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Research Article

HUMORAL RESPONSES TO *Plasmodium falciparum* BLOOD-STAGE AND PRE-ERYTHROCYTIC ANTIGENS IN INDIVIDUALS LIVING IN TWO SETTINGS WITH DIFFERENT EPIDEMIOLOGICAL STRATA IN CÔTE D'IVOIRE

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Abstract- Humoral immune responses play a pivotal role in naturally acquired immunity to malaria. Several *Plasmodium falciparum* antigens have been described as targets of the host immunity. We assessed antibody responses against a panel of *P. falciparum* antigens in patients with uncomplicated *falciparum* malaria in two urban malaria-endemic of Côte d'Ivoire with different epidemiological strata. A cross-sectional analysis of blood samples gathered from participants older than 6 months, in Man and Bouake in Côte d'Ivoire with different transmission strata was performed. Standardized ELISA was used to measure total IgG levels against 5 specific-*P. falciparum* antigens (CSP, LSA3, SALSA, GLURP, AMA1). A total of 151 sera were analyzed. The combined data from the two sites showed a proportion of responders greater than 50% for all the antigens tested. Proportion of IgG responders was greater than 50% except for SALSA (36.73%) at Man site. At Bouake site proportion of IgG responder was less than 50% with CSP (37.50%) and LSA3 (25%). The lowest proportion was observed in lowest age group. Proportion of IgG responder increased with increasing age at the two sites. Variation in the proportion of responders across the two sites was similar. The levels of IgG against AMA1, LSA3, GLURP and SALSA increased with increasing age at Man site. In contrast only IgG level against AMA1 increased while IgG level against CSP decreased with increasing age at Bouake site. Overall, antibody levels against AMA1, CSP, GLURP and LSA3 were gender-independent. These findings may have significant implications in the design of future malaria vaccines trials.

Keywords- Humeral response, *Plasmodium falciparum*, antigens, Côte d'Ivoire

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Introduction

Despite notable progress in malaria control over the last decade, malaria is still a serious public health problem in sub-Saharan Africa. In 2016, an estimated 216 million cases of malaria occurred worldwide, compared with 237 million cases and 211 million cases in 2010 and 2015 respectively. In 2016, there were an estimated 445 000 deaths from malaria globally, accounted for 91% in Africa. Resistance of *P. falciparum* to the derivatives in South East Asia seriously jeopardizes the achievements of malaria control [1, 2]. Children and pregnant women in endemic areas such as Côte d'Ivoire are at high risk of clinical and severe malaria because of their low immunity. Immunity to malaria is acquired slowly in an age-dependent manner after repeated exposures [3, 4]. More than 40 merozoite proteins involved in the parasite invasion have been identified, and most of them have been considered as targets of acquired immunity [5]. The role of antibodies (Ab), particularly immunoglobulin G (IgG), in the protection against malaria infection has been demonstrated. A study including patients from Côte d'Ivoire and Burkina Faso infected with *Plasmodium falciparum* has demonstrated that a predominance of IgG1 and IgG3 cytophilic antibodies was associated with protection against the disease, whereas a predominance of IgG2 and IgG4 non-cytophilic antibodies were associated with increased susceptibility to malaria infection [6]. Passive transfer of purified immunoglobulin (Ig) G from hyper immune sera to infected subjects has demonstrated that IgG mediates anti-parasitic activity and overall protection against malaria [7]. Other studies have also demonstrated the role of IgG following passive plasma transfer experiments from individuals living in endemic areas to unexposed naïve individuals [8, 9]. Several sero-epidemiological and vaccines studies have suggested a potential role of antibodies against CSP, AMA1, GLURP, SALSA, LSA and in mediating protection from malaria [10-12], but this immunity may vary in intensity according to the level of malaria transmission [13, 14]. Despite many decades of intense research and development effort, there is no commercially available malaria vaccine at the present time. RTS, S/AS01 is the most advanced vaccine candidate against *P. falciparum*. More than 20 other vaccine constructs are currently being evaluated in clinical trials or are in advanced preclinical development. Monitoring malaria transmission intensity is a key element of monitoring changes in transmission and assessing the impact of malaria control and elimination interventions implemented by malaria control programs. Antibody responses to one or more malaria specific antigens offer an alternative measure to estimate post exposure to malaria [15]. In Côte d'Ivoire, malaria transmission is heterogeneous with different epidemiological strata in terms of their geographical and ecological characteristics, transmission pattern and endemicity level and main vectors transmitting malaria parasites. There are three main malaria vector species in Côte d'Ivoire: *Anopheles gambiae* s.s., *An. Funestus* s.s. and *Anopheles nili* s.s. [16]. The cities of Man and Bouaké, respectively located in the West and Center of Côte d'Ivoire; represent two areas of differing malaria endemicity.

Table-1 Antigens et peptides

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

Table-2 Characteristics of the study population

Characteristics	BOUAKÉ	MAN	Total	P value
Number of patients (%)	40 (26.49)	111 (73.51)	151 (100)	<0.0001
Age group (%)				
1-4 years	08 (20)	49 (44.14)	57 (37.75)	0.01
5-9 years	18 (45)	26 (23.42)	44 (29.14)	0.01
≥ 10 years	14 (35)	36 (32.43)	50 (33.11)	0.92
Mean DP. (parasites/µl, Min – Max)	92,748 (10,040-199,600)	26,338 (2,013-177,866)	-	<0.0001
Sex ratio (M/W)	13/27	58/53	71/80	0.04
Mean Age (years, ±SD)	8.78 (±7.44)	9.65 (±10.99)	-	0.19
Mean Temperature (°C, ±SD)	39.13 (±0.65)	38.26 (±0.40)	-	<0.0001
Hemoglobin S (n, %)	1 (2.50)	7 (6.31)	8 (5.30)	0.61
Mean Hemoglobin (g/dl, ±SD)	10.79 (±1.00)	10.99 (2.03)	-	0.84

DP; density parasitemia, Sex Ratio (M/W); Sex Ratio (men/women), SD; Standard Deviation Min; Minimum, Max; Maximum

Table-3 Distribution of the proportion of IgG response stratified by Age and Site

Antigen	Response	< 5 n(%)			5-9 n(%)			≥ 10 n(%)			
		Man	Bouake	p-value	Man	Bouake	p-value	Man	Bouake	p-value	TOTAL
		25 (51.02)	03 (37.50)	0.74	15 (57.69)	10 (55.56)	1	27 (75.00)	11 (78.57)	1	91(60..26%)
	Negative	24 (48.98)	05 (62.50)		11 (42.31)	08 (44.44)		09 (25.00)	03 (21.43)		60 (39.74)
AMA1	Positive	38 (77.55)	05 (62.50)	0.63	19 (73.08)	15 (83.33)	0.66	26 (72.22)	13 (92.86)	0.22	116 (76.82)
	Negative	11 (22.45)	03 (37.50)		07 (26.92)	03 (16.67)		10 (27.78)	01 (07.14)		35 (23.18)
LSA3	Positive	26 (53.06)	02 (25.00)	0.27	16 (61.54)	12 (66.67)	0.97	19 (52.78)	08 (57.14)	1	83 (54.97)
	Negative	23 (46.94)	06 (75.00)		10 (38.46)	06 (33.33)		17 (47.22)	06 (42.86)		68 (45.03)
GLURP	Positive	27 (55.10)	06 (75.00)	0.5	21 (80.77)	18 (100)	0.13	32 (88.89)	14 (100)	0.47	118 (78.15)
	Negative	22 (44.90)	02 (25.00)		05(19.23)	00 (00)		04 (11.11)	00 (00)		33 (21.85)
SALSA	Positive	18 (36.73)	04 (50)	0.74	15 (57.69)	13 (72.22)	0.5	26 (72.22)	10 (71.43)	1	86 (56.95)
	Negative	31 (63.27)	04 (50)		11 (42.31)	05 (27.78)		10 (27.78)	04 (28.57)		65 (43.05)

Table-4 Mean of IgG levels against antigens tested stratified by Age and Site

Antigen	< 5 mean (±SD)			5-9 mean (±SD)			≥ 10 mean (±SD)		
	Man	Bouake	p	Man	Bouake	p	Man	Bouake	p
CSP	2.68 ±1.89	4.65 ±8.32	0.66	4.86 ±7.43	3.37 ±2.98	0.58	4.72 ±3.54	2.90 ±1.43	0.14
AMA1	4.42 ±3.25	2.62 ±0.95	0.11	3.98 ±3.33	5.72 ±4.73	0.22	4.08 ±4.51	5.82 ±4.20	0.04
LSA3	3.22 ±3.37	3.98 ±6.40	0.59	2.78 ±1.67	4.79 ±6.07	0.45	5.16 ±4.73	3.77 ±6.91	0.52
GLURP	6.89 ±11.67	12.41 ±13.94	0.27	10.13 ±15.96	16.65 ±11.52	0.02	19.01 ±21.83	14.40 ±10.70	0.86
SALSA	2.32 ±2.04	2.27 ±1.00	0.61	3.09 ±3.19	3.42 ±2.67	0.16	3.65 ±2.96	2.68 ±1.21	0.44

Table-5 Effect of gender, age and parasite density on IgG mean level in all participants (Logistic regression)

	AMA_1			CSP			GLURP			SALSA			LSA3		
Factor	OR	CI 95%	p	OR	CI 95%	p	OR	CI 95%	p	OR	CI 95%	p	OR	CI 95%	p
Gender															
Female	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-
Male	1.06	0.49-2.31	0.87	0.98	0.49-1.95	0.96	0.55	0.23-1.30	0.17	0.78	0.39-1.55	0.47	1.42	0.74-2.78	0.29
Age (Yrs)															
01-Apr	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-
05-Sep	1.21	0.45-3.28	0.7	1.33	0.56-3.16	0.51	6	1.99-20.8	0.002	2.34	0.96-5.77	0.06	2.16	0.92-5.19	0.07
≥10	1.9	0.50-7.90	0.35	2.5	0.67-9.13	0.16	9.95	1.59-78.1	0.01	2.03	0.50-7.52	0.29	1.74	0.56-5.58	0.34
Log(PD)	1.04	0.78-1.39	0.75	0.92	0.72-1.19	0.55	0.9	0.66-1-23	0.54	0.95	0.74-1.22	0.71	0.92	0.72-1.17	0.53

CI, Confidence Interval ; Log (PD), Parsite Density Log ; p, p-value ; OR, Odds Ratio

Table-6 Effect of gender, age and parasite density on IgG mean level at Man site (Logistic regression)

	AMA_1			CSP			GIURP					LSA3			
Facteurs	OR	CI95%%	p	OR	CI 95%	p	OR	CI 95%	p	OR	IC 95%	p	OR	IC 95%	p
Gender															
Female	1	-	-	1	-	-	-	-	-	1	-	-	1	-	-
Male	0.97	0.92-1.03	0.42	1.10	0.50-2.46	0.80	0.70	0.27-1.76	0.45	0.54	0.23-1.21	0.13	1.65	0.76-3.63	0.20
Age (Years)															
1-4	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-
5-9	0.88	0.28-2.83	0.82	1.29	0.46-3.61	0.62	3.79	1.16-14.0	0.03	1.98	0.68-5.87	0.26	1.87	0.68-5.37	0.23
≥10	1.10	0.24-5.50	0.89	1.91	0.39-9.22	0.40	5.39	0.71-47.0	0.10	1.71	0.29-8.60	0.52	1.89	0.47-8.24	0.37
Log(PD)	0.82	0.62-1.28	0.53	0.85	0.61-1.17	0.34	0.72	0.49-1.02	0.07	0.81	0.57-1.13	0.22	0.81	0.59-1.12	0.22

CI, Confidence Interval; Log (PD), Parsite Density Log; p, p-value; OR, Odds Ratio

In Bouake, malaria transmission is moderate in dry season and high during the rainy season while Man experiences high-intensity malaria transmission throughout the year. The two sites are malaria clinical trials and sentinels sites, respectively, for Institut Pasteur of Côte d'Ivoire and the National Malaria Control Program since 2005. In Côte d'Ivoire, malaria prevention and control strategies are based on the use of long lasting insecticidal nets (LLINs), intermittent preventive treatment with sulfadoxine-pyrimethamine (IPT-SP) and environmental sanitation, prompt diagnosis and treatment of malaria with Artemisinin-based Combination Therapy (ACTs). Sero-epidemiological studies in Côte d'Ivoire are limited and no study has been conducted in the cities of Man and Bouake. There is a need to collect more information on natural immune responses in patients living in the two sites. The aim of this study was to assess the humoral immune response against AMA1, CSP, GLURP, LSA3, and SALSA in patients with uncomplicated *falciparum* malaria in Man and Bouake, two urban areas with different epidemiological strata. Data from this study may be useful for designing future malaria vaccines trials at the two sites

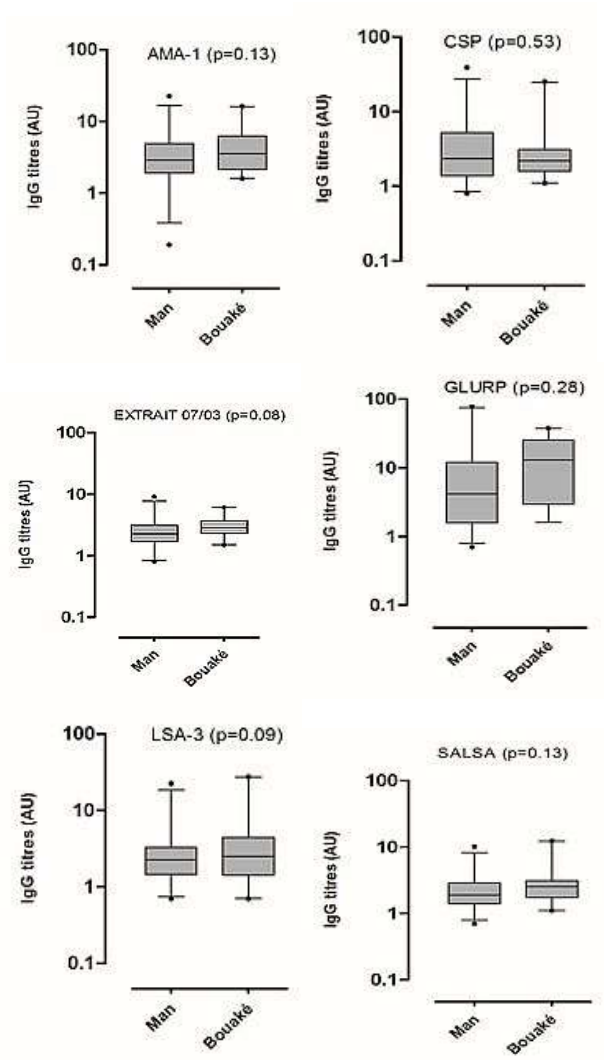

Fig-1 Box plot depicting the variation of mean antibody level (log-transformed) with study site

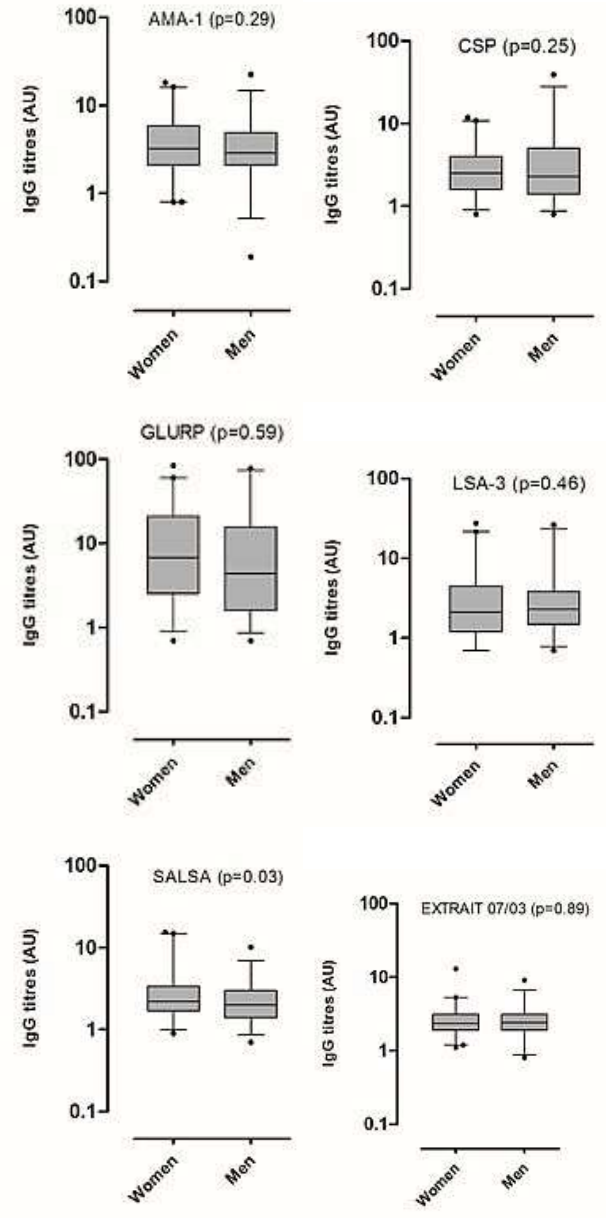

Fig-2 Box plot depicting the variation of mean antibody levels (log-transformed) with gender

Material and methods

Study areas

Two malaria epidemiological strata (the cities of Bouake and Man) in Côte d'Ivoire were selected.

Man site

Man (7°24'N, 7°33'), is a city located in the Western zone of the country. The climate in the western part is subequatorial (mountain climate) consisting of tropical rainy forest with hills and mountains. Four seasons characterize the Western region: a long rainy season from April to mid-July and a short rainy season from September to November, alternating with a short dry season from mid-July to September and a long dry season from December to March. The average annual rainfall ranged from 1600 to 2500 mm and the annual temperature averaged is 24.5°C. *Plasmodium falciparum* transmission is intense and perennial, with recrudescence during the rainy season. Malaria transmission in Man can be described as hyper-endemic. The main vectors are *Anopheles gambiae* and *Anopheles funestus* [16]. The Human biting rate (HBR) has been estimated at 4.21 b/p/n. *Plasmodium falciparum* is the predominant malaria species encountered (80–95 % of infections), followed by *Plasmodium malariae* (7–10 %) and *Plasmodium ovale* (1–3 %) [17,18]. Malaria control strategies mainly based on insecticide treated nets and treatment of clinical cases with ACTs.

Bouaké site

Bouaké (7°44'N, 5°41'W), has an humid tropical climate and opens up to a transition between the south and north Characterized by daily mean temperature between 23.7 and 33.8°C, an average annual rainfall of 1334mm. Vegetation is characterized by Guinean forest-savannah mosaic belt with four seasons: a long rainy season from March to June and a short rainy season from September to October. The long dry season starts in November through to February and a short dry season from July to August. Malaria transmission is perennial with maximal transmission occurring at the beginning and towards the end of the rainy season. The main vectors for malaria transmission in this area are *Anopheles gambiae* and *Anopheles funestus* [19].

The Human biting rate (HBR) has been estimated at 0.33 b/p/n (unpublished data). As in Man, the predominant parasite species is *P. falciparum* [18] and the same malaria control strategies have been implemented.

Study design and duration

The study was part of a clinical trial conducted in the two sites. It was a cross-sectional survey performed in the 2 epidemiological strata. Samples were collected in the study two sites from January to May 2016 and from May to July 2016 at Man and Bouake sites respectively.

Study Population

We randomly selected children and adults with uncomplicated *P. falciparum*. The following criteria were used for participant selection: i) older than 6 months, body weight ≥5 kg; ii) history of fever in the previous 24 hours or measured fever (axillary temperature ≥37.5°C or rectal ≥38°C); iii) mono-infection with *P. falciparum* with parasite density between 2,000–200,000 asexual parasites/µl of blood; iv) no other cause of fever than suspected malaria; v) no general danger signs or signs of severe and complicated *falciparum* malaria as per WHO guidelines [1]; vi) signed informed consent (by patient or responsible caregiver) to participate to clinical trial. A second written informed consent was required for immunological assay participation.

Thick/thin blood smears

Thick/thin blood smears were collected at the time of screening and were performed as previously described [19]. Briefly, Blood smears were prepared and stained with 10% Giemsa. Thick films were examined with a binocular microscope with an oil immersion objective lens to determine parasite density. Parasite density was measured by counting the number of asexual parasites and leucocytes in 200 high-power fields based on a putative count of 8,000 leucocytes per microliter of blood. Two qualified independent microscopists read all Giemsa-stained slides. Discordant readings were re-examined by a third qualified independent microscopist. Final parasite density was averaged of the two most concordant counts.

Blood samples collection

A total of 4 ml of venous blood was collected from participants in dry tubes on day 0 before treatment. The collected blood was centrifuged at 2000 rpm and around 500µl of serum was collected and preserved in cryotubes and stored at -20°C.

Antigens and peptides

The same antigens and peptides have been used in previous studies [20, 21]. Peptides derived from liver (CSP, LSA3, SALSA) and blood stage antigens (GLURP, AMA1) of *P. falciparum* were used for immunological evaluation. The peptides used in this study were designed as already described [22]. A N-terminal cysteine residue was added to allow a unidirectional coupling to BSA and was done by the manufacturer (GenScript HK Inc.,Hong Kong, China). Purity of each BSA-peptide was estimated >85 % by HPLC and mass spectrometry. The sequence of the peptides used was presented in [Table-1].

Antibody measurements

We followed procedures described in our previous studies [20, 21]. Briefly, total IgG responses were quantified by ELISA in duplicate plasma samples diluted 1:100 as previously described using whole parasite extract Ag from schizonts 07/03 Dielmo strain adapted to culture [11, 21]. The positive and negative controls were used in each assay as standards for plate comparability: 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 sera. Each ELISA plate (Maxisorp Nunc, Deanemark) were coated with 100 µL of specific antigens (1 µg : mL) or crude Schizont extract 07/03 (1 µL : 1.2 mL) diluted in carbonate bicarbonate buffer at pH = 9.6. Plates were incubated at 4°C overnight before washing 3 times with PBS-Tween (PBS 0.1% + Tween 20) and blocking for 1h at room temperature with110 µl of PBS-Tween (blocking buffer). Following this, plate were washed again 3 times and incubated at 37°C for 1 h with 100 µl of serum 1 : 100 in dilution buffer (1X PBS 0.1% Tween 20 1% BSA). The plates were then washed 4 times and incubated for 30 minutes at room temperature with100 µl of conjugate anti-human IgG diluted at 1 :7000 in dilution buffer before final washing and development using 100 µl substrate solution TetraMethylBenzidine (TMB Eurobio®).The appearance of a blue coloris stopped by adding 100 µL per well of ortho phosphoricacid (H_3PO_4) to 1M (stop solution). The OD was measured at 490 nm with 655 as reference. Results were expressed as OD ratio = OD sample/OD naive serum pool [23]. Sera showing an OD ratio >2 corresponding to the signal of naive controls + 2 SD were considered sero-positive for prevalence calculations.

Statistical analysis

The software GraphPad Prism 5 software was used for data analysis. Mann Whitney test was used to compare the mean level of Ab-Ag response between Man and Bouake. The Chi2 test was used to compare the proportions of responders in Man and Bouaké by Ag tested. The Significance threshold was set at 5%.

Ethical Statement

A first written informed consent for participation in the clinical trial was obtained from adult's patients or children's parents or guardians, in accordance with the Declaration of Helsinki. A second written informed consent was required for participation in the immunological assay. Approval of the study was granted by National Research Ethic Committee of Côte d'Ivoire. (N°049/MSLS/CNER-dkn). Assessment was required from young children (10-17 years) in addition to their parent's or guardian consent. A second written informed consent was required for immunological assay.

Results

Characteristic of study population

A total of 151 patients were included in this study, with 111 patients (73.50%) from Man and 40 patients (26.50%) from Bouake. Among Bouake's participants, there were more females (13/27), while males in Man were slightly more represented (58/53) (p=0.04). Mean age was 9.66 years ± 11 (Man) versus 8.78 years ± 7.44 (Bouake). However, the majority of participants were less than 5 years at Man's site (44.14%) and between 5 and 9 years at Bouake's site (45%). There was a statistically significant difference in mean parasite density and mean temperature, which were all higher at Bouake site. The geometric mean (GM) of the parasite density (PD) in the study participants was 92,748 and 26,338parasites/µl at Bouake and Man site, respectively (p<0.0001). The mean temperature from Man's participants was 39.32°C versus 38.26°C for Bouake's participants [Table-2].

Distribution of antibody seropositivity in the study population

Sero-positivity in this study was defined as serum antibody levels higher than the cutoff value, which is the mean of the antibody concentrations of the negative control plus 2 standard deviations. The combined data from the two sites showed a proportion of responders greater than 50% for all the antigens tested with highest proportion for GLURP (78.15%) following by AMA1 (76.82%), CSP (60.26%), SALSA (56.95%) and LSA3 (54.97%) (Table 3). Proportion of IgG responders was greater than 50% except for SALSA (36.73%) at Man site. At Bouake site proportion of IgG responder was less than 50% with CSP (37.50%) and LSA3 (25%). The lowest proportion of responder was observed in lowest age group. Proportion of IgG responder increased with increasing age at the two sites. Proportion of responders stratified by age varied with age. The highest proportion of responders was found in participants ≥ 10 years and the lowest proportion observed in children less than 5 years at both study sites. Distribution of the proportion of IgG response stratified by age and site was similar [Table-3].

Total IgG antibody levels

IgG levels against GLURP in patients between 5 to 9 years was higher at Bouake site (p=0.02). The level of IgG against AMA1 in patients ≥10 years was higher in Bouake (p=0.04). IgG level against the others antigens in old participants from Man compared to Bouake but the difference was not statistically significant.

When analyzed as a whole population IgG levels increased with increasing age for AMA1, CSP and GLURP and the parasite density does not influence the antibody production of any of the antigens tested [Table-5]. IgG levels varied from site to site. The highest level was observed with GLURP at Man site in older patients. The levels of IgG against CSP, AMA1, LSA3, GLURP and SALSA increased with increasing age at Man site. The trend increasing antibody level with age was confirmed using regression analysis [Table-6]. In contrast only IgG level against AMA1 increased with increasing age while IgG level against CSP decreased with increasing age at Bouake site. For LAS3, GLURP and SALSA the highest level was observed in patients aged from 5 to 9 years at this site [Table-4]. Overall, antibody responses against AMA1, CSP, GLURP and LSA3 were independent of gender ($p>0.05$), meanwhile responses against SALSA were significantly higher for females ($p = 0.03$) than for males [Fig-2].

Discussion

In malaria endemic regions, burden of *Plasmodium falciparum* infection and clinical disease are closely related to the level of malaria transmission. Individuals living in malaria-endemic areas naturally develop immunity to clinical malaria over several years [24-26]. This study described data on antibodies responses to AMA1, CSP, LSA3, SALSA and GLURP antigens of *P. falciparum* infected individuals living in two epidemiological stara of Côte d'Ivoire. When analyzed as a whole population proportion of responders was over than 50% with all antigens tested with highest proportion for GLURP followed by AMA1, CSP, SALSA and LSA3. The proportion of IgG responders against all antigens tested was higher in the two sites in patients over than 5 years due probably to the constant exposure to infective bites of mosquitoes or sustained an immune response after active infection [27]. The lowest proportion was observed in children under-fives years with SALSA (36.73%) at Man site and CSP (37.50%) and LSA3 (25%) at Bouake site. In this study, the proportion of responders to AMA1, CSP, LSA3, SALSA and GLURP increased with increasing age at both study sites, reflecting cumulative exposure to malaria parasites and, possibly, gradual maturation of the immune system over time. These findings confirm those of earlier studies of blood-stage antigens carried out in areas where malaria is endemic [25, 28-30]. This study reports increased IgG levels against all antigens tested with increasing age at Man site which is similar to the previous reports [28-30]. In contrast level of IgG level to CSP decreased with increasing age at Bouake site. The reason for lack of correlation with age for anti CSP antibodies may be due to the less immunogenicity of CSP. This current study has shown that the immunogenicity of the antigens tested often targeted as malaria vaccine candidates are not the same. Antibody responses against GLURP and AMA1 indicated that these two blood-stage antigens are more immunogenic and offer good prospects for a vaccine. Results from the current study also indicate that none association between parasite density and antibody levels in contrast to the previous findings which demonstrated that high antibody levels against GLURP(R0) and MSP3 are associated with lower parasite density [29,31,32] Statistically significant differences for the proportion of responders and antibody levels between the two study sites (Man and Bouake) were not observed. However, children who are highly exposed to malaria usually have higher antibody levels compared with children exposed to low- to -moderate transmission intensity although antibody levels generally increase with age. Over the age of 5 years, the proportion of responders in the majority of cases was higher in Bouake than in Man. Previous observations at both study sites have revealed that malaria transmission in both site is hyper-endemic but Man site is characterized by a high-intensity of transmission throughout the year compared to Bouake site [19, 33]. Therefore, we expected, given the transmission levels previously described in the study areas, to see higher proportions of responders in Man than in Bouake. However, several factors could explain the similar results at both study sites despite a different level of malaria transmission. The first factor could be the period of the study. At Bouake site, the study took place during the high rainy season while at the Man site, the study took place from January to April corresponding to a drop in the level of transmission. This level of antibody is important during or just after the period of high transmission and fall during the period of low transmission [26, 34]. The study was conducted at the health Center of Dar Es Salam at Bouake site, where the entomological studies found an HBR of 0.05b/ p/n (unpublished data), contrasting with the high prevalence of malaria in this area. Patients included in this immunoassay may be from other areas where transmission is higher or may be infected in areas of high transmission. Finally, the size of the samples is small in Bouaké due to the low proportion of patients who consented to participate in this immunological study and the quality of the samples. At the Man study site, the majority of the samples were from patients under 5 years of age (44.14%) while at the Bouake study site, only 20% of the patients were less than 5 years. Other factors including ethnicity [35], parasites strains [34], red blood cell defects [36] or use of mosquito nets, could greatly influence exposure to malaria parasites and modulate the levels of antibodies in different populations [34]. The variation between the immune responses between site may be also due to coinfection particularly with helminths such as Schistosoma haematobium. Coinfections with helminths and malaria parasites are frequently observed, and helminth infections might affect the immune response against malaria parasites. Helminth infections are prevalent throughout Man and Bouaké but with high prevalence observed in Man compared to Bouaké [37]. This co-infection with helminths could alter immmune response to malaria [38-41]. The IgG antibody levels against SALSA were higher in females but no gender disparity was observed with CSP, AMA1, GLURP and LSA3. These findings suggest that in the study area females were more prone to infection with malaria parasites than males. The immunological dimorphism of anti-SALSA antibody observed in the patients of Man suggested that female sexual hormones could directly promote the induction of antibodies. Indeed, sexual steroids receptors or female sexual hormones such as estrogen, progesterone could be expressed on many immune cells [42]. Despite these interesting results, we recognize that our study has several limitations. First, the period of study was different between the two study sites. Second, co-infections, particularly with helminths or HIV, the rate of use of mosquito nets and residence time in the two study sites have not been assessed in this study. Third, the difference in sample size and the lack of information in ethnicity could also be limiting our interpretations. In addition, only total IgG antibody was measured and not the IgG sub-types. However, the ultimate goal of this study was to have baseline data on anti-malaria immunity in preparation for vaccine trials. Our future research plan will take into account the limiting factors of this study. These findings may have significant implications in the design of future trials to test malaria vaccine candidates in the two sites.

Conclusion

A high proportion of responders to the different antigens tested were observed in both study sites despite a difference in transmission level. However, the proportion of the responders and IgG levels was higher with GLURP antigen. The proportion of the responders as well as the IgG levels increased with age in both sites.

Application of research: These data supplemented with those from asymptomatic and healthy subjects will be useful for the implementation of future vaccine trials in both sites.

Research Category: Humoral immune responses

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Author Contributions: All authors participated in the design, implementation, and analysis or data interpretation of the study. OAT was involved in all phases of the study and has full access to all the data. Assi SB, Beourou S and Djaman J Assi SB and Djaman J supervised the study. Tiacoh NL, Kone AA, Ouattara O, Kone B, Gbessi, Beourou S, Yao SS and Lawson K Tiacoh NL, Kone AA, Ouattara O, Kone B, Gbessi, Beourou S, Yao SS and Lawson K conducted field work and coordinated study procedures. Yao SS and David K performed the immunological analyzes. Analysis of data was led by Gbessi AE and Ako AAB. All authors critically reviewed the report and approved the final version of the report for submission.

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