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Full Length Research Paper

Associations between IgG antibody responses to multiple *Plasmodium falciparum* antigens and treatment outcomes with ACTs in Man, Cote d'Ivoire

Yao SS[1], Offianan AT[1], Tiacoh NL[1], Ako AAB[1], Koffi D[1], Kouame E[1], Tuo K[1], Beourou S[1], Djaman J[2]

[1]Malariology Department, Institut Pasteur Côte d'Ivoire.
[2]Biochemestry Department Institut Pasteur/UFR Biosciences Félix Houphouët Boigny University.

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Successful antimalarial drugs treatment might be due to intrinsic susceptibility of *P. falciparum* and host's factors. In this study we assessed relationship between host IgG antibody responses and treatment outcome with Artesunate+Amodiaquine (AS+AQ) and Artemether+Lumefantrine (AL) in high transmission setting of Cote d'Ivoire. The study was done as part of a clinical drug-efficacy trial of AS+AQ and AL conducted at Man in western Côte d'Ivoire. Serum samples from the time of malaria diagnosis and 7 days later were analyzed for total IgG against schizonte extract (07/03) and 5 specific-*P. falciparum* Ags using a quantitative indirect ELISA. IgG antibody responses were related to treatment outcome. A total of 56 and 55 serum samples in AL and AS + AQ group respectively were available for antibody analysis, on day 0 and day 7. The levels IgG to CSP, GLURP and SALSA increased significantly with age (p<0.05). Antibody responses against all antigens decreased significantly, except SALSA and sch07/03 in day 7 after treatment, with the greatest decreasing seen in IgG response to GLURP (p= 0.0013). Adequate Clinical and Parasitological Response PCR corrected (ACPR) was of 98.3% (AS +AQ) and 100% (AL). Prevalence and levels of anti-Glutamate-rich Protein (GLURP) and anti-circumsporozoite (CSP)-specific IgG antibodies were significantly higher (P < 0.001) in Patients with ACPR in AS+AQ and AL group respectively. GLURP and CSP IgG antibody responses in Man region may contribute to malaria treatment success and support hypothesis that acquired immunity enhances clinical efficacy of antimalarial dugs.

Keywords: IgG Antibody responses, treatment outcome, ACT, Man, Côte d'ivoire

INTRODUCTION

Naturally acquired immunity to malaria has been shown to protect these individuals against malaria (Crompton et al., 2010; Gupta et al., 1999; Schofield and Mueller, 2006). Passive transfer of sera from chronically exposed individuals eliminated blood parasites in *P. falciparum*-infected patients (Bouharoun-Tayoun et al., 1995; Cohen et al., 1961).

Currently, artemether-lumefantrine (AL) and artesunate-amodiaquine (AS-AQ) are the only widely available drugs, which are recommended by most malaria endemic countries in the treatment of uncomplicated falciparum malaria (WHO; 2010). In Côte d'Ivoire, AS-AQ and AL have been recommended respectively in first- and second line treatment since 2005.

There is a considerable inter-individual variability in parasite clearance mechanism. Efficacy of antimalarials is influenced by factors such as parasite life stage, pharm-

Corresponding Author E-mail: yaosergestephane@yahoo.fr
Tel. (00225) 40 33 57 83/ (00225) 49 45 91 03

acokinetic profile and patient immune response levels (Nébié et al., 2008; White, 2011).
This immunity is slowly established after repeated exposure of the infection (Bouharoun-Tayoun et al., 1990). In addition, parasite clearance is slower in low transmission and relatively quick in high-transmission areas, suggesting that acquired immunity contributes to parasite elimination (Stepniewska et al., 2010). However, the association between the levels of immunoglobulins (IgG) specific to *P. falciparum* and the reduction of the risk of infection of malaria is debated (Biswas et al., 2014; Bejon et al., 2011; Wilson et al., 2013). Antibodies against *P. falciparum* antigens can target the sporozoite stage leading to a subsequent reduction in transmission and infection: the multiplication of parasites is reduced, as are the clinical signs of malaria (Fowkes et al., 2010; Chan et al. 2014). Treatment efficacy improves with age and intensity of transmission, suggesting that acquisition of immunity may play a role in determining the efficacy of antimalarial treatment (Staedke et al., 2004; Francis et al., 2006; Greenhouse et al., 2009). In this regard, previous work in Africa, mainly in high-transmission areas, has noted various associations between immunity and failure of treatment with sulfadoxine pyrimethamine, chloroquine and ACTs (Van Geertruyden et al., 2009 Borrmann et al., 2011; Diarra et al., 2012). The aim of this study was to assess contribution of immunity to treatment outcomes in patients treated with two ACTs by comparing total IgG responses of patients with failure, or adequate clinical and parasitological response.

MATERIALS AND METHODS

Study population and samples

The study was done as part of a clinical drug-efficacy trial in collaboration with the National Malaria Control Programme in Côte d'Ivoire. The trial was conducted from January to May 2016 in Man. Man islocated in western Côte d'Ivoire. *Plasmodium falciparum* transmission is intense and perennial, with recrudescence during the rainy, season. The main vectors are *Anopheles gambiae* and *Anopheles funestus*. *Plasmodium falciparum* is the predominant malaria parasite, accounting for more than 95% of infections in children under five years of age (Offianan et al., 2014). The number of bites per person per night (Entomological Inoculation Rate, EIR) due to An. Gambiae is 4,21 b/p/n.
Malaria control strategies mainly based on insecticide-treated nets and treatment of clinical cases. The use of insecticide-treated nets in this area remains low.
The efficacy study was designed to enroll 120 patients aged at least six months to presenting with uncomplicated malaria if they met the criteria as defined in the standard efficacy testing protocol by WHO (WHO; 2003) body weight ≥5 kg; a history of fever in the previous 24 hours or measured fever (axillary temperature ≥37.5°C or rectal ≥38°C); mono-infection with *P. falciparum*, with parasite density between 2,000–200,000 asexual parasites per microlitre of blood; no other cause of fever than suspected malaria; and no general danger signs or signs of severe and complicated falciparum malaria as per WHO guidelines (WHO; 2000); able to take study drugs by the oral route; able to attend clinic on stipulated days for follow-up; and signed informed consent (by patient or responsible caregiver).
Exclusion criteria consisted of: presence of severe and complicated malaria as defined by WHO; a mixed plasmodial infection, or concomitant disease masking assessment of the response to anti-malarial treatment; full course of AS-AQ or AL in the past 7 days; and known hypersensitivity to any of the study drugs.
Patients were randomly assigned to receive either AS-AQ or AL. Both treatments were three-days oral regimens dosed by weight according to the manufacturer's instructions: AS-AQ 5 to <9 kg: one tablet/day of artesunate (AS) 25 mg/amodiaquine (AQ) 67.5 mg; 9 to <18 kg: one tablet/day of AS 50 mg/AQ 135 mg; 18 to <36 kg: 1 tablet/ day of AS 100 mg/AQ 270 mg; ≥36 kg: 2 tablets/day of AS 100 mg/AQ 270 mg.
AL tablet strength was 20 mg artemether/120 mg lumefantrine: 5 to <15 kg: 1 tablet/dose; 15
to<25 kg: 2 tablets/dose; 25 to <35 kg: 3 tablets/dose; ≥35 kg 4 tablets/dose. AL was administered twice a day.
Treatment outcomes were classified according to the WHO guidelines for areas of intense transmission as adequate clinical and parasitological response (ACPR), early treatment failure (ETF), late clinical failure (LCF) and late parasitological failure (LPF) (Who, 2003). Failure was defined as the sum of ETF, LCF and LPF. For the purpose of this study, patients with ETF, LCF and LPF were grouped as treatment failures (TF). In order to distinguish recrudescence from reinfection, blood samples collected on filter paper were assessed with nested PCR technique as described previously (Offianan et al., 2014).
Five ml of venous blood was withdrawn on Day 0 and day 7 into an EDTA tube from each participant and the plasma obtained was aliquoted and stored at -20°C until further analysis

Antigens and peptides

Peptides derived from liver (CSP, LSA3, SALSA) and blood stage antigens (GLURP, AMA1) of *Plasmodium falciparum* were used to measure antibody responses. The antigen description is summarized in Table 1.

IgG Antibody Testing by Enzyme-Linked Immunosorbent Assay (ELISA)

IgG responses were quantified by ELISA in duplicate sera samples diluted 1:100 as previously described using

Table 1. Antigens et peptides.

Antigen	Protein or peptides	Sequence and/or Reference	Stage of expression	Antigenfrom
CSP	Peptide	ANPNANPNANPNANPNVDPNVDPC	sporozoite/hepatic stage	*P. falciparum*
LSA3	Peptide	VLEESQVNDDIFNSLVKSVQQEQQHNVC	hepatic stage	*P. falciparum*
SALSA	Peptide	SAEKKDEKEASEQGEESHKKENSQESAC	sporozoite/hepaticstage	*P. falciparum*
GLURP	Peptide	EDKNEKGQHEIVEVEEILC	merozoite/schizont	*P.falciparum*
AMA1	Peptide	YKDEIKKEIERESKRIKLNDNDDEGNKKIIAPRIFISDDKDSLKC	merozoite/schizont	*P.falciparum*

specific Ags (peptides) and whole parasite extract sch 07/03 (Dielmo strain adapted to culture) (Niang et al., 2017; Diop et al., 2014; Osier et al., 2008; Bonnet et al., 2006; Aribot et al., 1996). In each plate, pool of sera from adults living in the village of Dielmo, immune IgG (kind gift from Prof M Hommel) and pools of European and African non-immune was used as positive and negative controls respectively. Results were expressed as OD ratio = OD sample/OD naive serum pool (Aribot et al., 1996; Perraut et al., 2003). Sera showing an OD ratio >.2 corresponding to the signal of naive controls + 2 SD were considered sero-positive for prevalence calculations.

Statistical Analysis

Antibody levels and prevalence of responders in different groups were compared using respectively the Mann–Whitney, the Spearman rank correlation test for non normally distributed paired data and the Fisher exact test. Statistical analyses were performed using Prism (graph pad….). For all the tests, *p value* < 0.05 was considered significant.

Ethical Statement

Written informed consent for participation in the study was obtained from adult's patients or children's parents or guardians, in accordance with the Declaration of Helsinki. Approval was granted by National Research Ethic Committee of Côte d'Ivoire. As sentiment was required from young children (10-17 years) in addition to their parent's or guardian consent.

RESULTS

Study profile

Over the course of the study, 843 total patients were screened and 120 (60 in each group) patients were randomized in the clinical trial. Patients were followed up to 42 days. Samples from 56 and 55 participants in AL and AS + AQ group respectively, meeting inclusion criteria were available for antibody analysis, on day 0 and day 7 (Figure 1).

Characteristics of Patients

The mean age was 6.8 and 12.14 years in AS +AQ and AL group respectively (p=0.001).
Body temperature (°C) mean was 38.42 ± 0.4710 and 38.31 ± 0.4446 in AS+AQ and AL group respectively. The mean parasiteamia was high in AS+AQ group (37 935 asexual parasites/µl) than in AL (16031 asexual parasites/µl) group with a range between 2100 and 177866 asexual parasites/µl. A total of 12 patients with Sickle cell trait (HbAS) were included in the study (Table 2).

Drug efficacy in the treatment of uncomplicated Plasmodium falciparum malaria

The overall failure rate for both drugs was 6.8% (4 patients) and 6.9% (4 patients) for AS+AQ and AL group with an Adequate Clinical and Parasitological Response PCR corrected (ACPR) of 98.3% (AS +AQ) and 100% (AL).

Relationship between age, parasiteamia and antibody levels at D0

In this study, the relationship between antibody levels and age were assessed. The levels of IgG to CSP, GLURP and SALSA increased significantly with age (p<0.05) (Table 3).

Levels of IgG to AMA1 decreased with age

There was no correlation between parasitaemia observed at recruitment with antibody responses against all antigens except a significant negative correlation with IgG responses to AMA1 (p<0.05) rho=-0.194 and GLURP (p<0.05), rho=-0.265.

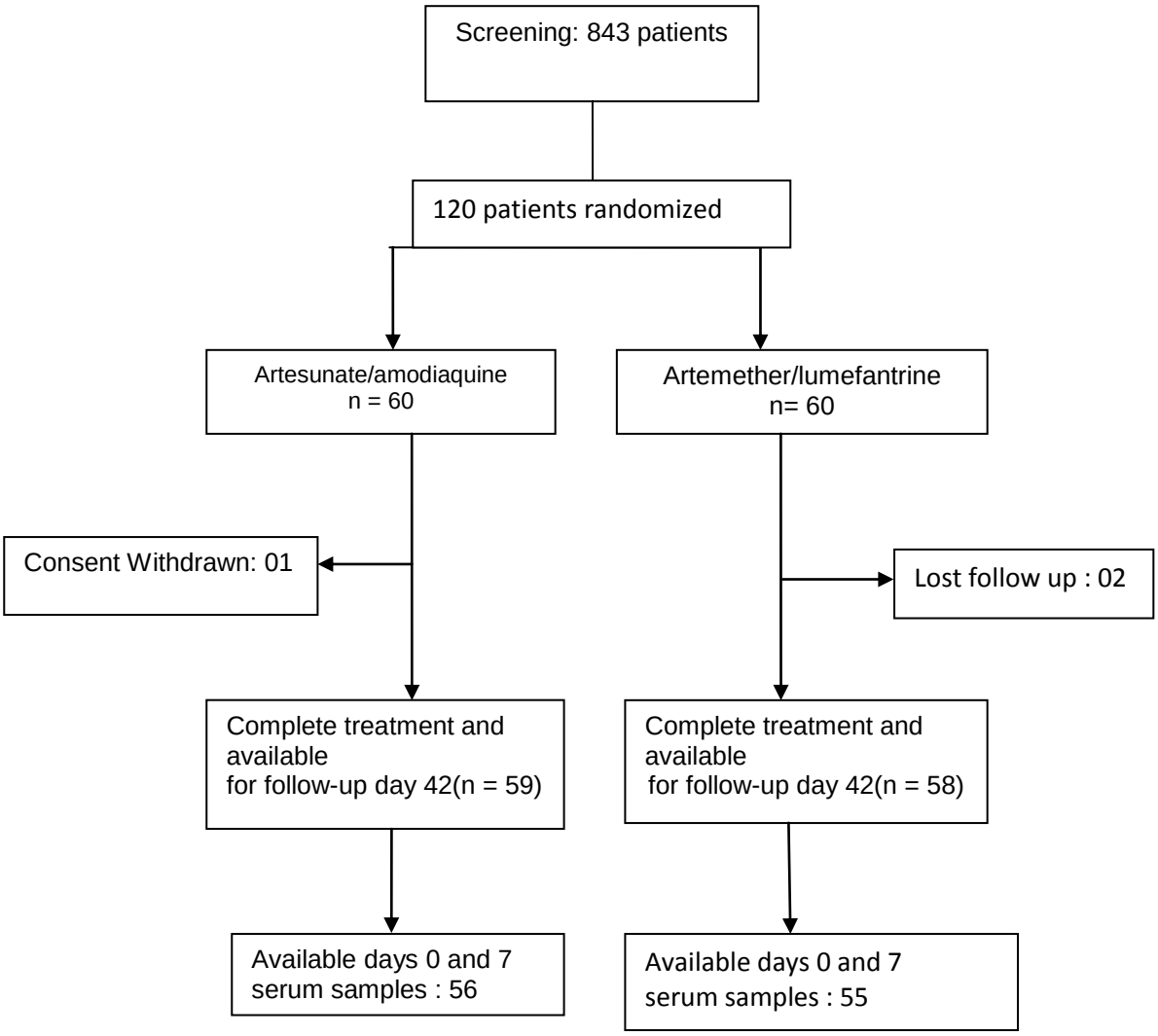

Figure 1. Trial Profile.

Antibody Responses at the Time of Malaria Diagnosis and 7 Days Later

Level and prevalence of antibody responses against the 6 peptides are summarized in Table 2. Prevalence levels were highly variable, ranging from 53.1 % (IgG to SALSA) to 74.7 % (IgG to AMA1).
Levels of antibody responses at days 0 and 7 are stated as Mean values and range [minimum–maximum] ODratio. The level of Ab responses against all antigens decreased significantly, except SALSA and sch07/03 in day 7 after treatment, with the greatest decreasing seen in IgG response to GLURP (p= 0.0013) (Table 4).

Association of antibody levels and efficacy of each drug in the treatment of uncomplicated *Plasmodium falciparum* malaria

To investigate whether the treatment efficacy for each of the drugs was associated with a background of immunity, antibody responses against all *falciparum* antigens (CSP, AMA-1, LSA 3, GLURP, SALSA, and sch07/03) were compared according to each drug's efficacy.
The relationship between immune responses and treatment efficacy was analyzed by stratifying outcome as RCPA and Failure. Table 5 and 6 are shown level of Ab responses according treatment outcome with each drug.
Prevalence and levels of anti-Glutamate-rich Protein (GLURP) and anti-cirsumsporozoite (CSP)-specific IgG antibodies were significantly higher ($P < 0.001$) in Patients with ACPR in AS+AQ and AL group respectively. Level of Glurp IgG antibodies at day 0 was 4.35 [3.3.-5.73] and 1.92 [0.48-7.77] in Patients with ACPR and failure in AS+AQ group (Table 6). In AL group level of CSP IgG antibodies was also higher in patients with RCPA (2.76; 2.24-3.40) than those with treatment failure (1.17; 1.03-1.33).

DISCUSSION

The study was designed to assess, using standardized

Table 2. Baseline characteristics of study participants.

PARAMETERS	ASAQ (n=60)	AL (n=60)	p-value
Meanage (Years)	6,8	12,14	0,001
6 months—<5 years	34	20	0,14
5-<15 yrs	21	22	1
≥15 yrs	5	18	0,004
Gender (M/F)	32/28	29/31	0,58
Male [n (%)]	32 (53.33)	29 (48.33)	0.6988
Female [n (%)]	28 (46.67)	31 (51.67)	0.7037
Meanweight	23.76	32.47	0.29
Body temperature [°C, mean (SD)]	38.42± 0.4710	38.31± 0.4446	0.60
Range	(38.20-38.43)	(38.30-38.55)	
Parasite density [parasite/µL, mean (SD)]	37 935±6271	16031± 3058	0,0092
Range	(2100-177866)	(2013–156230)	
Sicklecell trait (HbAS)[n (%)]	7 (11.66)	5 (8.33)	

Table 3. Relationship between age and antibody.

Antigen	Age groups (year)	N	RtDO (D0) Geo.means (95% IC)
AMA1	[0 - 5[	56	3.50 (2.92 – 4.19)
	[6 - 15[	33	2.96 (2.18 – 4.02)
	≥ 15	22	2.61 (1.80-3.77)
	p		0.1716
CSP	[0 - 5[	56	2.37 (1.94 – 2.90)
	[6 - 15[	33	2.83 (2.20 – 3.65)
	≥ 15	22	3.93 (2.77 – 5.58)
	p		**0.0241**
GLURP	[0 - 5[	56	3.43 (2.54 – 4.62)
	[6 - 15[	33	7.11 (4.66 – 10.86)
	≥ 15	22	9.64 (15.49 – 16.92)
	p		**0.0009**
LSA3	[0 - 5[	56	2.35 (1.92 – 2.86)
	[6 - 15[	33	2.34 (1.88 – 2.90)
	≥ 15	22	2.45 (1.59 – 3.79)
	p		0.9115
SALSA	[0- 5[	56	1.91 (1.67 – 2.18)
	[6 - 15[	33	2.74 (2.13 – 3.52)
	≥ 15	22	2.92 (2.19 – 3.91)
	p		**0.0025**
sch07/03	[0 - 5[	56	2.20 (1.97 – 2.45)
	[6 -15[	33	2.55 (2.26 – 2.88)
	≥ 15	22	2.40 (1.80 – 3.18)
	p		0.3096

Geo.means: geometric means The Kruskal-Wallis test was used.

methods, association of antibody levels to six malaria antigens (CSP, AMA-1, LSA 3, GLURP, SALSA, and EXTRAIT) with response to antimalarial drugs therapy.

The impact of immunity on treatment outcome have however resulted in conflicting findings (Robert et al., 2000; Mayxay et al., 2001; Mawili-Mboumba et al., 2003; Pinder et al.,

Table 4. Antibody prevalence and level at day 0 and day 7.

	Antibody Prevalence % (95% CI)			**Antibody level Geometric mean (range)**		
Antigen	**D0**	**D7**	**P**	**D0**	**D7**	**p**
CSP	60.3 (50,3 - 69.5)	56.7 (47.0 - 66.1)	0.2926	2.7 (2.4 - 3.2)	2.5 (2.2- 2.9)	0.1235
AMA1	74.7 (65.6 -82.5)	65.7 (56.1 -74.5)	0.0921	3.1 (2.7 - 3.6)	2.7 (2.3 - 3.0)	0.0002
LSA3	54.9 (45.2- 64.4)	44.1 (34.7 - 53.8)	0.0699	2.3 (2.0 - 2.7)	1.8 (1.6 - 2.1)	< 0.0001
GLURP	72.0 (62.7 - 80.1)	55.8 (46.1 -65.2)	0.004	5.2 (4.1 - 6.6)	2.8 (2.2 - 3.6)	0.0013
SALSA	53.1 (43.4 - 62.6)	61.2 (51.5 - 70.3)	0.111	2.3 (2.0 - 2.6)	2.5 (2.2 - 2.9)	0.0152
sch07/03	66.6 (57.0 -75.3)	76.5 (67.5 - 84.0)	0.0692	2.3 (2.1 - 2.6)	2.7 (2.5 - 3.0)	< 0.0001

Table 5. Association between antibody response and treatment outcome in AL group.

	AL group		
Antigen	Treatmentfailure Geo. means (95% IC) N =4	ACPR Geo. means (95% IC) N=52	p
Ac-AMA1 D0	3.81 (1.15-12.60)	2.82 (2.21-3.60)	0.2571
Ac-AMA1 D7	2.80 (0.93-8,41)	2.79 (2.25-3.45)	0.4494
Ac-CSP D0	1.17 (1.03-1.33)	2.76 (2.24-3.40)	0.0097
Ac-CSP D7	1.75 (0.74-4.07)	3.10 (2.46-3.86)	0.0807
Ac-LSA3 D0	2.42 (0.54-10.69)	2.11 (1.72-2.60)	0.4431
Ac-LSA3 D7	2.08 (0.47-9.15)	1.74 (1.37-2.21)	0.3572
Ac-GLURP D0	3.93 (0.30-52.18)	6.92(4.68-10.22)	0.1865
Ac-GLURP D7	1.39 (0.70-2.74)	2.09 (1.40-3.13)	0.2728
Ac-sch07/03D0	2.33 (1.37-3.96)	2.58 (2.24-2.96)	0.3995
Ac-sch07/03D7	3.17 (1.21-8.26)	3.00 (2.62-3.44)	0.3632
Ac-SALSA D0	2.42 (1.17-5.01)	2.48 (2.08-2.96)	0.4620
Ac-SALSA D7	2.08 (1.20-3.63)	2.40 (1.97-2.94)	0.3513
Age (years)	3.40±1.40	13.76±1.86	0.1250
Parasite density D0 (asexual parasites/µl)	22296±10367	15677±3423	0.4375

2006; Aubouy et al., 2007).We examined in an area of intense transmission of P. falciparum in Côte d'Ivoire during a drug trial with Artesunate + amodiaquine (AS+AQ) or Artemether + lumefantrine (AL) the hypothesis that there is a synergy between drug treatment and acquired malaria immunity.

The study showed that antibody levels to IgG to CSP, GLURP and SALSA increased with age as demonstrated by

Table6. Association between antibody response and treatment outcome in AS + AQ group.

Antigen	ASAQ group		
	Treatment failure Geo. means (95%IC) N=4	**ACPR Geo. means (95%) N=51**	**p**
Ac-AMA1 D0	3.46 (1.23-9.74)	3.44 (2.9-4.13)	0.4871
Ac-AMA1 D7	2.21 (1.01 4.81)	2.62 (2.15-3.18)	0.3020
Ac-CSP D0	2.40 (0.7-8.28)	3.00 (2.40-4.82)	0.5595
Ac-CSP D7	2.82 (0.55-14.57)	2.1 (1.67-2.63)	0.5380
Ac-LSA3 D0	5.6 (4.67-6.71)	2.49 (2.01-3.07)	0.0079
Ac-LSA3 D7	2.13 (0.80-0.71)	1.90 (1.58-2.29)	0.3075
Ac-GLURP D0	1.92(0.48-7.77)	4.35 (3.30-5.73)	0.0659
Ac-GLURP D7	1.25 (0.85-1.84)	4.33 (314-5.97)	0.0259
Ac-sch07/03 D0	2.67(2.06-3.47)	2.11 (1.88-2.356)	0.0561
Ac-sch07/03 D7	2.82 (2.12-3.74)	2.51 (2.49-2.80)	0.1424
Ac-SALSA D0	1.73 (1.13-2.65)	2.21 (1.85-2.64)	0.2743
Ac-SALSA D7	1.88 (0.46-7.43)	2.78 (2.22-3.47)	0.1574
Age (years)	4 ± 0.91	6.40 ± 0.98	0.3125
Parasite density D0 (asexual parasites/µl)	20791 ± 12893	37958 ± 6849	0.1250

others results (Tongren et al., 2006; Dodoo et al., 2008; Segeia et al., 2010).

Our results confirmed the hypothesis that malaria immunity is largely effected through antibody-mediated mechanisms and that protective antibody levels to relevant antigens increase with age related exposure to the parasites.

During this study, eight (8) patients failed treatment with four (4) per treatment arm. To investigate whether the level of antibody induced by each antigen tested in our study was associated with treatment failure (ASAQ orAL), we compared the antibody levels produced by each antigen on day 0 and day 7 of patients whose treatment failed to those whose treatment succeeded. The results showed that with AL, only the level of antibody produced by the CSP antigen on day 0 was high in the patients with ACPR compared to that induced by those with treatment failure. In the ASAQ arm, it was on D7 that there was a remarkable difference. Same observation has been made with anti-GLURP antibodies in AS+AQ group.

These results suggested that anti-CSP and GLURP antibodies would have a synergistic effect and would be associated with a low recrudescence (re-infestation) during the treatment of malaria with these two ACTs. Several studies in malaria endemic areas in Africa have reached the same conclusion. The work of the Enevold and Diarra teams respectively in 2007 and 2012 showed that the anti-GLURP antibody would be associated with a low recrudescence (re-infestation) during the treatment of malaria, from which it would play a protective role (Enevold et al., 2007; Diarra et al., 2012). Adu and collaborators have shown during their study that anti-GLURP-R2 antibody is associated with protection against malaria (Adu et al., 2016). However, our results differ from those of Keh et al.(2012) who have shown that only the anti-AMA1 antibody would protect against treatment failures among eight antigens tested during malaria treatment with either Amodiaquine or Sulfadoxine-pyrimethamine. Other studies are demonstrated the role of anti-MSP1-19 antibody in treatment outcome with chloroquine (Aubouy et al., 2006; Pinder et al., 2006).

Differences in result between studies could be due to varying efficacy and pharmacokinetics; differences in the epidemiological setting and patient characteristics, different means of assessing treatment outcome and the use of different methods.

Genetic factors such as sickle cell trait (HbAS), haemoglobin E (Hutagalung et al., 2000, Adjei et al., 2014) and αthalass-

aemia (Terlouw et al., 2002; Mockenhaupt et al., 2001) could also affect antimalarial treatment outcome. In this study only sickle cell trait test has been done in all the patients. The twelve patients in our study with sickle cell trait had ACPR. Sickle cell genotype did not influence the treatment efficacy of artemisinin-based combination Therapy as demonstrate by studies conducted in Ghana and DRC (Adjei et al., 2014; N'Dounga et al., 2015) In a study conducted in Kenya, efficacy of sulfadoxine–pyrimethamine in children with HbAS was increased compared with children with normal haemoglobin (Terlouw et al., 2002). Moreover for intermittent preventive treatment of malaria in children, studies have shown that sulfadoxine-pyrimethamine (SP) is more effective than CQ in paediatric carriers of HbAS (Nakibuuka et al., 2009).

Our study have some limitations. Firstly HIV test was not done. Studies on effect of infection with HIV on the outcome of treatment with antimalarial drugs showed sometimes that HIV infection decreased response to treatment with antimalarial drugs (Brentlinger et al., 2007).

Secondly Measuring IgG1-subclass or IgG3-subclass responses to recombinant antigens might provide more information than total IgG responses (Rogerson et al., 2010).

CONCLUSION

Ac-anti GLURP and CSP may be associated with AL and ASAQ treatment outcome respectively in Man region of Côte d'Ivoire.

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RFERENCES

Adjei GO, Goka BQ, Enweronu-Laryea CC, Rodrigues OP, Renner L, Sulley AM, Alifrangis M, Khalil I, Kurtzhals JA (2014). A randomized trial of artesunate-amodiaquine versus artemether-lumefantrine in Ghanaian paediatric sickle cell and non-sickle cell disease patients with acute uncomplicated malaria. Malar J, 13:369.

Adu B, Cherif MK, Bosomprah S, Diarra A, Arthur FKN, Dickson EK, Corradin G, Cavanagh DR, Theisen M, Sirima SB, Nebie I, Daniel Dodoo D (2016). Antibody levels against GLURP R2, SP1 block 2 hybrid and AS202.11 and the risk of malaria in children living in hyperendemic (Burkina Faso) and hypo-endemic (Ghana) areas Bright Malar J 15:123 DOI 10.1186/s12936-016-1146-4.

Aribot G, Rogier C, Sarthou JL, Trape JF, Balde AT, Druilhe P, Roussilhon C (1996). Pattern of immunoglobulin isotype response to Plasmodium falciparum bloodstage antigens in individuals living in a holoendemic area of Senegal (Dielmo, West Africa). Am J Trop Med Hyg. 54:449–57.

Aubouy A, Migot-Nabias F, Deloron P (2007). Correlations between treatment outcome and both anti-MSP119 antibodyresponse and erythrocyte-related genetic factors in Plasmodium falciparum malaria. Infect Genet Evol 7: 147– 154. doi:10.1016/j.meegid.2006.07.001.

Bejon P, Cook J, Bergmann-Leitner E, Olotu A, Lusingu J, Mwacharo J, Vekemans J, Njuguna P, Leach A, Lievens M, Dutta S, von Seidlein L, Savarese B, Villafana T, Lemnge MM., Cohen J, Marsh K, Corran PH, Angov E, Riley EM, Drakeley CJ, (2011). Effect of the pre-erythrocytic candidate malaria vaccine RTS,S/AS01E on blood stage immunity in young children. J. Infect. Dis. 204, 9–18.

Biswas S, Choudhary P, Elias SC, Miura K, Milne K.H, de Cassan SC, Collins KA, Halstead FD, Bliss CM., Ewer KJ, Osier FH, Hodgson SH, Duncan CJA, OHara GA, Long CA, Hill AVS, Draper SJ (2014). Assessment of humoral immune responses to blood-stage malaria antigens following ChAd63-MVA immunization controlled human malaria infection and natural exposure. PLoS One 9, http://dx.doi.org/10.1371/journal.pone.0107903.

Bonnet S, Petres S, Holm I, Fontaine T, Rosario S, Roth C, Longacre S (2006). Soluble and glyco-lipid modified baculovirus*Plasmodium falciparum* C-terminal merozoite surface protein 1, two forms of a leading malaria vaccine candidate. Vaccine. 24:5997–6008.

Borrmann S, Sasi P, Mwai L, Bashraheil M, Abdallah A, Muriithi S, Fruhauf H, Schaub B, Pfeil J, Peshu J, Hanpithakpong W, Rippert A, Juma E, Tsofa B, Mosobo M, Lowe B, Osier F, Fegan G, Lindegardh N, Nzila A, Peshu N, Mackinnon M, Marsh K (2011). Declining responsiveness of *Plasmodium falciparum* infections to artemisinin-based combination treatments on the Kenyan coast. PLoS One 6(11):e26005.

Bouharoun-Tayoun H, Attanath P, Sabchareon A, Chongsuphajaisiddhi T, Druilhe P (1990). "Antibodies that protect humans against *Plasmodium falciparum* blood stages do not on their own inhibit parasite growth and invasion in vitro, but act in cooperation with monocytes." J Exp Med. 172(6): 1633-41.

Bouharoun-Tayoun H, Oeuvray C, Lunel F, Druilhe P (1995). Mechanisms underlying the monocyte-mediated antibody-dependent killing of *Plasmodium falciparum* asexual blood stages. J Exp Med. 182: 409–418. Epub 1995/08/01. PMID: 7629503.

Brentlinger PE, Behrens CB, Kublin JG (2007). Challenges in the prevention, diagnosis, and treatment

of malaria in human immunodeficiency virus infected adults in sub-Saharan Africa. Arch Intern Med. 167: 1827–36.

Chan JA, Fowkes FJI, Beeson JG (2014). Surface antigens of *Plasmodium falciparum* infected erythrocytes as immune targets and malaria vaccine candidates. Cell Mol Life Sci 71(19):3633–3657.

Cohen S, McGregor IA, Carrington S (1961). Gamma-globulin and acquired immunity to human malaria. Nature 192:733–737.

Crompton PD, Miura K, Traore B, Kayentao K, Ongoiba A, Weiss G, Doumbo S, Doumtabe D, Kone Y, Yu Huang C, Doumbo OK, Miller LH, Long CA, Pierce SK (2010). In vitro growth-inhibitory activity and malaria risk in a cohort study in Mali. Infect Immun. 78: 737–745. Epub 2009/11/18. doi: 10.1128/IAI.00960-09 PMID: 19917712.

Diarra A, Nebie I, Tiono A, Soulama I, Ouedraogo A, Konate A, Theisen M, Dodoo D, Traore A Sirima SB (2012). Antibodies to malaria vaccine candidates are associated with chloroquine or sulphadoxine/ pyrimethamine treatment efficacy in children in an endemic area of Burkina Faso Malar J. 11:79 http://www.malariajournal.com/content/11/1/79.

Diop F, Richard V, Diouf B, Sokhna C., Diagne N, Trape JF, Faye MM, Tall A, Diop G, Balde AT (2014). Dramatic declines in seropositivity as determined with crude extracts of *Plasmodium falciparum*schizonts between 2000 and 2010 in Dielmo and Ndiop, Senegal Malar J. Mar 6;13:83. doi: 10.1186/1475-2875-13-83.

Dodoo D, Aikins A, Kusi KA, Lamptey H, Remarque E, Milligan P, Bosomprah S, Chilengi R, Osei YD, Akanmori BD, Theisen M (2008). Cohort study of the association of antibody levels to AMA1, MSP119, MSP3 and GLURP with protection from clinical malaria in Ghanaian children. Malar J. 7:142

Enevold A, Alifrangis M, Sanchez JJ, Carneiro I, Roper C, Børsting C, Lusingu J, Vestergaard LS, Lemnge MM, Morling N, Riley E, &Drakeley CJ (2007). Associations between alpha+-thalassemia and *Plasmodium falciparum* malarial infection in northeastern Tanzania. J Infect Dis. Aug 1; 196(3):451-9.

Fowkes FJ, Richards JS, Simpson JA, Beeson JG (2010). The relationship between antimerozoite antibodies and incidence of *Plasmodium falciparum* malaria: A systematic review and meta-analysis. PLoS Med 7(1):e1000218.

Francis D, Nsobya SL, Talisuna A, Yeka A, Kamya MR, Machekano R, Dokomajilar C, Rosenthal JP Dorsey G (2006). Geographic differences in antimalarial drug efficacy in Uganda are explained by differences in endemicity and not by known molecular markers of drug resistance. J Infect Dis.;193:978–86.

Greenhouse B, Slater M, Njama-Meya D, Nzarubara B, Maiteki-Sebuguzi C, Clark TD, Staedke SG, Kamya MR, Hubbard A, Rosenthal PJ, Dorsey G (2009). Decreasing efficacy of antimalarial combination therapy in Uganda is explained by decreasing host immunity rather than increasing drug resistance. J Infect Dis. 199:758–65.

Gupta S, Snow RW, Donnelly C, Newbold C (1999). Acquired immunity and postnatal clinical protection in childhood cerebral malaria. ProcBiol Sci. 266: 33–38. Epub 1999/03/19. PMID: 10081156.

Hutagalung R, Wilairatana P, Looareesuwan S, Brittenham GM, Gordeuk VR (2000). Influence of hemoglobin E trait on the antimalarialeffect of artemisininderivatives. J Infect Dis; 181: 1513–16.

Keh CE, Jha AR, Nzarubara B, Lanar DE, Dutta S, Theisen M, Rosenthal PJ, Dorsey G, Nixon DF, Greenhouse B (2012). Associations between Antibodies to a Panel of *Plasmodium falciparum* Specific Antigens and Response to Sub-Optimal Antimalarial Therapy in Kampala,Uganda PLoS ONE 7(12):.https://doi.org/10.1371/journal.pone.0052571.

Mawili-Mboumba DP, Borrmann S, Cavanagh DR, McBride JS, Matsiegui PB, Missinou MA, Kremsner PG, Ntoumi F (2003). Antibody responses to *Plasmodium falciparum* merozoite surface protein-1 and efficacy of amodiaquine in gabonese children with *P.falciparum* malaria. J Infect Dis. 187: 1137-1141.

Mayxay M, Chotivanich K, Pukrittayakamee S, Newton P, Looareesuwan S, White NJ (2001). Contribution of humoral immunity to the therapeutic response in falciparum malaria. Am J Trop Med Hyg. 65: 918-923.

Mockenhaupt FP, May J, Bergqvist Y, Meyer CG, Falusi AG, Bienzle U (2001). Evidence for a reduced effect of chloroquine against *Plasmodium falciparum* in alpha-thalassaemic children. Trop Med Int Health. 6: 102–07

Nakibuuka V, Ndeezi G, Nakiboneka D, Ndugwa CM, Tumwine JK (2009). Presumptive treatment with sulphadoxine-pyrimethamine versus weekly chloroquine for malaria prophylaxis in children with sickle cell anaemia in Uganda: a randomized controlled trial. Malar J. 8:237.

Ndounga M, Mayengue PI, Casimiro PN, Koukouikila-Koussounda F, Bitemo M, Matondo BD, Diakou LAN, Basco LK, Ntoumi F (2015). Artesunate-amodiaquine versus artemether-lumefantrine for the treatment of acute uncomplicated malaria in Congolese children under 10 years old living in a suburban area: a randomized study. Malar J. 14:423

Nebie I, Diarra A, Ouedraogo A, Soulama I, Bougouma EC, Tiono AB, Konate AT, Chilengi R, Theisen M, Dodoo D, Remarque E, Bosomprah S, Milligan P, Sirima SB (2008). Humoral responses to *Plasmodium falciparum* blood-stage antigens and association with incidence of clinical malaria in children living in an area of seasonal malaria transmission in Burkina Faso, West Africa. Infect Immun, 76:759-766.

Niang M, Niass O, Diagne N, Sarr FD, Michel Matar Faye MM, Diop F, Diouf B, Faye J, Badiane A, Perraut R, Sokhna C, Trape JF, Tall A, Toure-Balde A (2017).

Temporal analysis of IgG antibody responses to *Plasmodium falciparum* antigens in relation to changing malaria epidemiology in a West African setting. 16:283 DOI 10.1186/s12936-017-1928-3.

Offianan AT, Assi SB, Tiacoh LN, Gbessi EA, Ako AB, Brou MJ, Ehouman MF, Gnamien LA, Coulibaly MAA, Coulibaly B, Beourou S, Bassinka I, Soumahoro A, Kadjo F, Mea A Tano MA. 2014. Open label, randomized, non-inferiority clinical trial of artesunate-amodiaquine versus artemether lumefantrine fixed-dose combinations in children and adults with uncomplicated falciparum malaria in Cote d'Ivoire. Malar J. 13:439

Osier FH, Fegan G, Polley SD, Murungi L, Verra F, Tetteh KK, Lowe B, Mwangi T, Bull PC, Thomas AW, Cavanagh DR, McBride JS, Lanar DE, Mackinnon MJ, Conway DJ, Marsh K, (2008). Breadth and magnitude of antibody responses to multiple *Plasmodium falciparum* merozoite antigens are associated with protection from clinical malaria. Infect Immun 76, 2240–2248.

Perraut R, Marrama L, Diouf B, Fontenille D, Tall A, Sokhna C, Trape JF, Garraud O, Mercereau-Puijalon O (2003). Distinct surrogate markers for protection against Plasmodium falciparum infection and clinical malaria identified in a Senegalese community after radical drug cure. J Infect Dis. 188:1940–50.

Pinder M, Sutherland CJ, Sisay-Joof F, Ismaili J, Mccall MBB, Ord R, Hallett R, Holder AA, Milligan P (2006). Immunoglobulin G antibodies to merozoite surface antigens are associated with recovery from chloroquine-resistant *Plasmodium falciparum* in gambian children. Infect Immun. 74: 2887-2893.

Robert V, Roeffen W, Brasseur P, Aribot G, Verhave JP, Roussilhon C (2000). Anti-NANP antibody and treatment efficacy in patients with acute uncomplicated falciparum malaria attacks. Parasite Immunol. 22: 589-593.

Rogerson SJ, Wijesinghe RS, Meshnick SR (2010). Host immunity as a determinant of treatment outcome in *Plasmodium falciparum* malaria. Lancet Infect Dis 10: 51–59. doi:10.1016/S1473–3099(09)70322–6.

Schofield L, Mueller I (2006). Clinical immunity to malaria. CurrMol Med. 2006; 6: 205–221. Epub 2006/03/07. PMID: 16515511.

Segeja MD, Mmbando BP, Seth MD, Lusingu JP, Lemnge MM (2010). Acquisition of antibodies to merozoite surface protein 3 among residents of Korogwe, north eastern Tanzania. BMC Infectious Diseases, 10:55.

Staedke SG, Sendagire H, Lamola S, Kamya MR, Dorsey G, Rosenthal PJ (2004). Relationship between age, molecular markers, and response to sulphadoxine-pyrimethamine treatment in Kampala, Uganda. Trop Med Int Health.; 9:624–9.

Stepniewska K, Ashley I, Lee SJ, Anstey N, Barnes KI, Tran Quang B, D'Alessandro U, Day PJN, de Vries JP, Dorsey G, Guthmann JP, Mayxay M, Newton NP, Olliaro P, Osorio L, Price NR, Rowland M, Smithuis F, Taylor JRW, Nosten F, White JN, (2010). In Vivo Parasitological Measures of Artemisinin Susceptibility J Infect Dis; 201:570–579.

Terlouw DJ, Aidoo MA, Udhayakumar V, Kolczak MS, Oloo AJ, Kager PA, Lal AA, Nahlen BL, Kuile FO (2002).Increased efficacy of sulfadoxine-pyrimethamine in the treatment of uncomplicated falciparum malaria among children with sickle cell trait in western Kenya. J Infect Dis. 186: 1661–68.

Tongren JE, Drakeley CJ, McDonald SL, Reyburn HG, Manjurano A, Nkya WM, Lemnge MM, Gowda CD, Todd JE, Corran PH, Riley EM (2006). Target antigen, age, and duration of antigen exposure independently regulate immunoglobulin G subclass switching in malaria. Infect Immun. 74:257-264.

Van Geertruyden JP, Van Eijk E, Yosaatmadja F, Kasongo W, Mulenga M, D'Alessandro U, Rogerson S (2009). The relationship of *Plasmodium falciparum* humeral immunity with HIV-1 immunosuppression and treatment efficacy in Zambia. Malar J 8:258.

White NJ (2011). The parasite clearance curve. Malar J. 10(1):278.

WHO (2010).World Health Organization: Guidelines for the treatment of malaria. 2nd edition. Geneva: WHO Press.

WHO (2000). Communicable diseases cluster. Severe falciparum malaria. Trans R Soc Trop Med Hyg. 94:S1–90.

WHO (2003). http://www.who.int/malaria/publications/atoz/whocdsmal20031093

Wilson PT, Malhotra I, Mungai P, King CL, Dent AE (2013). Transplacentally transferred functional antibodies against *Plasmodium falciparum* decrease with age. Acta Trop. 128, 149–153, http://dx.doi.org/10.1016/j.actatropica.2013.07. 018.