was to quantify the dynamic antibody responses to multiple blood-stage antigens (merozoite and PfVAR2CSA) during pregnancy to determine which, if any, of the antibody response(s) are biomarker(s) of exposure to malaria in pregnancy that could subsequently be targeted by sero-surveillance. Antibody responses to both *P. falciparum* and *P. vivax* were measured repeatedly in pregnant women attending antenatal clinics on the Thai-Myanmar border, a low malaria transmission setting, and were jointly analysed to account for the correlations between the antibodies to inform sero-surveillance approaches in pregnant women.

Methods

Study population and design

The study population was pregnant women attending antenatal clinics (ANCs) at the Shoklo Malaria Research Unit (SMRU) (20, 21), where malaria transmission is low and peaks between May and September. The ANCs were located in the Maela refugee camps (22).

Details of the nested case-control study design and procedures have been published previously (18). In brief, participants were identified from 1000 Karen women who were enrolled in a

placebo randomized controlled trial of chloroquine prophylaxis against *P. vivax* infection during pregnancy from November 1998 through January 2000 (23). Samples were obtained weekly from the women for *Plasmodium* species infection detection by microscopic examination of blood smears and fortnightly for serum sample collection. Case subjects were women with *Plasmodium* infection detected by light microscopy at any time during pregnancy during the trial (n = 136). Of the 864 women with no detectable parasitemia at any time while pregnant during the trial, 331 were randomly selected to be control subjects (3:1 ratio).

Antibody determination

All available serum samples from the 136 case subjects were selected. A subset of 115 control subjects was selected for longitudinal antibody determination based on IgG responses to schizont extract at enrollment. The 115 controls were selected as follows. All available control enrollment samples (320 of the 331 randomly selected controls had serum samples measured at enrollment) were tested for total IgG in response to schizont extract. A cut-off threshold for seropositivity to schizont extract was set to the mean + 3 standard deviations of the IgG responses to schizont extract for 8 negative controls (non-exposed Melbourne donors). The subset of 115 controls consisted of 78 individuals seropositive to schizont extract at enrollment together with 37 randomly selected individuals that were seronegative to schizont extract at enrollment (Fig. 1). See Supplementary material, Fowkes et al. 2012 (18) for further details. High throughput enzyme-linked immunosorbent assay (ELISA) was used to determine the total IgG titer (measured as optical density (OD) values) of *P. falciparum* merozoite antigens (apical membrane antigen, PfAMA1), erythrocyte binding antigen 175 (PfEBA175; region 3–5, merozoite surface protein, PfMSP2, PfMSP3), schizont extract, PfVAR2CSA (DBL5 domain), and *P. vivax* merozoite antigen (PvAMA1) (Supplementary material, Fowkes et al. 2012 (18)).

Statistical Analysis

Patient characteristics at baseline were summarised using median (25th – 75th percentiles) for continuous variables or frequency (%) for categorical variables.

A multivariate mixture linear mixed model was used to identify clusters (i.e., latent classes) of pregnant women that have similar antibody responses to all six antigens over gestational age (24). To construct a multivariate mixture linear mixed

effects model, first a linear mixed effects model was specified for each of the six antibody responses. The following covariates were included as fixed effects for each antibody response: age (years), primigravidae (1 if primigravidae and 0 if multigravida), treatment arm (1 if given chloroquine (CQ) as prophylaxis at enrolment and 0 if given a placebo) and having a history of malaria prior to enrolment (1 if exposed to malaria at least once prior to enrolment and 0 otherwise). To capture the between subject variability in the six antibody responses over gestational age, a random intercept and random slope for gestational age were included in the linear mixed effects model for each antibody response. The random intercepts and slopes for each response and woman (i.e., 12 random effects per woman) are assumed to follow a mixture of multivariate normal distributions.

The mixAK package in R (25) was used to fit the multivariate mixture linear mixed effects model to the six antibody response profiles available from each of the 250 women (135 cases and 115 controls) in this study. The mixAK package adopts a Bayesian approach to inference and implements a block Gibbs sampler with Metropolis-Hastings steps to sample parameter values from the posterior distribution (Supplementary material, Komárek and Komárková (2013, Appendix B) (24)). Weakly informative prior hyperparameters in the mixAK package were used (Supplementary material, Komárek and Komárková (2013, Appendix A) (24)). Two chains were initialised. The first 500 parameter values sampled for each chain were discarded as burn-in and an additional 5,000,000 parameter values (in total for both chains) were sampled after burn-in. Every 50th iteration after burn-in was kept, resulting in 100,000 (50,000 per chain) samples per parameter for calculation of posterior summaries. Results are presented as the posterior median (50th percentile) and 95% credible interval, calculated as the 2.5th and 97.5th percentiles of the 100,000 samples for each parameter. Traceplots were examined to assess whether the 50,000 parameter draws from each chain had appropriately converged.

The number of clusters was selected by fitting a mixture model assuming each of 1–4 clusters. The number of clusters was selected according to the model that produced the lowest penalized expected deviance and/or greatest shift of the posterior distribution of deviances to lower values (24).

The posterior probability of a woman belonging to a cluster (posterior class probability) was calculated at each iteration of the fitting algorithm and these probabilities were used to assign a woman to a cluster as follows. First, a woman was assigned to the cluster which had the highest median posterior class probability. Second, the woman remained in the cluster if the lower limit of the 95% credible interval for the posterior class probability exceeded 0.5; otherwise, the woman was considered unclassified (Supplementary methods, Sect. 4).

The variable-specific entropy was calculated to identify the antibody responses that have the highest influence on the classification of the women into clusters. The variable-specific entropy indicates how well the antibody response to a single antigen predicts the classification based on antibody responses to all six antigens, and ranges from 0–1 (26). Antibody responses with

variable-specific entropy values close to 1 drive the classification of women into clusters (Supplementary methods, Sect. 6).

To compare and identify the best antibodies for classifying women as being a case (exposed to malaria during pregnancy) or control, additional univariate and pairwise multivariate mixture linear mixed-effects modelling were performed with the number of clusters set at two groups, and the proportion of cases and controls classified in the high and low antibody response groupings calculated.

Results

Patient characteristics

A total of 1692 samples were included in the analysis; 727 samples were available from the 135 cases (1 case excluded because only an enrolment sample was available) and 965 from the 115 controls.

Table 1 presents the baseline characteristics of the 135 cases (women with *P. falciparum* and/or *P. vivax* detected by microscopy during pregnancy) and 115 controls (women without detected *Plasmodium* infection). The proportion of cases in their first pregnancy (22.2%) at enrolment was around twice of that in the control group (13.9%). Anaemia was more prevalent among cases at enrolment (23.5%), compared to controls (9.6%). The proportion of women who had received chloroquine prophylaxis (only to prevent vivax episodes) (23) was slightly higher for the control group (47.8% vs 41.5%). For both cases and controls, most women were enrolled in the trial during their first trimester (cases 76% and controls 85%). Nearly all controls were residing in refugee camps (99.1%), while less than half of the cases (44.9%) were living in refugee camps during pregnancy (the remaining cases were residing in villages south of the Mae Sot township).

Table 1
Distribution of patient characteristics at enrollment for cases and controls

Characteristic	Cases (n = 135)	Controls (n = 115)
Age (years) median (IQR)	24 (20–30)	26 (22–32)
Gravidity, median (IQR)	3 (2.0–4.5)	3 (2.0–5.0)
Primigravida, n (%)	30 (22.2)	16 (13.9)
Multigravida, n (%)	105 (77.8)	99 (86.1)
Parity, median (IQR)	1 (0, 3)	2 (1, 4)
Haematocrit (%), median (IQR)	32.5 (30–35)	34.0 (32–36)
Anaemia ^a , n (%)	32 (23.7)	11 (9.6)
Residence in refugee camp	61 (45.2)	114 (99.1)
Received chloroquine prophylaxis, n (%)	56 (41.5)	55 (47.8)
Estimated Gestational Age ^b , median (IQR)	9.7 (7–14)	9.4 (7.6–11.6)
Trimester		
1(< 14wk), n (%)	102 (75.6)	98 (85.2)
2 (14 to < 28wk), n (%)	31 (23)	17 (14.8)
3 (28 wk or more), n (%)	2 (1.5)	0 (0.0)
<i>Plasmodium</i> spp. before enrolment ^c , n (%)	75 (55.6)	42 (36.5)
<i>P. falciparum</i>		
Proportion women infected, n (%)	50 (37.9)	32 (27.8)
<i>P. vivax</i>		
Proportion women infected, n (%)	32 (24.2)	13 (11.3)
Follow up (weeks), median (range)	28.9 (22.6–32.1)	30.9 (28.3–32.4)
^a Haematocrit < 30%.		
^b Determined at enrolment.		
^c Any microscopically confirmed <i>Plasmodium</i> infection documented at SMRU before enrolment into the study.		

More than half of the cases (55.6%) had a documented history of malaria at the SMRU ANCs (any *Plasmodium* spp.; 37.9% *P. falciparum* and 24.2% *P. vivax*) prior to enrollment compared with 36.5% of the controls (27.8% *P. falciparum* and 11.3% *P.*

vivax).

Antibody dynamics over gestation

For all six antigens, the antibody response profiles of pregnant women who were infected with malaria during the trial (cases) tended to be maintained at higher levels compared to women who were free from malaria (controls) (Fig. 2). However, substantial variation in the antibody profiles of *Pf*AMA1, *Pf*EBA175, *Pf*MSP2 and *Pf*VAR2CSA was observed, irrespective of exposure to malaria (Figs. 2A-C, 2F). For *Pf*MSP3 and *Pv*AMA1, the antibody profiles tended to remain low over gestation for most controls, while the antibody responses for the cases exhibited greater variability (Figs. 2D-E). *Pf*MSP2 had the highest antibody responses at conception and remained highest over the gestational period compared to the other five antibodies, irrespective of exposure to malaria.

Classification of pregnant women

The best fitting multivariate mixture linear mixed effects model according to both the penalized expected deviance and the posterior distribution of the deviances classified the six antibody profiles from each of the 250 pregnant women into two clusters (Supplementary methods, Sect. 1–5): 186 into Cluster 1 and 55 into Cluster 2, with 9 unclassified. The antibody response profiles allocated to each cluster are shown in Fig. 3 and demonstrate that the clustering method has clearly differentiated Cluster 1: a group of pregnant women with antibody profiles maintained at relatively low levels or dynamic low-medium levels across all 6 antibodies, from Cluster 2: those who had relatively high or dynamic medium-high antibody levels over gestational age. As such, herein clusters 1 and 2 are referred to as “low immune” and “high immune” groups, respectively.

Association between maternal factors and antibody responses

Posterior summaries for the fixed effect and population average intercept and slope parameters of the multivariate mixture linear mixed effects model are presented in Table 2. For most antigens, the mean change in antibody responses increased with age (per year) and were higher for those pregnant women that received chloroquine prophylaxis; the exceptions were the mean change in antibody titer to *Pv*AMA1 decreased by -0.001 per year (95% Credible Interval (CrI): -0.002,0.0004)) and the mean antibody titer to *Pf*MSP3 were - 0.003 (95% CrI: -0.019,0.013) lower for those that received chloroquine prophylaxis compared to those that did not. Mean antibody responses to *Pf*AMA1, *Pf*MSP2 and *Pf*MSP3 were increased for primigravidae compared to multigravida women. For pregnant women with a history of malaria, only mean antibody responses to the antigen *Pv*AMA1 were increased (0.012 (95% CrI: -0.003, 0.029)) compared to women without a history of prior infection(s).

Table 2

Multivariate linear mixed-effects modelling of the combined IgG responses (measured as optical density (OD) values) to *Plasmodium falciparum* and *Plasmodium vivax* recombinant antigens. Posterior medians (95% credible interval) for the fixed effects, population average intercept and population average slope parameters.

Variable	<i>Pf</i> AMA1	<i>Pf</i> EBA175	<i>Pf</i> MSP2	<i>Pf</i> MSP3	<i>Pv</i> AMA1	<i>Pf</i> VAR2CSA
Fixed effects ^a						
Age (years)	0.001 (-0.006,0.008)	0.007 (-0.001,0.015)	0.005 (-0.001,0.01)	0.001 (0.0001,0.003)	-0.001 (-0.002,0.0004)	0.003 (-0.0001,0.007)
Gravidity						
Primigravidae	0.064 (-0.05,0.179)	-0.017 (-0.153,0.118)	0.013 (-0.075,0.102)	0.004 (-0.018,0.027)	-0.005 (-0.029, 0.02)	-0.012 (-0.069,0.046)
Intervention group						
Chloroquine	0.054 (-0.021,0.13)	0.048 (-0.041,0.136)	0.009 (-0.05,0.067)	-0.003 (-0.019,0.013)	0.019 (0.002,0.036)	0.031 (-0.007,0.069)
History of malaria						
Yes	-0.04 (-0.116,0.036)	-0.024 (-0.113,0.065)	-0.02 (-0.08,0.039)	-0.002 (-0.018,0.013)	0.012 (-0.005,0.028)	-0.014 (-0.053, 0.024)
Average random effects for a woman						
Low immune						
Constant ^b	0.31 (0.231,0.388)	0.469 (0.373,0.564)	0.704 (0.631,0.777)	0.069 (0.048,0.09)	0.095 (0.073,0.117)	0.235 (0.189,0.281)
Gestation (weeks) ^c	-0.001 (-0.002,0.0001)	-0.002 (-0.003, -0.0004)	-0.003 (-0.004, -0.001)	0.00004 (-0.001,0.001)	-0.0003 (-0.001,0.0003)	-0.001 (-0.002,0.0004)
High immune						
Constant ^b	0.864 (0.67,1.062)	1.076 (0.897,1.264)	1.000 (0.893,1.111)	0.603 (0.404,0.808)	0.448 (0.283,0.608)	0.634 (0.498,0.766)
Gestation (weeks) ^c	-0.0004 (-0.006,0.005)	0.0001 (-0.005,0.005)	0.001 (-0.002,0.005)	-0.006 (-0.012, -0.001)	-0.003 (-0.008,0.002)	0.002 (-0.003,0.006)
^a Fixed effects interpretation: change in mean antibody levels per year increase in age and difference in mean antibody levels for primigravidae vs. multigravida (reference group), chloroquine vs. placebo (reference group) and history vs. no history of malaria (reference group).						
^b Population mean antibody levels for women of average age (25 years old), multigravida, in the placebo group and with no history of malaria.						
^c Population average change in antibody levels for a one week increase in gestation.						

The posterior medians for the population average slope indicate that, on average, antibody responses to *Pf*AMA1 and *Pv*AMA1 decreased with gestational age in both the low and high immune groups. For *Pf*EBA175, *Pf*MSP2 and *Pf*VAR2CSA,

the posterior medians for the population average slope indicated that, on average, antibody responses decreased with gestational age in the low immune group but increased with gestational age in the high immune group; these trends were reversed for antibody responses to *PF*MSP3.

Influence of specific antibodies on the classification into high and low immunity groups

The variable-specific entropy for the antibody responses to the six antigens are given in Table 3. The relatively high entropy values for *PF*MSP3 (0.949) and *Pv*AMA1 (0.935) indicate that the allocation of women to the low or high immune group was predominantly determined by their antibody responses to these antigens, whereas antibody responses to the antigen *PF*MSP2 played a lesser role in cluster allocation (lowest entropy = 0.763). The biological mechanisms of *PF*MSP3 and *Pv*AMA1 are quite different. *Pv*AMA1 is a *P. vivax* antigen while *PF*MSP3 does not connect directly to the merozoite surface, unlike the other four *P. falciparum* related antibodies. Therefore, additional analyses were performed to assess the importance of including both *PF*MSP3 and *Pv*AMA1 antibodies in allocating a woman to the low or high immune group. These analyses found that at least one of the two antigens, *Pv*AMA1 or *PF*MSP3, is necessary for correctly grouping pregnant women with low antibody profiles (Supplementary methods, Sect. 7).

Table 3
Influence of specific antibodies on the classification into high and low immunity profiles.

Antibody	Entropy value ^a
<i>PF</i> MSP3	0.949
<i>Pv</i> AMA1	0.935
<i>PF</i> VAR2CSA	0.873
<i>Pf</i> AMA1	0.844
<i>Pf</i> EBA175	0.817
<i>PF</i> MSP2	0.763

^aThe entropy is a value between 0 and 1 and values closer to 1 indicate that an antibody highly contributes towards the classification of women to cluster 1 or 2. The influence on classification declines as the entropy value declines.

Antibodies that best identify malaria cases

Using all the six antibody responses, the multivariate model performed well for the controls by classifying them into the low immune group (107 of 111 controls, 96.4%); whereas the cases were poorly identified by the antibody responses to the six antigens, 55 cases and 79 cases classified in high and low immune groups.

Analyses including each antigen separately (i.e. univariate linear mixed-effects modelling), found antibody responses to the antigen *Pf*AMA1 were best able to discriminate between cases and controls and classified 66% of controls in the low immune group (77 out of 115 controls) and 55% of cases in the high immune group (74 out of 135 cases). The model fit to antibody responses to *Pv*AMA1 was best at identifying the controls (95%, 109 of 115 controls), followed by *PF*MSP3 (90%, 103 of 115 controls). The model fit to antibody responses to *Pf*EBA175 was best at identifying the majority of the cases (60%, 81 of 135 cases), followed by *Pf*AMA1 (55%, 74 of 135 cases). Although the model fits to antibody responses to *Pv*AMA1 and *Pf*EBA175 were best at identifying controls and cases, respectively, they were unable to accurately classify the pregnant women in the other exposure group (Table 4).

Table 4
Performance of classifying controls to Cluster 1^a and cases to Cluster 2^b based on univariate analyses^c.

	Number of controls ^d classified into Cluster 1 (%)	Number of cases ^e classified into Cluster 2 (%)	Total women classified into the expected Cluster ^f (%)
PfAMA1	76 (66.1)	74 (54.8)	150 (60)
PfVAR2CSA	86 (74.8)	57 (42.2)	143 (57.2)
PfEBA175	61 (53.0)	81 (60.0)	142 (56.8)
PvAMA1	109 (94.8)	28 (20.7)	137 (54.8)
PfMSP3	103 (89.6)	33 (24.4)	136 (54.4)
PfMSP2	63 (54.8)	67 (49.6)	130 (52)
^a Cluster 1 (low immunity group)			
^b Cluster 2 (high immunity group)			
^c Antibodies are ordered from the highest to the lowest percentage of classifying pregnant women into the expected cluster.			
^d Out of 115 controls			
^e Out of 135 cases			
^f Computed based on the decision made that Cluster 1 would represent controls and Cluster 2 would represent cases. The sum of controls classified into Cluster 1 and cases classified into Cluster 2 were then divided by the total pregnant women, i.e. 250 to obtain the percentage.			

The results of selected pairwise multivariate analyses indicate that antibody responses to both *PfAMA1* and *PfVAR2CSA* were best at identifying the cases as most women were classified into the high immune group. This combination classified 63% (73 of 115) of the controls into the low immune group and 63% (85 of 135) of the cases into the high immune group.

Discussion

Pregnant women may serve as an accessible sentinel population to estimate the burden of malaria (27) and potentially for sero-surveillance studies to detect infections. In this cohort, antibody responses to the malaria parasite were highly dynamic, varying greatly within and between women during pregnancy. Modelling the longitudinal antibody response to six different antigens simultaneously found that pregnant women were classified into two major clusters high immune and low immune response groupings, where 97% of the women who did not have a malaria infection detected during pregnancy had a low immune response. Antibody responses to *PfMSP3* and *PvAMA1* were the least dynamic and did not boost with infection, influencing the classification of pregnant women into the low immunity cluster suggesting that they are not suitable serological markers of recent exposure to malaria during pregnancy. Antibody responses to the antigens *PfAMA1* and *PfVAR2CSA* were best for identifying exposure to malaria during pregnancy, suggesting that these two antibodies may be good candidates for sero-surveillance of malaria infections in pregnant women.

Antibodies specific for *Plasmodium* spp. blood-stage antigens, in particular *PfVAR2CSA*, are an attractive candidate for sero-surveillance of malaria in pregnancy as they have been demonstrated as a biomarker of recent exposure to malaria during pregnancy (systematically reviewed in (17)) and to monitor changes in malaria transmission over time (28). Our results show that the overall immunity corresponding to the *P. falciparum* parasite is positively correlated with increasing age, potentially due to increased lifetime exposure to malaria (29, 30). No strong correlation with gravidity was observed, even with the pregnancy-specific immunity (*PfVAR2CSA*), which may reflect the specific epidemiology of malaria in pregnancy in low endemic settings (31). Regardless, *PfVAR2CSA* did predict exposure to malaria in pregnancy in this setting, and in

combination with antibodies specific for the non-pregnancy specific blood-sage antigen *Pf*AMA1, was the most accurate indicator of exposure to malaria during pregnancy. This is the first study to incorporate and combine pregnancy and non-pregnancy specific antibodies, so our findings provide a novel avenue for improved sero-surveillance studies to be validated in a range of transmission settings.

This study has several limitations. First, the availability of only one antibody marker for *P. vivax*, *Pv*AMA1, limits what can be understood about the immune response of pregnant women exposed to vivax malaria. The antibody responses to *Pv*AMA1 remained relatively low compared to other antibodies (except for *Pf*MSP3, the only synthetic peptide antigen included in the study which may explain the lower immunogenicity of this *P. falciparum* antigen). Parasite density is believed to have a proportional relationship with antibody maintenance and boosting (32–34). That these densities are lower in vivax infections compared to falciparum infections could possibly explain the maintenance of *Pv*AMA1 antibody responses at low levels. Administration of chloroquine, preventing vivax episodes, could be a significant confounding factor in disrupting antibody responses to *Pv*AMA1 (23) and may explain the association of chloroquine prophylaxis with *Pv*AMA1 responses we observed (albeit in a counterintuitive direction).

Another limitation of this study was that the detection of exposure to malaria was based on microscopy, thereby, failing to detect low density sub-microscopic infections (35). Indeed, as visualised in Fig. 1, there were some controls (78 of 115) who recorded high responses to schizont lysate suggesting potential submicroscopic infections that were not detected by light microscopy at fortnightly routine testing. Therefore, the generalizability of our antigen combinations to accurately detect submicroscopic infections remains to be determined but is important given the high rates of submicroscopic infections in malaria endemic regions (36).

Conclusions

This study demonstrates that the combination of anti-*Pf*AMA1 and anti-*Pf*VAR2CSA antibodies could be used as a potential biomarker of exposure to malaria during pregnancy. This combined antibody diagnostic tool may therefore facilitate the detection of microscopic infections as an alternative to standard diagnostic methods, particularly in low transmission settings. This proposed malaria sero-surveillance tool may also enhance malaria control and progress efforts to eliminate malaria.

Abbreviations

AMA1: apical membrane antigen 1; ANCs: antenatal clinics; CQ: chloroquine; CrI: credible interval; CI: confidence interval; CSA: chondroitin sulphate A; EBA: erythrocyte binding antigen; IEs: infected erythrocytes; IQR: inter-quartile range; LOESS: locally estimated scatterplot smoothing; MCMC: markov chain Monte Carlo; MSP: merozoite surface protein; *P*: *Plasmodium*; *Pf*: *P. falciparum*; *Pv*: *P. vivax*; SMRU: shoklo Malaria Research Unit; VAR2CSA: variant surface antigen 2 – CSA.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Faculty of Tropical Medicine of Mahidol University, the London School of Hygiene & Tropical Medicine, and the Walter and Eliza Hall Institute of Medical Research.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Funding

Tharkeshi T. Dharmaratne is funded by the University of Melbourne (Melbourne Research Scholarship) for education. The funder had no role in the design of the study, collection, analysis and interpretation of data or writing the manuscript. This work is supported in part by the Australian Centre for Research Excellence in Malaria Elimination, funded by the NHMRC (1134989). JAS is funded by an Australian National Health and Medical Research Council of Australia (NHMRC) Investigator Grant (1196068) and FJIF by a NHMRC Career Development Fellowship (1166753).

Authors' contributions

TTD analysed the data, interpreted the findings and wrote the initial draft of the manuscript; JAS designed the study, supervised the entire study and reviewed the manuscript; FJIF and KO contributed to study design, data collection, provided immunological advice and reviewed the manuscript; SD coordinated interpretation of the findings and reviewed the manuscript; SZ contributed to the statistical analyses and reviewed the manuscript, DJP contributed to R coding and reviewed the manuscript, and FN and RM contributed to study design, data collection and reviewed the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We would like to thank the staff of the Shoklo Malaria Research Unit for supporting data collection activities and technical assistance provided in all stages of the study. We are also grateful to all the mothers for their participation.

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Figures

Figure 1

Flow diagram of cohort selection for the current study.

Figure 2

Longitudinal antibody levels against six antigens for all women. Spaghetti plots A–F represent the antibody profiles of PfAMA1, PfEBA175, PfMSP2, PfMSP3, PvAMA1 and PfVAR2CSA, respectively. The antibody levels of the pregnant women exposed to malaria (cases) and free from malaria (controls) are represented by orange and blue, respectively. LOESS curves for all pregnant women (in black) and for each exposure group are superimposed on each spaghetti plot. The shaded area around each LOESS curve represents the 95% confidence interval (CI). Of note, the Y axes of the plots of A, B and D are truncated at 0; the CIs did extend to negative values due to limited information in the early period of gestation. OD: Optical Density.

Figure 3

Trajectory plot of the antibody responses versus gestation (weeks) for each individual by cluster (cluster 1 (low immune) - green, cluster 2 (high immune) - red). The trajectories in blue are the pregnant women who were not classified with certainty into either cluster. OD: Optical Density.

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