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Correlation Between Malaria-Specific Antibody Profiles and Responses to Artemisinin Combination Therapy for Treatment of Uncomplicated Malaria in Western Kenya

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Background. The impact of preexisting immunity on the efficacy of artemisinin combination therapy must be examined to monitor resistance, and for implementation of new treatment strategies.

Methods. Serum samples obtained from a clinical trial in Western Kenya randomized to receive artemether-lumefantrine (AL) or artesunate-mefloquine (ASMQ) were screened for total immunoglobulin G against preerythrocytic and erythrocytic antigens. The association and correlation between different variables, and impact of preexisting immunity on parasite slope half-life ($t_{½}$) was determined.

Results. There was no significant difference in $t_{½}$, but the number of individuals with lag phase was significantly higher in the AL than in the ASMQ arm (29 vs 13, respectively; $P < .01$). Circumsporozoite protein–specific antibodies correlate positively with $t_{½}$ (AL, $P = .03$; ASMQ, $P = .09$), but negatively with clearance rate in both study arms (AL, $P = .16$; ASMQ, $P = .02$). The $t_{½}$ correlated negatively with age in ASMQ group. When stratified based on $t_{½}$, the antibody titers against circumsporozoite protein and merozoite surface protein 1 were significantly higher in participants who cleared parasites rapidly in the AL group ($P = .01$ and $P = .02$, respectively).

Conclusion. Data presented here define immunoprofiles associated with distinct responses to 2 different antimalarial drugs, revealing impact of preexisting immunity on the efficacy of artemisinin combination therapy regimens in a malaria-holoendemic area.

Clinical Trials Registration. NCT01976780

Keywords. malaria infections; malaria antigens; malaria immunity; artemisinin combination therapy; Western Kenya; malaria-holoendemic areas; drug treatment.

Malaria prevalence has declined in the last decade [1], and artemisinin combination therapy (ACT) has been one of the critical tools kit used for medical intervention and strategic elimination. Unlike in Southeast Asia, where resistance to ACT is now established and spreading, thus far there is no evidence of validated resistance in Africa [2]. This is important because Africa still bears majority of malaria burden, accounting for about 90% of reported cases and deaths [2].

The reduction in malaria transmission has varied widely across Africa. As a result, malaria prevalence has become more heterogeneous and fragmented, leading to increased heterogeneity of immunity in the population [3, 4]. The level of immunity affects the ability to assess drug resistance, because patients with strong immunity respond to treatment even when infected by parasites with known genetic mutations associated with treatment failure [5, 6]. This presents a problem, because emergence of genetic mutations may go unnoticed in populations with high immunity but decreased antimalarial drug efficacy may become more apparent as immunity wanes.

Residents of malaria-endemic areas acquire natural immunity, with rapid rates of acquisition occurring in high- compared with low-transmission settings [7], and in an age-dependent fashion in which older children and adults tend to have higher parasite density yet a lower incidence of clinical malaria [8, 9]. The types and specificities of immune responses that arise are closely linked to unique strain- and stage-specific antigens expressed during the asymptomatic preerythrocytic and the symptomatic erythrocytic (blood-stage) stages of parasite development. Although the specific targets or patterns of responses are important in natural infections, individuals with

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a greater repertoire of responses have a lower risk of developing clinical malaria [10–12].

It is important to monitor and assess the effect of preexisting immunity on the efficacy of the different ACT regimens, especially because it has been suggested that deploying multiple first-line antimalarial therapies or rotating regimens may be the best strategy to prevent the development of resistance in Africa to available ACT [13, 14]. Understanding potential synergistic interaction between immunity and the different drug responses could allow for rational selection of drug regimens based on population or patient's immunoprofile, and/or other parameters such age, parasite load, and parasite and host genetic factors, which may affect the effectiveness of the antimalarial drugs [15–17].

The present study set out to determine the interplay between preexisting patient immunoprofile by examining preerythrocytic and erythrocytic antigens, parasite density, and the clearance rates after 2 different ACT regimens, artemether-lumefantrine (AL and or artesunate-mefloquine (ASMQ), in Kenya where AL has been the first-line treatment for uncomplicated malaria for more than a decade [18]. These drugs were selected for 2 reasons. First, the use of AL would provide current status on the efficacy of artemisinin based first-line treatment of malaria in Kenya. Second, ASMQ would allow for accurate evaluation of artemisinin derivative without the confounding influence of the partner drug because it was administered sequentially, starting with artesunate followed by mefloquine 72 hours later. We hypothesized that the breadth of antibodies would influence treatment outcomes in an age-dependent fashion, with adults having a larger repertoire of antigens leading to a more robust response to drug treatment, and that ASMQ would be a more effective drug treatment with more rapid clearance rates than AL.

MATERIALS AND METHODS

Ethical Considerations

The study was approved by the scientific and ethics review boards of the Kenya Medical Research Institute, Nairobi Kenya (approved protocol 2518) and the Institutional Review Board of the Walter Reed Army Institute of Research, Silver Spring, MD (WRAIR; approved protocol 1935). All participants and/or their legal guardians gave a voluntary written informed consent before any study procedures commenced.

Study Site, Population, and Sample Collection

The study was conducted in Kombewa district hospital in Kisumu County, Western Kenya, from June 2013 through November 2014. Detailed clinical study findings will be reported elsewhere. Kisumu County is a malaria-holoendemic lake region with intense malaria transmission throughout the year, with annual entomological inoculation rates of 31.1 infected bites per year [19]. This was a 2-arm randomized open-label study that recruited patients aged 6 months to 65 years who presented with uncomplicated malaria at the Kombewa district hospital. Study participants were randomized to receive AL or ASMQ using block randomization schemes with varying block sizes. Venous blood samples were collected at hours 0, 4, 8, 12, 18, 24, and then every 6 hours until 2 consecutive smears tested negative. Giemsa-stain films were prepared according to World Health Organization guidance and read by 2 independent expert microscopists. The geometric mean parasite count (per microliter) was then calculated for each participant at each sampling time point. Participants were followed up for a total of 42 days. A total of 118 participants were enrolled in the study, 59 from each arm. From these, 96 samples were randomly selected for analysis, and complete data were obtained for 82 samples, including 40 from the AL arm and 42 from the ASMQ arm (Figure 1).

Parasite Clearance Rates Calculation

The statistical models used to estimate the parasite clearance measures and lag phase duration were fitted using the Parasite Clearance Estimator tool developed by the Worldwide Antimalarial Resistance Network [20]. The following parameters were estimated: parasite clearance half-life, parasite clearance rate constant (K), and the estimated time to reduce parasitemia by 50%, 90%, 95%, and 99%. Log-transformed parasite density was plotted against time in hours to generate slope half-life ($t_{½}$), defined as the time needed for parasitemia to be reduced by half [21]. This constant is independent of starting value of parasitemia. The slope $t_{½}$ was calculated as follows: $t_{½} = \log_e(2)/K = 0.692/K$, where K is the clearance rate constant and represents the rate of parasite clearance after start of drug treatment.

Multiplex Assay for Antibody Determination

Baseline serum samples from 82 enrolled participants (40 in the AL and 42 in the ASMQ arm) were analyzed for total immunoglobulin G against (1) preerythrocytic antigens circumsporozoite protein (CSP), liver-stage antigen 1 (LSA-1), and the cell-traversal protein for ookinetes and sporozoites (CelTOS) and (2) blood-stage merozoite surface protein 1 (MSP-1) and apical membrane antigen 1 (AMA-1); Luminex technology was used for analysis. These antigens are associated with natural immunity, are considered important vaccine targets (reviewed in [22] and [23] for preerythrocytic and erythrocytic respectively), and have been extensively studied in field settings. The purification, characterization and use of these proteins (antigens) have been published elsewhere [24–28]. A multiplex assay with coupled beads was performed as described elsewhere [29].

Samples were analyzed on a Multiplex MAGPIX system (Millipore) using xPONENT 4.1 software, following the manufacturer's instructions. Antibody response was considered positive for median fluorescence intensity values if the signal

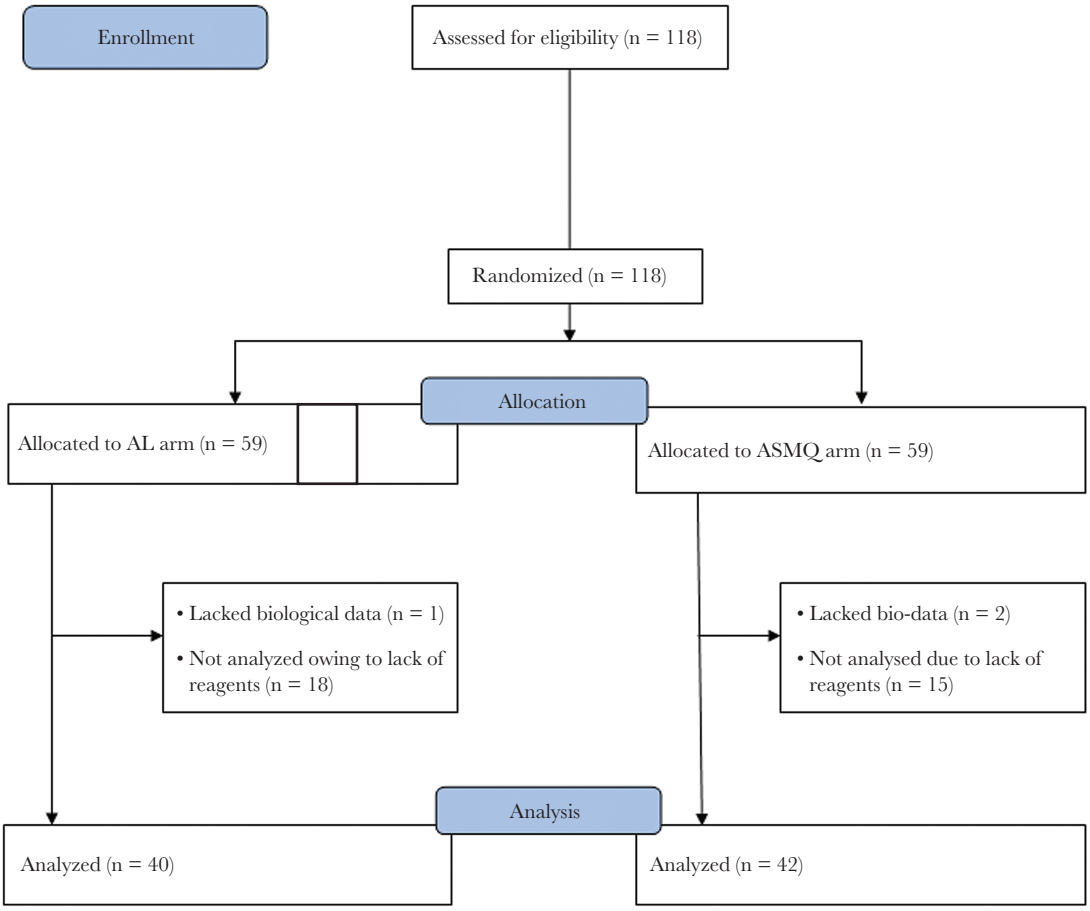

Figure 1. Consort flow diagram of the study and samples analyzed.

was greater than twice the background signal (mean of 6 determinations plus 2 standard deviations of the negative pool of nonimmune control serum samples). Pooled serum samples from malaria-naive (US) donors were used as negative controls (Gemini Bio-Products) and pooled serum samples from malaria-experienced (Western Kenya) donors as positive controls.

Statistical Analysis

Statistical analysis was performed using Stata (version 13; StataCorp) and Minitab (version 17; Minitab) software. Significance for categorical variables was determined using the χ^2 test. Bivariate analyses were performed using Kruskal-Wallis or analysis of variance (ANOVA) tests for multiple comparisons; Mann-Whitney or 2-sided *t* tests were used for comparisons between 2 groups. Sample size determinations were performed using a *z* test comparison of proportions (SigmaPlot, version 13; Systat Software). The ratio of patients with to those without lag phase was 29:40 (0.725) for AL and 13:42 (0.309) for ASMQ. The power of the test was set at 0.8, and the α value at .05. The parasite clearance $t_{½}$ was obtained from an in vivo efficacy study, wherein $t_{½}$ was derived using the Parasite Clearance Estimator [21]. The $t_{½}$ was obtained for the 82 participants whose samples were analyzed in this study. The median $t_{½}$ was 2.40 hours (interquartile range, 1.95–2.80 hours). Parasites with $t_{½}$ ≥5 hours after artemisinin treatment are considered resistant [30].

Because all the parasites in this study were fast clearing ($t_{½}$ ≤5 hours), parasites were stratified based on the 25th quartile $t_{½}$ (2.02 hours), which was used as a cutoff, with participants with $t_{½}$ ≤2.02 hours classified as rapid clearers, and those with $t_{½}$ >2.02 hours as fast clearers. Correlations between 2 factors were determined by using the Pearson correlation tests (for normally distributed data sets). Correlation matrices (correlograms) were computed and plotted using R software (www.sthda.com). The degree of correlation between each pair of variable is visualized through dots; the color, color intensity, and size of the dots indicate the level of correlation between the respective variables. Multivariate analysis was performed by clustering variables and plotting them as a dendrogram.

RESULTS

Characterization of Patient Population

The descriptive characteristics of participants enrolled in the AL and ASMQ study arms are summarized in Table 1. The prevalence of malaria-specific antibodies was high (ie, >92% of

Table 1. Descriptive Characteristics of Patient Population

Characteristic	No. With Characteristic/Total No. (%)[a] AL Arm	ASMQ Arm	*P* Value[b]
Age			
<5 y	24/40 (60)	33/42 (78.5)	.53
>5 y	16/40 (40)	9/42 (21.5)	
Sex			
Male	22/40 (56.4)	23/42 (54.8)	.98
Female	17/40 (43.6)	19/42 (45.4)	
Antibody prevalence, %[c]			
Preerythrocytic			
CSP	95.1		.06
CelTOS	91.6		.24
LSA-1	96.3		.11
Erythrocytic			
AMA-1	97.6% (3D7), 97.5% (HB3)		.79
$MSP\text{-}1_{p42}$	100% (3D7), 96.8% (FVO)		.78
Parasite density, median (IQR), parasites/μL (parasites per micro liter of blood)	69 109 (10 825–112 700)	60 623 (7610–97 765)	.49
$t_{½}$, median (range), h	2.46 (1.48–4.22)	2.25 (0.97–3.56)	.39
Participants with lag phase	29/40 (72.5)	13/42 (30.9)	<.01
Time to parasite clearance, median (IQR)			
PC50	7.41 (0.5–15.29)	3.9 (0.28–11.08)	<.001
PC90	12.87 (4.09–21.85)	9.32 (3.24–14.52)	<.001
PC95	15.77 (6.12–25.07)	11.67 (4.22–17.85)	<.001
PC99	21.55 (9.95–33.02)	16.83 (6.49–25.59)	<.001

Abbreviations: AL, artemether-lumefantrine; AMA-1, apical membrane antigen 1; ASMQ, artesunate-mefloquine; CelTOS, cell-traversal protein for ookinetes and sporozoites; CSP, circumsporozoite protein; IQR, interquartile range; LSA-1, liver-stage antigen 1; $MSP\text{-}1_{p42}$, merozoite surface protein-highly conserved C-terminus of the MSP-1 protein after it undergoes two successive proteolytic cleavage events; NS, not significant; PC50, PC90, PC95, and PC99, estimated time to reduce parasitemia by 50%, 90%, 95%, and 99%, respectively; $t_{½}$, half-life.

[a]Data represent No. with characteristic/total No. (%) unless otherwise specified.

[b]*P* values were calculated using χ^2 tests for categorical and *t* tests for continuous variables, and they represent differences between study arms.

[c]Antibody prevalence was measured using Luminex technology in samples collected at enrollment before randomization into study arms (see Supplementary Figure 1).

the study participants had antigen-specific antibodies) against both preerythrocytic (CSP, LSA-1, and CelTOS) and erythrocytic (MSP-1 and AMA-1) antigens. There was significant difference in the number of participants with a lag phase (29 in the AL and 13 in the ASMQ arm; $P < .01$) and the time to clearance. However, there was no significant difference in $t_{½}$ between the study arms ($P = .2$; 2-sided *t* test); $t_{½}$ excludes lag phase. The mean $t_{½}$ for both study arms combined was 2.38 hours (95% confidence interval, 2.23–2.52 hours), the median was 2.40 hours (interquartile range, 1.95–2.80 hours), and the range was 0.97–4.22 hours.

Association Between Age, Antibody Fine Specificities, and Parasite Density

The immunological profile of malaria-specific antibodies for study participants for both treatment arms is shown in Figure 2. The data for the study population were stratified by age group: 1–5, 6–15, or >15 years. When the ratio of antibodies specific to preerythrocytic (CSP, LSA-1, and CelTOS) versus erythrocytic (MSP-1 and AMA-1) antigens within the total antibody were analyzed, data revealed that the majority (approximately 80%) of the response was targeted to erythrocytic antigens (Figure 2A). There was no significant difference in the profiles between the age groups (Figure 2B and 2C). However, there was a trend wherein the adults (aged >15 years) had a higher proportion of antibodies specific to preerythrocytic antigens than children (<5 years) or adolescents (aged 6–15 years) ($P = .16$; ANOVA). Finally, one needs to consider the fact that all study participants were symptomatic at the time of analysis, which could skew their immunoprofiles compared with those of asymptomatic individuals.

Correlation Between Antibody Levels, Parasite Density, and Age

The data sets for the different measurements (variables) were used to generate a dendrogram that visualizes the statistical relationships between the variables (each consisting of a data set of individual measurements) shown in a graph (Figure 3). The dendrogram shows significant correlation between antibodies directed to the 2 tested blood-stage antigens, AMA-1 and MSP-1 ($R^2 = 0.688$; $P < .001$), and the 2 tested preerythrocytic antigens, CSP and CelTOS ($R^2 = 0.605$; $P < .001$). There was a weak positive correlation between LSA-1– and AMA-1–specific antibodies, which was not significant ($P = .16$; Pearson correlation). There was no significant correlation between any of the antibody

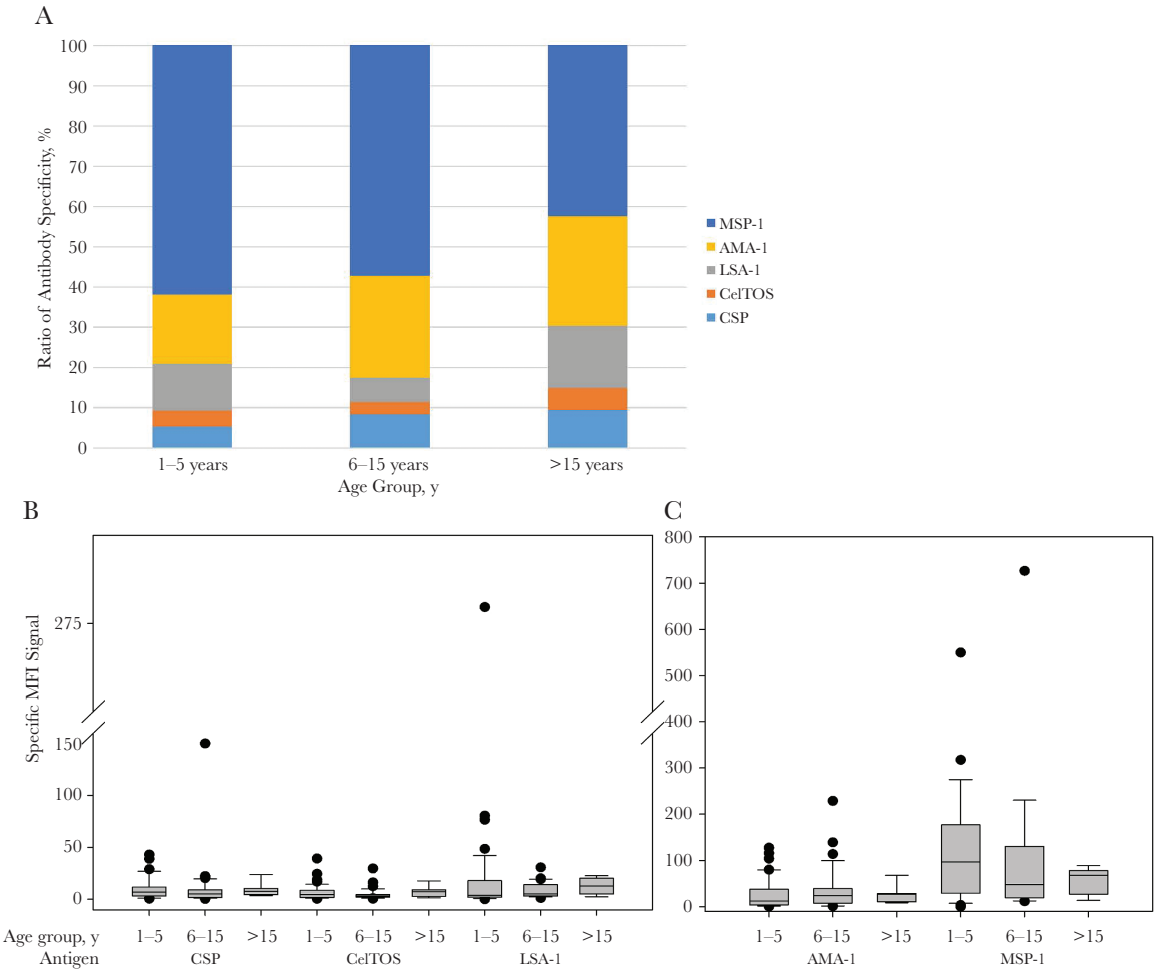

Figure 2. Antibody specificities by age group: in years: 1–5 years (n = 44), 6–15 years (n = 33), and >15 years (n = 4). Data obtained with Luminex technology are expressed as the ratio of the antigen-specific response to the total measured anti-*Plasmodium* response (100%). *A,* Stage-specific antibodies represent the majority of the anti-*Plasmodium* serological response. *C, D,* Levels of antibody to selected antigens tested did not change significantly with age in study participants. Abbreviations: AMA-1, apical membrane antigen 1; CelTOS, cell-traversal protein for ookinetes and sporozoites; CSP, circumsporozoite protein; LSA-1, liver-stage antigen 1; MFI, median fluorescence intensity; MSP-1, merozoite surface protein 1.

specificities and the parasite density or clearance rate (as shown by the separated clades in Figure 3). There was a significant correlation between age and clearance rate ($R^2 = 0.393$; $P = .002$).

Impact of Preexisting Immunity on Parasite Clearance Induced by Drug Treatment

We next sought to determine potential interactions between antibody titers, clearance rate (K) and $t_{½}$ in the 2 treatment arms (Figure 4 and Supplementary Table 1). There were 3 key observations. First, CSP-specific antibodies were positively correlated with $t_{½}$ (AL arm: $R^2 = 0.212$; $P = .09$; ASMQ arm: $R^2 = 0.357$; $P = .03$; Pearson correlation for all comparisons) but negatively with K (AL arm: $R^2 = -0.228$; $P = .16$; ASMQ arm: $R^2 = -0.391$; $P = .02$). Second, there was strong negative correlation between $t_{½}$ and K in both study arms (AL arm: $R^2 = -0.963$; $P < .001$; ASMQ arm: $R^2 = -0.95$; $P < .001$). Finally, $t_{½}$ was negatively correlated with age in the ASMQ study arm ($R^2 = -0.340$; P = .03).

Differences in the Antibody Profiles of Participants with Lag Phase

Monitoring the drug response and clearance rate revealed that some individuals in both study arms displayed a lag phase, that is, a period after the start of treatment when parasitemia continued to increase or, at a minimum, failed to decrease. The serological data were stratified by study group and within each study arm (AL vs ASMQ), for participants with and those without lag phase (Figure 5 and Supplementary Table 2). In Figure 5, correlograms summarize the relationship between the various parameters depending on the kinetic of response to drug treatment. Comparing the antibody levels to the 5 measured antigens in the 4 different groups revealed that (CSP antibody levels differed significantly ($P < .01$; ANOVA). In the AL group, although the difference did not reach significance, the

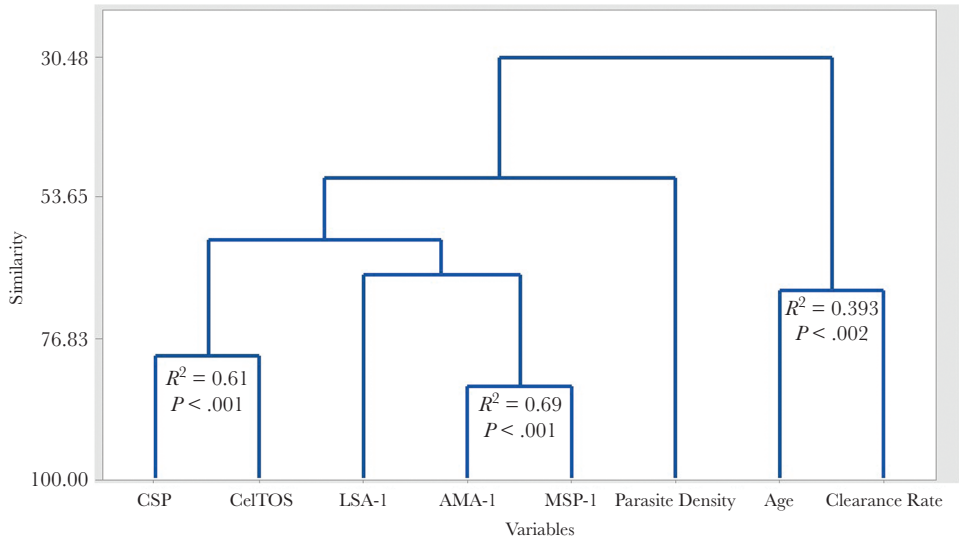

Figure 3. Impact of malaria-specific antibody levels on parasite density. Dendrogram demonstrates results of multivariate analysis investigating the relationship between age, pretreatment levels of antibodies specific to circumsporozoite protein (CSP), cell-traversal protein for ookinetes and sporozoites (CelTOS), liver-stage antigen 1 (LSA-1), apical membrane antigen 1 (AMA 1), merozoite surface protein 1 (MSP-1), clearance rate, and parasite density. The clades were arranged according to similarity between the variables. Clades that are close to the same height are similar to each other; clades with different heights are dissimilar—the greater the difference in height of the clades, the more dissimilar the variables.

levels of CSP-specific antibodies were higher in participants who did not have a lag phase than in those who did (P = .11; 2-sided t test). In the ASMQ group, there was no difference in the CSP antibodies between participants and those without a lag phase (P = .61; 2-sided t test). In addition, there were no significant differences in antibody levels specific for LSA-1, CelTOS, MSP-1, or AMA-1 between the AL and ASMQ arms, regardless of response to treatment (ie, lag phase).

Next, we sought to investigate the difference in the relationship between parasite clearance (ie, K and $t_{½}$) and antibodies. There was no correlation between CSP-specific antibodies from participants in the AL arm who had immediately responded to drug treatment (ie, no lag phase) and other specificities (Figure 5A). In participants with a lag phase, CSP-specific antibodies were correlated positively with CelTOS-specific antibodies (R^2 = 0.564; P < .001; Pearson correlation) and, at a nonsignificant level, with LSA-1–specific antibodies (R^2 = 0.178; P = .23; Pearson correlation) (Figure 5C). In the ASMQ arm, participants responding to treatment without a lag phase had significant positive correlations between all measured antibody specificities, including CSP (Figure 5B). In contrast, ASMQ-treated participants with a lag phase showed a strong negative correlation between MSP-1–specific and CSP-specific antibodies (R^2 = −0.755; P < .001, Pearson

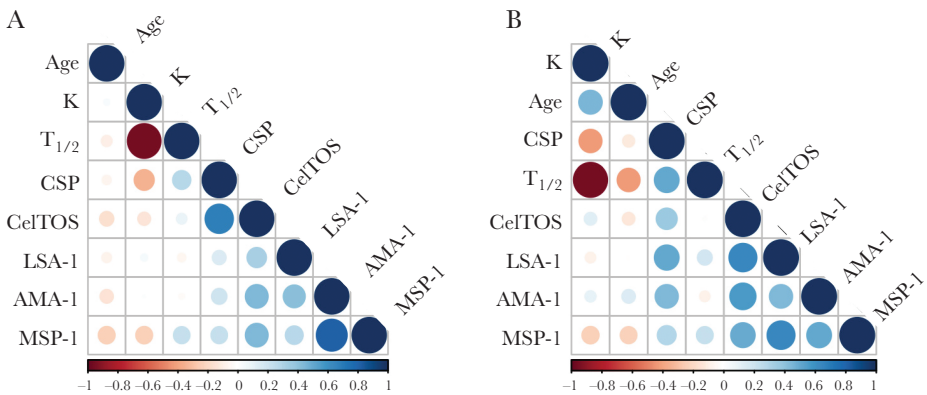

Figure 4. Impact of age and antibody titers on response to drug treatment. Correlograms summarize the relationship between prevalent antibodies, age and parasite clearance in participants treated with either artemether-lumefantrine (*A*) or artesunate-mefloquine (*B*). Color, size, and intensity of dots (scales below graphs) indicate level of correlation between factors. Abbreviations: AMA-1, apical membrane antigen 1; CelTOS, cell-traversal protein for ookinetes and sporozoites; CSP, circumsporozoite protein; *K*, clearance rate constant; LSA-1, liver-stage antigen 1; MSP-1, merozoite surface protein 1; $t_{½}$, half-life.

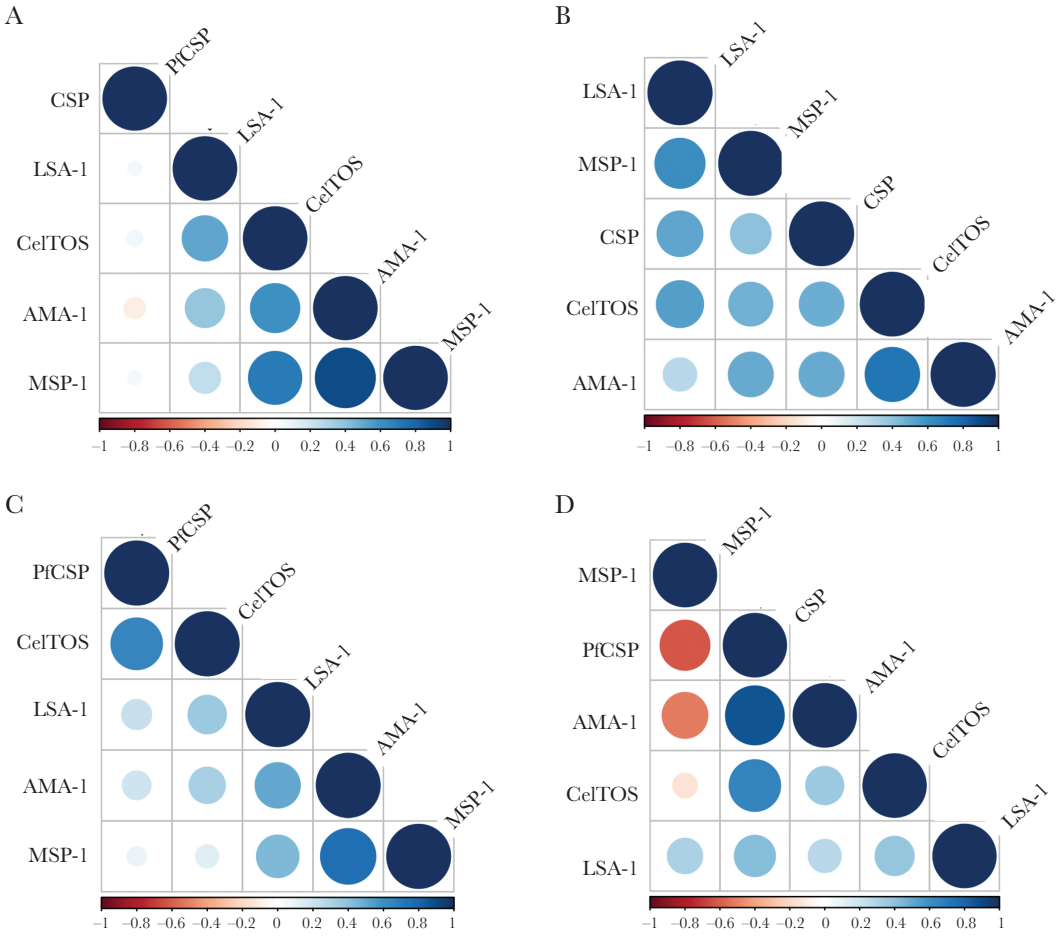

Figure 5. Correlation between antibody specificities in participants displaying lag phase after drug treatment. Data are stratified based on absence (*A, B*) or presence (*C, D*) of lag phase after treatment with either artemether-lumefantrine (*A, C*) or artesunate-mefloquine (*B, D*). Color, size, and intensity of dots (scales below graphs) indicate level of correlation between factors. Abbreviations: AMA-1, apical membrane antigen 1; CelTOS, cell-traversal protein for ookinetes and sporozoites; CSP, circumsporozoite protein; LSA-1, liver-stage antigen 1; MSP-1, merozoite surface protein 1.

correlation), and MSP-1– and AMA-1–specific antibodies ($R^2 = -0.46$; $P < .001$; Pearson correlation) (Figure 5D).

Differences in Antibody Profiles Depending on $t_{½}$ After Drug Treatment

Next, we sought to determine the impact of preexisting immunity on the $t_{½}$. Previous studies have used $t_{½}$ values as a cutoff in separating participants with distinct parasite clearance rates [31]. In the current study, participants were stratified as rapid or fast clearers based on their median $t_{½}$, as described elsewhere [32]. When we stratified participants based on their $t_{½}$ and compared antibody titers for the 5 tested antigens, the antibody levels to CSP and MSP-1 were significantly higher in rapid than in fast clearers in the AL group ($P = .01$ and $P = .02$, respectively; 2-sided *t* test). In the ASMQ arm, the difference between rapid and fast clearers was observed only for CSP-specific antibodies and did not reach statistical significance ($P = .07$; 2-sided *t* test).

We then investigated the correlations between antibodies in the different groups, using correlograms that summarize the interactions between the various parameters (Figure 6). We made 3 key observations: (1) the statistical relationship between the 5 antibody specificities for fast clearers are comparable between the 2 study arms (Figure 6A and 6B); (2) the statistical relationship between the 5 antibody specificities for rapid clearers differ between study arms (Figure 6C and 6D); and (3) there are negative correlations between MSP-1–specific antibodies and antibodies to CelTOS, MSP-1 and LSA-1 in rapid clearers treated with ASMQ (Figure 6D).

DISCUSSION

The present study identified the association between preexisting *Plasmodium falciparum*–specific immunoprofiles in individuals living in Western Kenya and the impact on parasite clearance mediated by treatment with AL or ASMQ. The patient immunoprofiles were established by measuring the levels of antibodies targeting preerythrocytic (CSP, LSA-1, and CelTOS) and erythrocytic (MSP-1 and AMA-1) antigens. AL has been the first-line treatment for uncomplicated malaria in Kenya

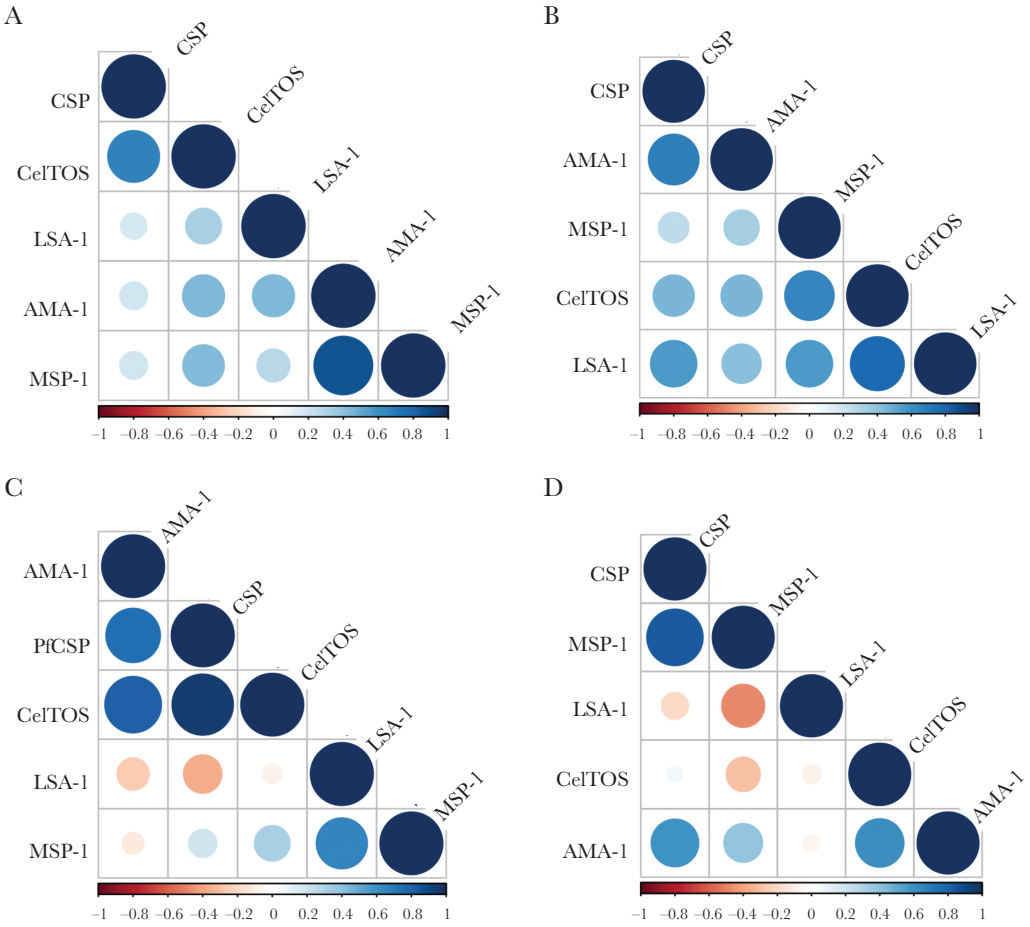

Figure 6. Profiles of preexisting antibodies differ in participants with rapid half-life. Participants were treated with either artemether-lumefantrine (*A, C*) or artesunate-mefloquine (*B, D*) and stratified, based on half-life ($t_{½}$), as rapid ($t_{½}$ ≤2.02 hours) (*C, D*) or fast ($t_{½}$ ≥2.02 hours) (*A, B*) responders to drug treatment. Color, size, and intensity of dots (scales below graphs) indicate correlation between factors. Abbreviations: AMA-1, apical membrane antigen 1; CelTOS, cell-traversal protein for ookinetes and sporozoites; CSP, circumsporozoite protein; LSA-1, liver-stage antigen 1; MSP-1, merozoite surface protein 1.

since 2006 [18], and it remains highly efficacious [33]. However, owing to the extensive use of this drug, it is important to monitor responsiveness to ensure there is no decline in its efficacy. Data presented here suggest that antibody profiles influenced treatment outcome differently after treatment with either AL or ASMQ, highlighting the importance of antibody profiling at a population level or level when considering implementation of new or different ACT options. For the first time, this study demonstrated the importance of CSP-specific antibodies to the $t_{½}$ in patients treated with different ACT regimens in malaria-holoendemic settings of sub-Saharan Africa.

Data from this study revealed a negative correlation between the clearance rate and the $t_{½}$, which may be due to the lag phase between treatment start and reduction of parasitemia. The number of individuals with lag phase was significantly higher in the AL than in the ASMQ study arm. Also notable was the strong positive correlation of CSP-specific antibodies with the $t_{½}$ but negative correlation with the clearance rate. This finding probably reflects an unrelated underlying association, because CSP is not expressed in blood-stage parasites. One possible explanation could be that individuals responding to drug treatment after a lag phase may harbor parasites that were not affected by CSP-specific antibodies at the time of infection. Alternatively, participants with a lag phase show a negative correlation between CSP- and MSP-1–specific antibody levels (Figure 4). Another striking difference in the lag-phase–stratified correlograms was a negative association between MSP-1– and AMA-1–specific antibodies in ASMQ-treated participants with a lag phase. In contrast, the level of antibodies to the 5 antigens tested correlated positively with each other in participants without a lag phase (both study arms), and in those with a lag phase after treatment with AL.

These results suggest that preexisting immunity modulates the response time after treatment with ASMQ, which significantly cleared parasites much faster than AL (R^2 = 0.7143; P = .004) (Supplementary Figure 1). Future studies should evaluate the interplay between CSP-, MSP-1–, and AMA-1–specific antibodies and why a potential antagonism is present when

participants are treated with ASMQ but not with AL. One hypothesis is that drug treatment stalls the maturation of infected cells, which may still allow MSP-1–specific antibodies to target the infected red blood cell for destruction. Because AMA-1 is expressed on mature merozoites at the time of rupture, these antibodies may have little access to their target during invasion.

The current study investigated the impact of preexisting immunity, as measured by malaria-specific antibody profiles, on the kinetics of antiparasite responses induced by either AL or ASMQ drug treatment. The $t_{½}$ was used for analysis because it is a standardized measure for parasite clearance not affected by the lag phase. Data revealed that the different antibody specificities were positively correlated with each other in participants who were fast clearers (with slower kinetics) in both study arms. In contrast, those who were rapid clearers (with faster kinetics) had a negative correlation with MSP-1, CSP, and LSA-1 in the ASMQ arm only.

The clear difference in the profile of participants treated with either AL or ASMQ, and the kinetics in which the drugs lead to clearance of the parasites suggest that different mechanisms are at play, owing to the difference in the pharmacokinetics of artemether and artesunate at administration [34–36]. Compared with artemether, which has a T_{max} of 3 hours, artesunate is quickly absorbed, with T_{max} detected within the first hour after dosing [35, 36], suggesting that artesunate is absorbed without appreciable delay. Individuals with a slower clearance rate may rely on the synergistic effect of antibodies supporting the clearance, as suggested elsewhere in an analysis of drug responses [37]. This assumes that AL and ASMQ affect the intraerythrocytic parasites differently; after treatment with ASMQ, parasites seem more susceptible to MSP-1–specific antibodies than after treatment with AL.

Existing reports on the immunological profiles of persons living in malaria-endemic areas have suggested that the breadth of the response and certain profiles are predictive of protection against the disease [11, 38, 39]. It has also been reported that the magnitude of the immunological response increases with age [40]. In the current study, data did not support age-dependent differences in the participants' antibody profiles (Figure 2). The high prevalence of malaria-specific antibodies even at a young age may be due to the holoendemicity of the study region, which has a high entomological inoculation rate [19, 41]. The lack of an age-distinct effect of malaria-specific antibody titers has also been reported elsewhere [42].

None of the 5 antibody specificities were negatively correlated with parasite density, which might be interpreted as evidence that none of these antigens play a role in the protecting against or reducing morbidity. However, it is important to consider that all the study participants were parasitemic and had clinical symptoms requiring drug treatment. The current findings do not disqualify any of the tested antigens as vaccine targets, because the fine specificity and the quality of the antibody (ie, avidity and isotype) play a critical role in determining its efficacy [43–46]. The current study was limited by the small number of *P. falciparum* antigens analyzed and the number of participants in subgroup analysis. Sample size calculations confirmed, however, that the subgroups were sufficiently large to validate conclusions. It will be important for future studies to address the impact of antibody isotypes and avidity and assess potential differences in the functional activity of antigen-specific antibodies.

In conclusion, the data presented here define immunoprofiles associated with distinct responses to different ACT drug regimens. The study showed that preexisting immunity can affect the efficacy of ACT regimens within a population living in a malaria-holoendemic area. A larger study in an area with changing and different transmission intensity, analyzing both symptomatic and asymptomatic individuals, would be important to further investigate the role of population immunoprofiles and different ACT regimens in malaria-endemic areas.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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