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Full Length Research Paper

In vitro efficacy of standard antimalarial drugs on parasitic isolates from patients with sickle cell disease (SCD) in Abidjan, Côte d'Ivoire

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Malaria infection triggers off vaso-occlusive attacks in sickle cell patients, leading drastically to death. Sickle-cell patients use artemisinin-based combination therapies (ACTs) as recommended by the WHO to treat malaria infections to prevent these crises. The survey of sensitivity of parasites collected from sickle-cell patients needs is important to be re-specified. Red cells from these patients differ from others in terms of redox potential and calcium flow. The aim of this study was to assess the *in-vitro* efficacy of standard antimalarials used by this fragile population in order to find out a suitable medical policy for them in Côte d'Ivoire. For this purpose, the standard isotope test for schizont maturation and the Ring-stage survival assay were used to ascertain the *in-vitro* sensitivity of 116 *Plasmodium falciparum* isolates from sickle cell patients, collected from Abidjan for dihydroartemisinin, chloroquine, amodiaquine, quinine and luméfantrine. All the isolates exhibited different responses with the different antimalarials. No cases of *in-vitro* resistance to Lumefantrine and Quinine were noticed, reappearance of chloroquine-sensitive and a strong cross activity of chloroquine and dihydroartemisinin were observed. Indeed, 87 (65.41%) isolates were dihydroartemisinin-sensitive *in-vitro* whereas 20 (15.04%) had a resistants after exposure to 700 nM. The majority of clinical isolates non-responding to dihydroartemisinin were from the sickle cell phenotype HbSS. To sum up, despite the decrease of the *in-vitro* sensitivity of dihydroartemisinin on some sickle cell isolates, this molecule is still efficient. However, it must be actively monitored by the National Malaria Control Programme, particularly for sickle cell patients where a decrease in sensitivity was observed.

Key words: Sickle cell anemia, malaria, Côte d'Ivoire, *Plasmodium falciparum*, anti-malarials, treatment resistance, *in-vitro* drug monitoring.

INTRODUCTION

Despite efforts made for many years, the latest WHO malaria report estimated in 2019 showed 228 million cases and 405,000 deaths, especially among under five year-old children (WHO, 2019).

Resistance of the parasite to antimalarial drugs has emerged as one of the greatest challenges for the control

programs (Ashley et al., 2014; Dondorp et al., 2009; Noedl et al., 2008). A high-quality diagnosis can help to limit the development or spread of resistance to antimalarial drugs (Ashley et al., 2014; Dondorp et al., 2009; Menard et al., 2016; Noedl et al., 2008; Piel et al., 2017).

Since WHO has recommended in 2005, the use of artemisinin-based combination therapies (ACTs) for the treatment against *Plasmodium falciparum* malaria, resistance to artemisinins and partner drugs have emerged in the Greater Mekong sub-region of South-East Asia (Ashley et al., 2014; Kyaw et al., 2013; Takala-Harrison et al., 2015; Takala-Harrison and Laufer, 2015). This resistance was associated with mutations in the K13 propeller gene (Huang et al., 2015; Wang et al., 2018, 2015). Since then, to confine the spread of artemisinin-resistant parasites, many countries in sub-Saharan Africa have improved their surveillance systems by assessing the efficacy of ACTs in clinical trials, *in-vitro* susceptibility testing or Pfkelch13 genotyping (Balikagala et al., 2017; Djaman et al., 2017).

In Côte d'Ivoire, the general use of ACTs such as Artemether-Lumefantrine and Artesunate-Amodiaquine combination for the management of uncomplicated *P. falciparum* malaria has undoubtedly contributed in improving malaria situation (Toure et al., 2018). However, after more than 15 years of use, clinical trials revealed the decrease in sensitivity of Artemether-Lumefantrine in the sentinel sites of NMCP (Serge-Brice et al., 2020; Toure et al., 2018; 2020; Yavo et al., 2015).

In the same time, sickle cell disease concerns nearly 120 million people worldwide, including more than 300,000 births in sub-Saharan Africa (Piel et al., 2013, 2017).

These red cells carrying abnormal hemoglobins are known to be very different from the others regarding especially redox potentials and calcium fluxes (Nebor et al., 2011; Wiley and McCulloch, 1982; Yamaja et al., 2002; Oniyangi and Omari, 2019), which can interfere with drug sensitivity. They can alter sensitivity of the parasites to ACT either by inactivation of the artemisine molecule (opening of the circle by oxidation), by modification of the protein functions of the parasites (transcriptomic regulation), or by selection of mutated proteins especially involved in the ionic regulation of the parasite (Naumann et al., 1992; Cheemadan et al., 2014). Thus, a modified intracellular media of the red blood cell could modulate the metabolic pathways involved in artesunate resistance, and select resistant parasites. However, no studies have been performed to access sensitivity of malaria parasites collected in sickle cell patients. This population is also at high risk, as malaria infection can trigger vaso-occlusive crises (VOCs) (Chemegni et al., 2018; Désiré et al., 2017; Komba et al., 2009; Makani et al., 2010). The sensitivity of parasites collected from sickle cell patients should therefore be re-specified to proposed new policy if needed. The purpose of this study was to address this question by investigating the parasite sensitivity to artesunate both using *in-vitro* RSA and schizont maturation tests.

METHODOLOGY

Study population

The study was conducted in Côte d'Ivoire (CI). It is a neighbouring country of Ghana, Burkina Faso and Mali with high rates of sickle cell disease with prevalence between 4 and 25% (Lainé et al., 2012; Sonia et al., 2017). Due to its strategic geographical position between Guinea golfe and Sahel, it is subject to a high migratory flow. Thus, crossbreeding and consanguineous marriages in Côte d'Ivoire are responsible for a sickle cell rate of around 14%, of which 2% are major forms (Tolo-Diebkilé et al., 2010; Sawadogo et al., 2014). Data obtained during clinical trials on the efficacy and tolerance of ACTs conducted in different regions in CI, highlighted a prevalence of sickle cell disease in malaria patients to be around 2% for major forms and 6% for the sickle cell trait (Tossea et al., 2018).

The recruitment of samples for this study took place from May 2017 to January 2020 on two sites, namely, the Clinical Haematology Department of Yopougon University Hospital and the Community Health Center (FSUCOM) of Anonkoua-Kouté in the district of Abobo. The sickle cell status of each patient enrolled in Yopougon was already known and they were already under care. Nevertheless, this status was validated by PCR using Fluorescence Resonance Energy Transfer (FRET) method (Sequences Profiles in Annex). At Anonkoua-Kouté patients were pre-selected among a cohort of febrile patients with suspected malaria infection. Malaria diagnostic was done firstly using a Histidine-rich protein (HRP-II) antigen immuno-chromatographic test for the qualitative detection of malaria *P. falciparum* in human whole blood. Subsequently, a thick smear and a blood smear were performed in the pre-selected subjects (Gupta and Singla, 2012). Patients older than 6 months whose Giemsa-stained thick blood smear was positive for *P. falciparum* mono-infection and a parasitaemia greater than or equal to 0.1% were pre-included in this study. Diagnosis of sickle cell disease was blindly done on participants. Patients with normal hemoglobin or with HbAS traits were enrolled. Parasitaemia was calculated after reading of methanol fixed and Giemsa staining smears.

The number of infected red cells containing viable parasites was counted in a total of at least 10,000 red blood cells. Only viable parasites were recorded to determine the parasitaemia.

Moreover, all patients with severe malaria according to WHO criteria, or requiring intensive care for other severe pathology, or who have received an antimalarial drug within 30 days prior to the medical consultation were excluded and directly sent back to the physician with their biological results.

The number of subjects required for this study was estimated to be able to highlight a 20% increase of resistance of parasites in RSA test in patients with SCD or trait in comparison with those with HbAA phenotype. Resistance in RSA for HbAA subjects was

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assumed to be 10% and ratio of patients enrolled with HbAA or SCD+trait was planned to be 1:2 as a case control study. With a power of 90% number of subjects to enroll should be 110 HbAA and 55 HbAS + HbSS.

Chemosensitivity tests

Three milliliters of blood were taken from each patient after informed consent, kept at 4°C until transportation and use at Institut Pasteur de Côte d'Ivoire. Parasites were cultured in RPMI-1640, L-glutamine (20 mM), HEPES buffer (25 mM), gentamicin (10 mg/ml), 5% Albumax II and 2% D-glucose, at 37°C in 5% O_2, 5% CO_2 and 90% N_2 atmosphere. RSA test was conducted as described by Witkowski et al. (2013) with 2% haematocrit inoculum (parasitaemia ranging between 0.1 and 1%). After 6 h of exposure to DHA at 37°C, the blood pellet was washed three times with a pre-warmed RPMI 1640 medium, resuspended and incubated under the same previous conditions for 66 h. After 72 h of incubation, blood smears were prepared from the blood pellet and examined under a light microscope by two independent microscopists. The number of infected red blood cells containing viable parasites was counted in a total of at least 10,000 red blood cells. After 72 h, the test was considered as interpretable when the proportion of viable parasites not exposed to DHA was higher than the initial parasitaemia (Witkowski et al., 2013). Two reference strains, K1 (artemisinin-sensitive strain) and IPC 3445 (artemisinin-resistant strain) were used as control.

In parallel, *in-vitro* maturation tests were conducted as described by Trager and Jensen (1976) using the same blood suspension, in 96-wells microtiter plate. Molecules (Dihydroartemisinin, Chloroquine, Lumefantrine, Quinine and Amodiaquine) were added in duplicate to the wells (100 µl/well). Drug concentrations inhibiting 50% parasite growth (IC_{50}) were graphically determined, using WWARN's *In-Vitro* Analysis and Reporting Tool (IVART) software (Le Nagard et al., 2011). The IC_{50} cut-off values retained for the evaluation of the *in-vitro* chemosensitivity were 10 nM (Pradines et al., 1998), 100 nM (Le Bras and Ringwald, 1990), 150 nM (David et al., 2013, 2014), 800 nM (Basco and Le Bras, 1993), 80-60 nM (Basco and Le Bras, 1993) for Dihydroartemisinin, Chloroquine, Lumefantrine, Quinine and Amodiaquine, respectively.

Statistical analysis

The results of this study were compiled as database in Excel® 2016 and analysed using GraphPad Prism 7 (GraphPad Inc., San Diego, CA, USA) and Statistica v9. The Shapiro-wilk test was used to check out data normality. For data that did not follow a normal distribution, medians with interquartile ranges were used. Mann-Whitney U test was used to determine whether differences observed in the *in-vitro* responses to antimalarial drugs were significant. Correlations were determined using the Spearman test. The potential for *in-vitro* cross-activity was assessed by standard linear regression analysis. For multiple comparisons, the significance level was adjusted using the Bonferroni correction. A comparison was statistically considered significant when p-value was ≤ 0.05.

Ethical considerations

The research protocol was approved by the National Committee for Ethics and Research (NCER). An informed consent was required from each participant and/or parents or legal guardians of children. For children over the age of 9, informed consent was required prior to their inclusion in the study.

RESULTS

General characteristics of participants

A total of 134 patients were enrolled and blood sampled. Among them, 72 had phenotypes HbAA, 26 were heterozygous (HbAC or HbAS) and 36 were double mutated (HbSC or HbSS) (Table 1). Sex ratio was almost one and body mass index was 19.10 and 15.6, respectively for minor (HbAS and HbAC) and major phenotypes (HbSS and HbSC). For the HbAA controls the index was 20.65. Most of the patients recruited in this study were children with a mean age of 11 years without difference between children with normal phenotype HbAA (13 years of age) and minor phenotypes HbAS and HbAC. For the major phenotypes HbSC and HbSS, the mean age was, respectively 13.15 and 10.17 years (no significant difference) (Table 2). The mean body temperature varies according to the different phenotypes of sickle cell disease (Table 1). It varies from 38.22 to 39.87°C, with significant differences between the phenotype HbAA and the phenotypes HbAS, HbSS and HbSC. Analyses of parasitaemia at inclusion showed significant difference (Kruskal-Wallis test, $P < 0.05$) between the phenotype HbAA and the phenotypes HbAS, HbSS and HbSC (Table 1).

In-vitro maturation test

The maturation test was performed only with 116 isolates out of the 134 samples collected due to small volume of blood. After 72 h of culture, successful maturation was obtained with 111 (95.69%) isolates. This rate slightly varied according to the drugs: 89/116 (80.18%) with Amodiaquine, 94/116 (84.68%) with Chloroquine, 95/116 (85.59%) with DHA, 98/116 (88.29%) with Lumefantrine and 92/116 (82.89%) with Quinine (Table 3).

The IC_{50} values ranged from 0.53 to 11.18 nM (geometric mean, 2.71 nM) for DHA, from 2.89 to 72.98 nM (geometric mean, 15.60 nM) for AQ, from 3 to 111.5 nM (geometric mean, 23.32 nM) for CQ, between 1.89 and 109.1 nM (geometric mean, 13.62 nM) for LUM and between 2.03 and 82.30 nM (geometric mean, 11.13 nM) for QN (Table 3). Six isolates were found to be resistant in culture to DHA (3/95 3.16%, IC_{50s} of 10.41, 11.18 and 11.01 nM). Resistance to Chloroquine and Amodiaquine occurred, respectively in 1.06% (1/94, IC_{50} of 111.45 nM) and 2.25% (2/89 IC_{50s} of 72.98 and 65.19 nM) (Figures 1 and 2).

A significant correlation between the *invitro* activity of artemisinin (DHA) and those of chloroquine ($n = 82$, Pearson $r = 0.478$; $p < 0.0001$) and amodiaquine ($n = 79$; Pearson $r = 0.326$; $p = 0.003$) was observed (Figure 3c and d). Accordingly, a positive correlation was detected between artemisinin and lumefantrine ($n = 86$, Pearson $r = 0.2318$; $p = 0.0317$) (Figure 3a). However, no resistance was found for lumefantrine and quinine (Figures 1 and 2).

Table 1. General characteristics of participants.

Characteristics	HbAA	HbAC	HbAS	HbSC	HbSS
Count	72	7	19	13	23
Body mass index (mean±SD)	20.65±5.77	21.67±4.54	19.06±5.40	18.75±4.22	14.41±3.41
Temperature (°C), (mean±SD)	39.58±1.16	39.87 ± 1.56	38.29 ± 0.92	38.28±0.71	38.22±1 .13
Parasitaemia (/µL of blood), Median (IQR)	38315 (19515-53543)	11200 (5610 - 57100)	13670 (8220 - 42600)	13250 (4805 - 21800)	16300 (7900 - 43900)
Hémoglobin level (G/100 mL), (mean±SD)	11.28±2.10	13.05±2.53	8.66±2.38	9.63±1.71	6.51±2.24
P-Values		**HbAA Vs HbAC**	**HbAA Vs HbAS**	**HbAA Vs HbSC**	**HbAA Vs HbSS**
Body mass index		0.677	0.359	0.262	< 0.001
Température (°C)		0.678	< 0.001	< 0.001	< 0.001
Parasitaemia (parasites/µL of blood),		0.061	0.012	0.002	0.014
Hémoglobin level (G/100 mL)		0.083	< 0.001	0.218	< 0.001

Hb: Hemoglobin, SD: standard deviation, IQR: interquartile range, µL: microliter, G: gram, mL: milliliter.
Source: Author

During this maturation test, parasites in HbAS phenotype seem more sensitive to all molecules tested compared to other sickle cell phenotypes (Figures 4, 5, 6, 7 and 8). For example, significant difference was observed for DHA between strains in red cells with phenotype HbAA and in HbAS (Mann Whitney test, P = 0.0021) and for strains in red cells with phenotype HbAS and HbSS (Mann Whitney test, P = 0.0034) (Figures 4 and 9). However, interestingly these differences were not found for LUM, QN, AQ and CQ (Kruskal-Wallis test, p > 0.05) (Figures 5, 6, 7 and 8).

RSA test

Among the 107 isolates with a parasite growth rate ≥ 1 in RSA, 87 (65.41%) were considered to be sensitive to DHA and 20 (15.04%) isolates borderline susceptibility (*in vitro* resistants) when exposed against 700 nM (Table 4). These 20 isolates had a median survival rate of 3.08% (IQR; range: 1.88-24.47; 1.28-33.75) and a median inclusion parasitaemia of 0.92% (IQR; range: 0.49-1.05; 0.25-1.94). were considered to have DHA IC_{50} maturation test values were not correlated with survival rates obtained with the RSA test (n=81, Spearman r = 0.100 [95% CI: -0.127; 0.318], p=0.374).

Parasites sensitive to DHA in RSA test and those with a borderline sensitivity also showed non-significant difference in sensitivity when exposed to AQ and CQ for 72 h (kruskalwallis test, p > 0.05) (Table 5).

However significant difference was found when exposed to LUM (15.92 nM versus 22.33 nM) or QN (11.01 nM versus 20.02 nM) (Kruskal-Wallis test, p < 0.05) (Table 5).

DISCUSSION

Côte d'Ivoire is one of the main endemic countries for *P. falciparum* infection in West-Africa. This study confirms that the different molecules used to treat uncomplicated malaria are still efficient against isolates circulating in this country.

Among amino-4-quinoleine, quinine remains an important drug to fit malaria in Côte d'Ivoire especially for pregnant women (Dondorp et al., 2010; Yavo et al., 2010), or to treat malaria during SCD vaso-occlusive crisis. It is still very active against the parasite. For chloroquine, a low prevalence of chloroquine-resistant *P. falciparum* was found during this work with isolates from sickle cell. These results are in line with recent reports indicating a decrease in the prevalence of chloroquine-resistant *P. falciparum* in several countries, such as Malawi, Zambia and Cote d'Ivoire (Dagnogo et al., 2018; Kublin et al., 2003; Mwai et al., 2009; Ndam et al., 2017). Parasites that carry the Lys-76 wild-type allele have a survival advantage in the absence of drug pressure (Laufer and Plowe, 2004). Indeed, when drug pressure is low, drug resistance is accompanied by a reduction in the genetic

Table 2. Distribution of the different haemoglobin phenotypes according to age groups.

Parameter		HbAA (N=72)	HbAS (N=19)	HbAC (N=7)	HbSS (N=23)	HbSC (N=13)
Meanage (years), [± SD]		12.90 [13]	13.47 [10.67]	12.43 [4.76]	10.17 [4.42]	19.15 [14.12]
< 5 years old	N (%) Patients	29 (40.28%)	6 (31.58%)	0 (0%)	5 (21.74%)	2 (15.38%)
	Mean age [± SD]	3.3 [1.44]	3.33 [1.36]		4.6 [0.55]	4 [0.0]
> 5 years old	N (%) Patients	43 (59.72%)	13 (68.42%)	7 (100%)	18 (78.26%)	11 (84.61%)
	Mean age [± SD]	19.37 [13.33]	18.15 [9.75]	12.43 [4.76]	11.72 [3.68]	21.91 [13.60]

P-value			HbAA Vs HbAS	HbAA Vs HbAC	HbAA Vs HbSS	HbAA Vs HbSC
< 5 years old	N (%) Patients	-	0.668	0.089	0.172	0.161
	Mean age [± SD]	-				
> 5 years old	N (%) Patients	-	0.668	0.089	0.172	0.161
	Mean age [± SD]	-				

Hb: Hemoglobin, SD: standard deviation, N: number of patients.
Source: Author

Table 3. Geometric mean of 5 antimalarial drugs IC_{50s} tested on clinical isolates of *Plasmodium falciparum* in sickle cell patients.

Antimalarials	Number of tested isolates	geometric mean of IC_{50} (nM)	Confidence interval 95% (nM)	Range (nM) Min-Max	Treshold value (nM)
DHA	95	2.71	2.32 - 3.17	0.53 - 11.18	10
AQ	89	15.6	13.07 - 18.62	2.89 - 72.98	60
CQ	94	23.32	19.62 - 27.71	03.0 - 111.5	100
LUM	98	13.62	11.00 - 16.86	1.89 - 109.1	150
QN	92	11.13	9.14 - 13.55	2.03 - 82.30	800

Dihydroartemisinin (DHA), Amodiaquine (AQ), Chloroquine (CQ), Luméfantrine (LUM) and Quinine (QN).
Source: Author

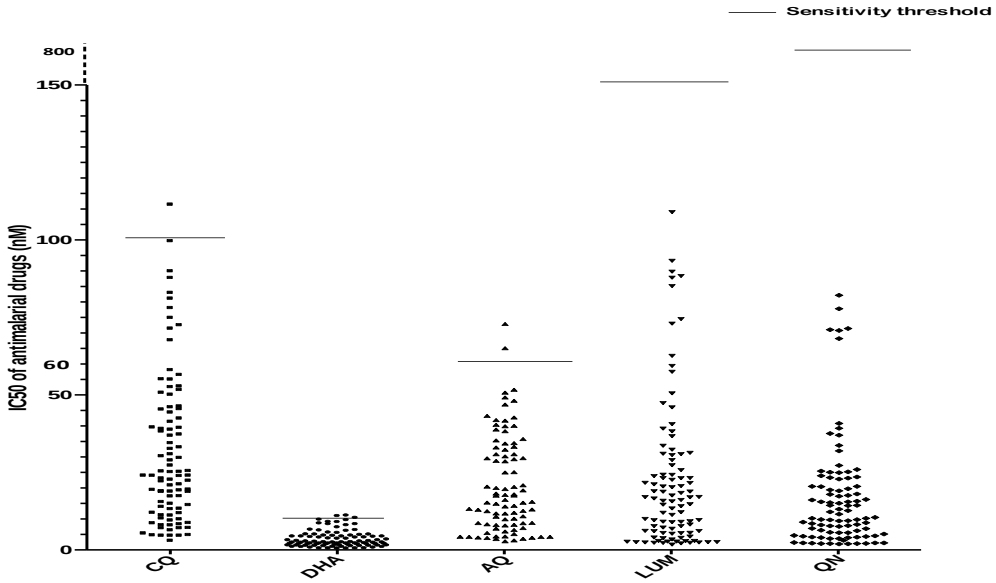

Figure 1. Response of *Plasmodium falciparum* according to the different antimalaria molecules.
Source: Author

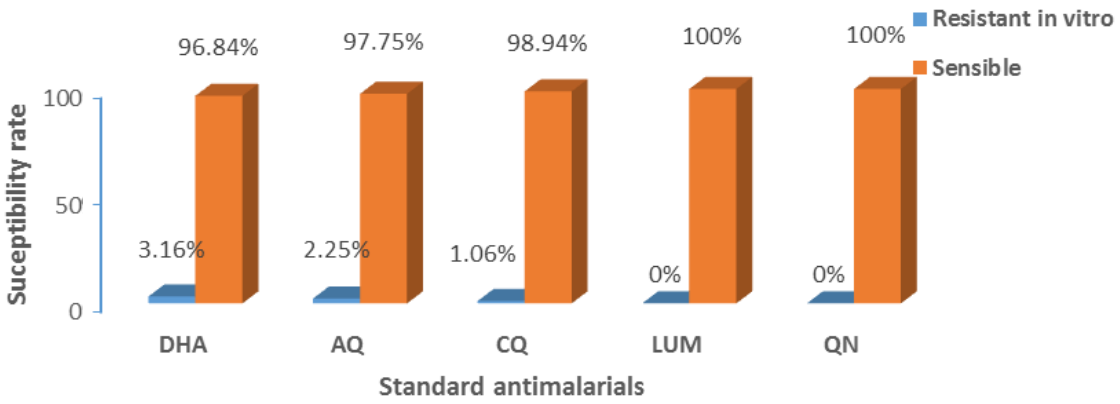

Figure 2. Distribution of the sensitivity rate according to the different molecules. The threshold value was 60, 100, 10, 150 and 800 nM, respectively for AQ, CQ, DHA, LUM and QN. Chloroquine (CQ), Amodiaquine (AQ), Quinine (QN), Lumefantrine (LUM), Dihydroartemisinin (DHA).
Source: Author

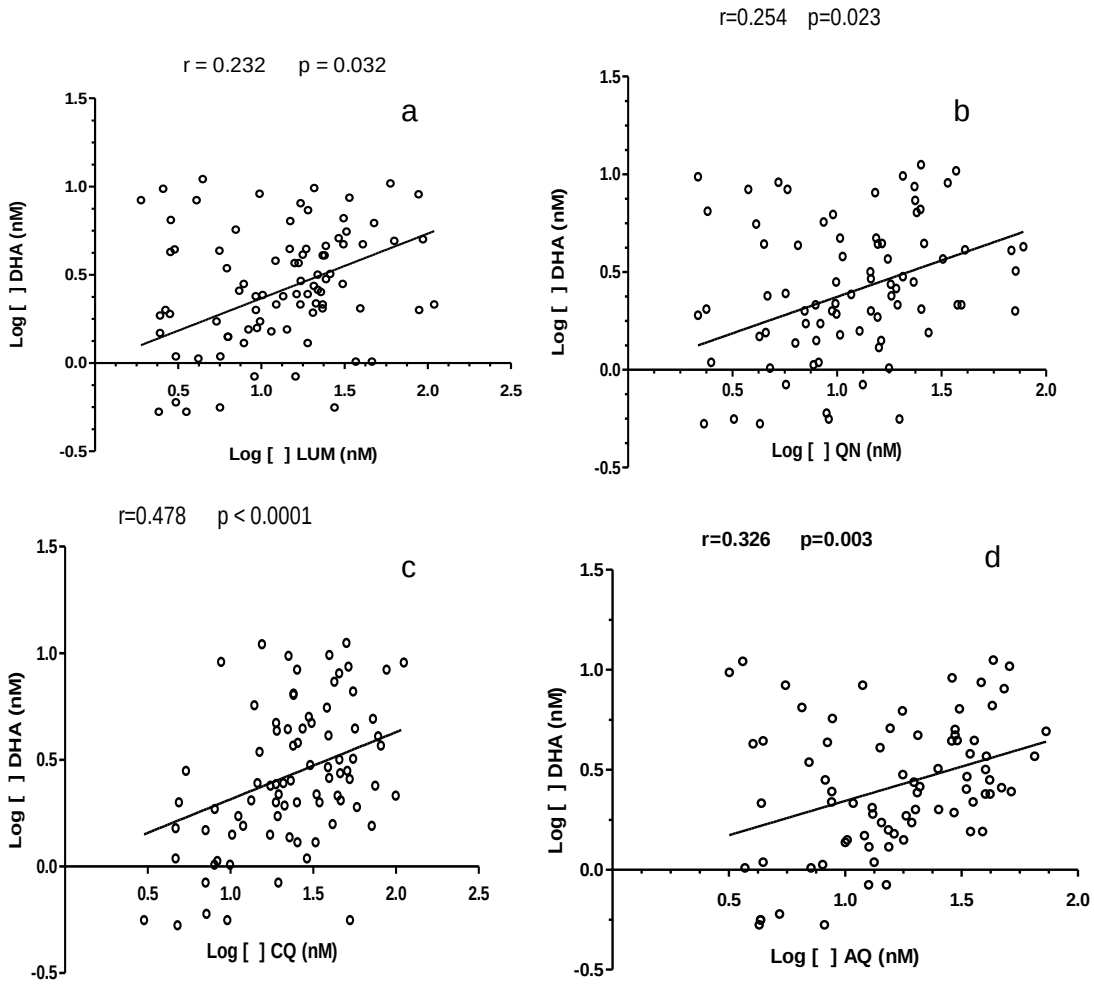

Figure 3. Plots of IC_{50} (nM) and regression line of *in-vitro* activity of DHA and other molecules (LUM, QN, CQ and AQ). Correlation and significance values for antimalarial drug couple (DHA/LUM, DHA/QN, DHA/CQ and DHA/AQ) were determined by Pearson. Dihydroartemisinin (DHA), Amodiaquine (AQ), Chloroquine (CQ), Luméfantrine (LUM) and Quinine (QN).
Source: Author

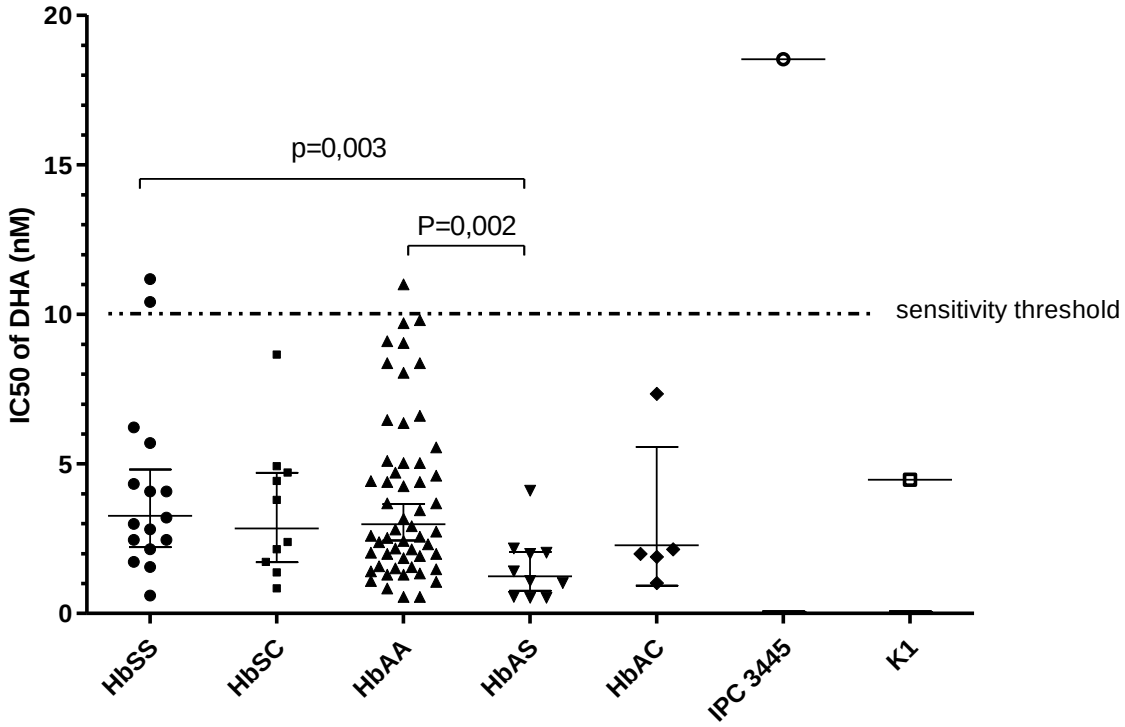

Figure 4, *In-vitro* response of sickle cell isolates to DHA. Geometric means with 95% confidence interval of the IC_{50} were represented. All assays were in duplicate. IC50, Inhibitory concentration 50%, DHA, Dihydroartemisinin; K1, artemisinin-sensitive strain; IPC 3445, artemisinin-resistant strain. A statistically significant difference was observed on DHA between phenotype HbAA and HbAS strains (Mann Whitney test, P = 0.0021) and between phenotype HbAS and HbSS strains (Mann Whitney test, P = 0.0034).
Source: Author

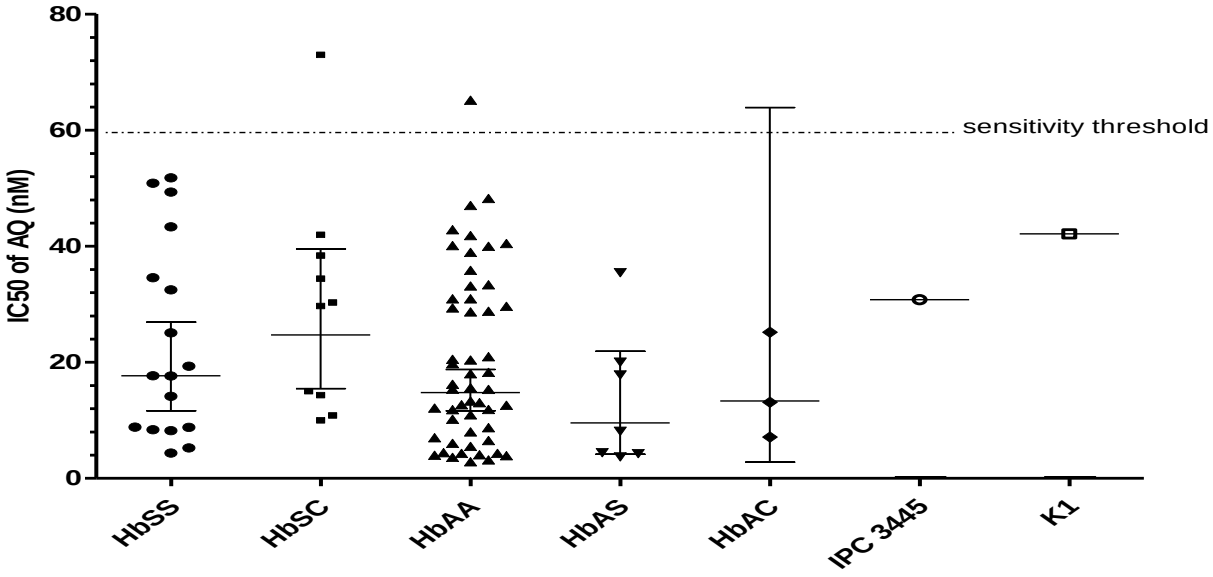

Figure 5, *In-vitro* response of sickle cell isolates to AQ. Geometric means with 95% confidence intervals of the IC_{50}were represented. All assays were in duplicate. IC50, Inhibitory concentration 50%; AQ, Amodiaquine. K1, artemisinin-sensitive strain; IPC 3445, artemisinin-resistant strain. No significant difference was observed between the different phenotypes for AQ (Kruskal-wallis test, $p > 0.05$).
Source: Author

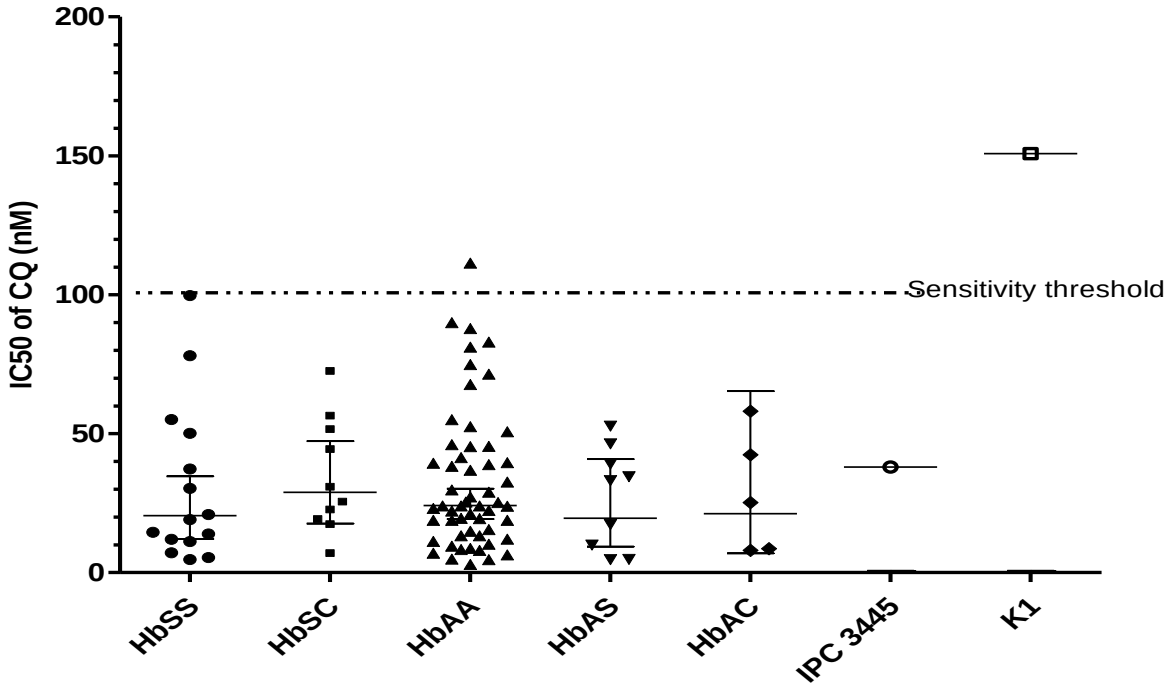

Figure 6. *In-vitro* response of sickle cell isolates on CQ. Geometric means with 95% confidence interval of the IC_{50} were represented. All assays were in duplicate. IC_{50}, Inhibitory concentration 50%; CQ, Chloroquine; K1, artemisinin-sensitive strain; IPC 3445, artemisinin-resistant strain. No significant differences were observed between the different phenotypes for CQ (Kruskal-wallis test, $p > 0.05$).
Source: Author

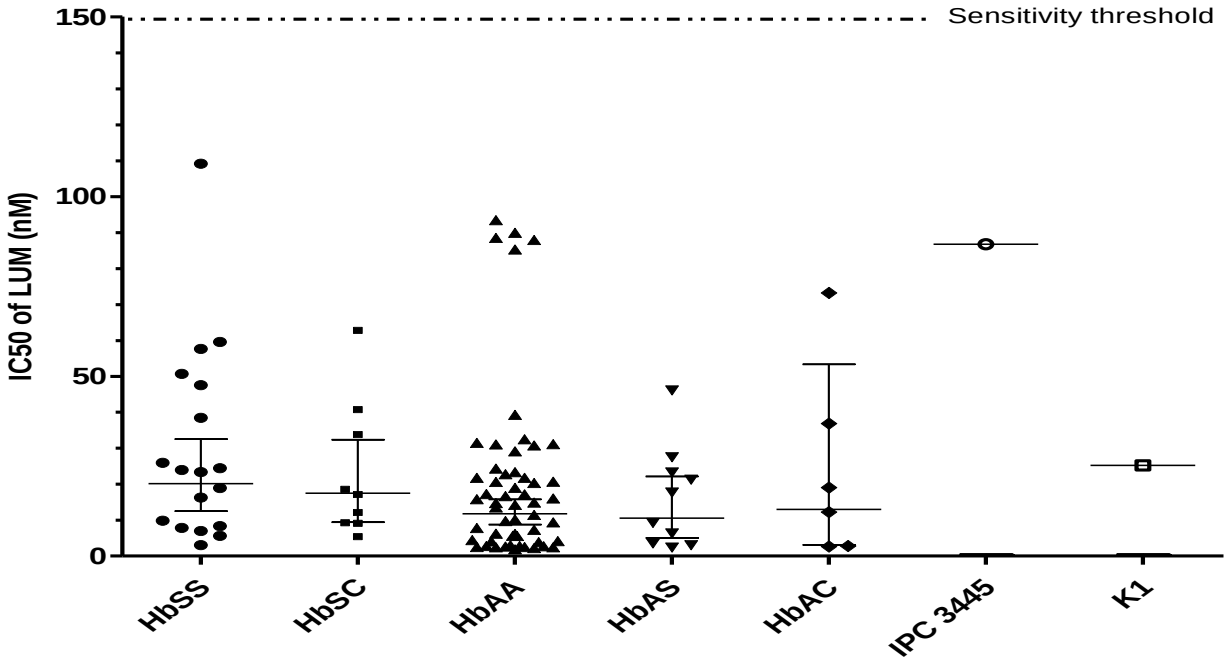

Figure 7. *In-vitro* response of sickle cell isolates on LUM. Geometric means with 95% confidence interval of the IC_{50} were represented. All assays are in duplicate. IC50, Inhibitory concentration 50%; LUM, Lumefantrine; K1, artemisinin-sensitive strain; IPC 3445, artemisinin-resistant strain. No significant difference was observed between the different phenotypes for LUM (Kruskal-wallis test, $p > 0.05$).
Source: Author

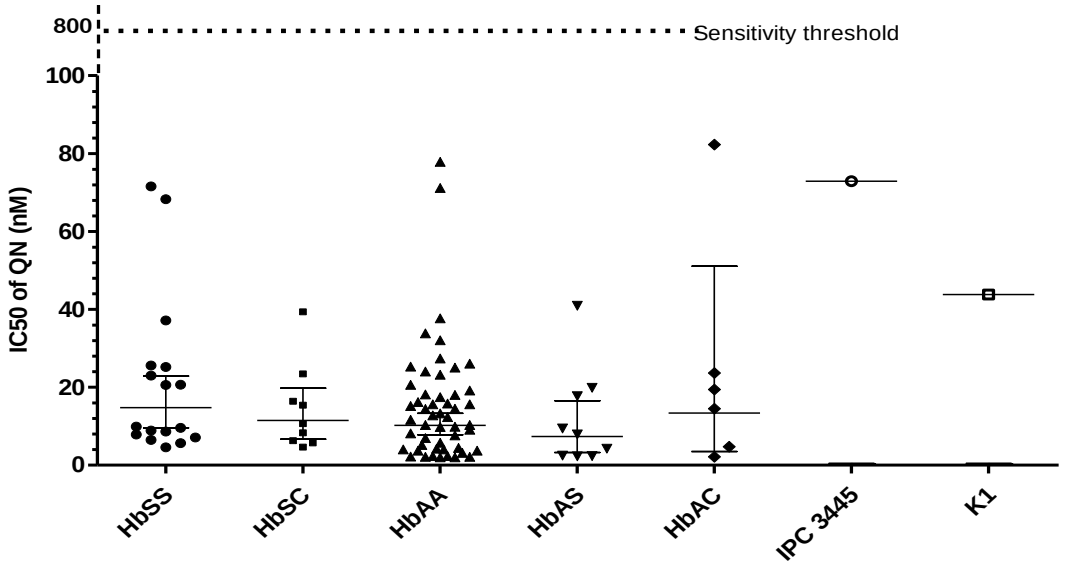

Figure 8. *In-vitro* response of sickle cell isolates on QN. Geometric means with 95% confidence interval of IC_{50} were represented. All assays were in duplicate. IC_{50}, Inhibitory concentration 50%; QN, quinine; K1, artemisinin-sensitive strain; IPC 3445, artemisinin-resistant strain. No significant differences were observed between the different phenotypes for QN (Kruskal-wallis test, $p > 0.05$).
Source: Author

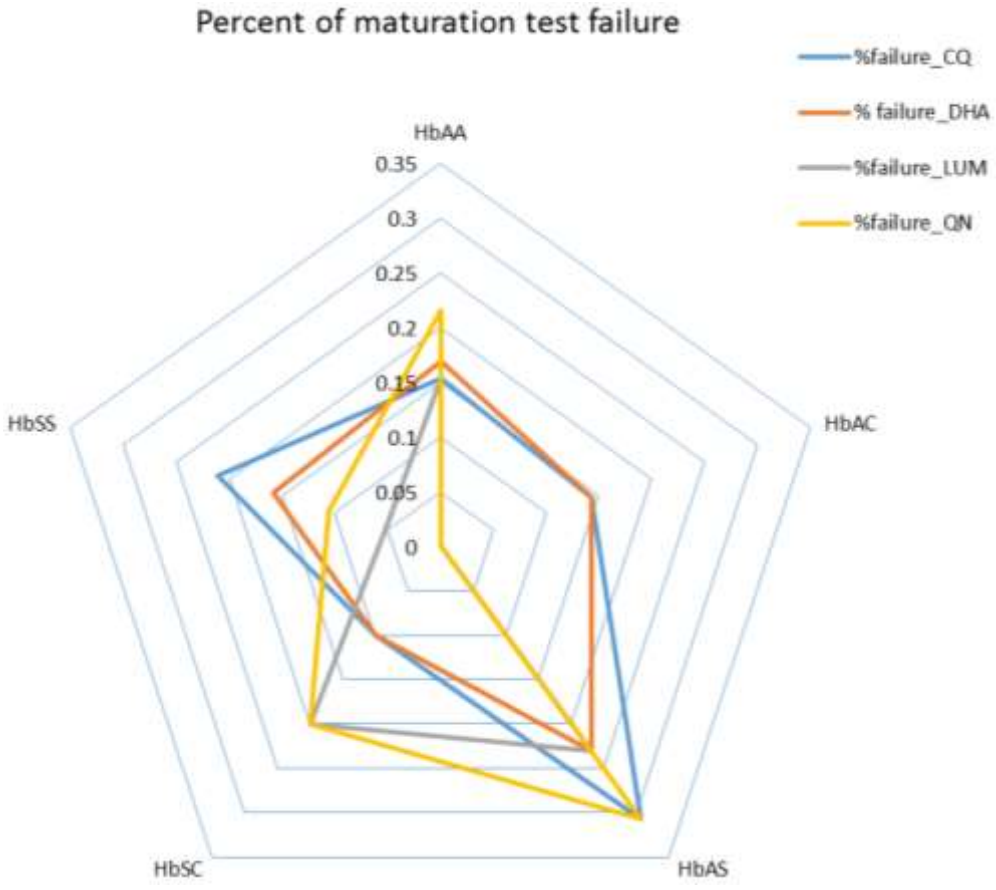

Figure 9. Percent of maturation test failure.
Source: Author

Table 4. Sensitivity of clinical isolates after exposure to 700 nM for 6 h to DHA according to sickle cell disease types.

Parameter	Growth failure n=26 (19.55)	RSA test results	
		Sensitivity to DHA *in-vitro* n=87 (65.41)	Resistance to DHA *in-vitro* n=20 (15.04)
Normal Hemoglobin (HbAA), n=72	9 (12.50)	53 (73.61)	10 (13.89)
Sickle cell traits, n=25	9 (36.00)	14 (56.00)	2 (08.00)
HbAS, n=18	9 (50.00)	8 (44.44)	1 (05.56)
HbAC, n=7	0 (00.00)	6 (85.71)	1 (14.29)
Abnormal Hemoglobin, n=36	8 (22.22)	20 (55.56)	8 (22.22)
HbSS, n=23	5 (21.74)	11 (47.83)	7 (30.43)
HbSC, n=13	3 (23.08)	9 (69.23)	1 (07.69)

n: Number of isolates, ( ) is the proportion of isolates in % of the RSA test results, DHA: dihydroartemisinin, Hb: Hemoglobin.
Source: Author

Table 5. Relationship between RSA test results and the ex vivo Conventional Drug-Susceptibility Assay results.

Parameter	*Invitro* RSA resistance test		p value	expected *In vivo* resistance		p value
	Resistant	Sensitive		Sensitive	Resistant	
	n (median)	n (median)		n (median)	n (median)	
IC50 AQ	17 (30.97)	60 (15.37)	0.314	71 (15.51)	7 (32.46)	0.147
IC50 CQ	15 (24.04)	64 (24.03)	0.851	73 (24.03)	7 (36.92)	0.658
IC50 DHA	15 (2.92)	66 (2.55)	0.412	78 (2.57)	4 (3.395)	0.445
IC50 LUM	20 (22.33)	64 (15.92)	0.031	78 (16.03)	7 (50.65)	0.019
IC50 QN	16 (20.02)	60 (11.01)	0.049	71 (13.06)	6 (16.48)	0.581

n represents the number of isolates, the numbers in brackets represent the median; RSA, ring-stage survival assay; IC_{50}: the effective concentration of drug needed to inhibit growth of *P. falciparum* by 50% ; AQ: Amodiaquine; CQ: Chloroquine; LUM: Lumefantrine; QN: Quinine; DHA : dihydroartemisinin.
Source: Author

performance of resistant parasites compared to susceptible parasites (Gadalla et al., 2010; Ord et al., 2007). Thus, when the drug pressure decreases, the proportion of sensitive parasites increases and that of the resistant parasites decreases (Kublin et al., 2003). This re-emergence of chloroquine-sensitive isolates may be due to a number of factors, the main one being the effective withdrawal of CQ since 2007 (15 years ago) in Côte d'Ivoire.

However, 15 years after CQ withdrawal from the national policy (alternative ACTs are still using), amino-4-quinoleine is still used as partner molecules in combinated drugs. It is the case of amodiaquine in Atesunate-Amodiaquine (ASAQ) which harbors cross-resistance mechanisms with CQ. This could explain persistence of resistance to CQ (Ferdig et al., 2004) observed in this study.

For plasmodial isolates from sickle cell patients, maturation test highlighs a reduced sensitivity to amino-4-quinolines (CQ and AQ) as well as DHA. These data are in line with previous studies conducted by Touré et al. (2008). Moreover, during clinical trials implemented so far in Côte d'Ivoire "warning signs" of delayed clearance of parasites after treatment with Artemether-Lumefantrine arm were noticed (Serge-Brice et al., 2020; Toure et al., 2018, 2020; Yavo et al., 2015). However, none of isolates revealed resistance to luméfantrine when tested *in-vitro* suggesting thus a decrease of sensitivity to artemisinin derivatives instead of lumefantrine.

Data also indicated no correlation between DHA IC_{50s} (maturation test) and RSA survival rates. These results were similar to those obtained in Uganda and Cambodia (Ikeda et al., 2018; Witkowski et al., 2013).

The standard parasite maturation test reveals easily resistance to conventional antimalarials drugs such as quinolines and antifolates but not resistance to artemisinins and its derivatives. Similarly, studies in Cambodia, Cameroon (Menard et al., 2016) and Uganda (Ikeda et al., 2018), showed that no polymorphisms in the PfKelch13 was found in clinical isolates with RSA test survival rates above 1% or 10% which confirm that molecular survey of polymorphism of this gene may not

be considered as a suitable method for Africa.

Patients with the HbSS phenotype harboured isolates with decreased sensitivity to DHA. The same result was obtained in Ghana where a study showed a slower parasite clearance in sickle cell patients compared to patients with normal phenotype HbAA (Adjei et al., 2014). However, despite this decrease of *in-vitro* sensitivity of DHA, clinical surveys showed that this drug still remains efficient to treat malaria in Ivory Coast, but this efficacity should be actively monitored, especially in sickle cell patients.

Overall, re-emergence of chloroquine-sensitive isolates in Côte d'Ivoire 15 years after the withdrawal of CQ from the national market, raises the hope that amino-4-quinoleines could be reintroduced in the near future, ideally as a partner drug for the treatment of uncomplicated malaria of for seasonal chemprophylaxis of malaria. Indeed, it has already been shown that artesunate-amodiaquine, still have a good activity against *P. falciparum* (Toure et al., 2020). This re-emergence of CQ sensitive isolates after a withdrawal of CQ has already been shown in Côte d'Ivoire and in other African countries (Dagnogo et al., 2018; Kublin et al., 2003; Mwai et al., 2009; Ndam et al., 2017; Ndiaye et al., 2012).
Nevertheless, it should be important to evaluate DHA-chloroquine combination should be evaluated *in-vitro* and *in-vivo* with a larger number of samples.

Conclusion

To address the problem of antimalarial drug resistance, the Ivorian National Malaria Control programme has developed standardised guidelines for the treatment of malaria by adopting a single therapy for the whole country. Thus, after fifteen years of intensive use of ACTs, the sensitivity of parasites to DHA must be monitored. Red cells with abnormal hemoglobin provide a specific biochemical environment which might be favorable to a descrease in drug efficacy. Indeed, preliminary observations obtained on isolates from sickle cell patients show, a decrease in DHA and amino-8-quinoline (CQ and AQ) in these red cells but no significant difference for LUM, QN, AQ and CQ, which support the hypothesis and encourage further studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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