MOLECULAR EPIDEMIOLOGY OF MALARIA IN YAOUNDE, CAMEROON.VII. ANALYSIS OF RECRUDESCENCE AND REINFECTION IN PATIENTS WITH UNCOMPLICATED FALCIPARUM MALARIA

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Abstract. In an endemic area where malaria transmission is intense and continuous, reappearance of asexual parasites may be ascribed to either recrudescence or reinfection. To distinguish between recrudescence and reinfection after oral treatment with chloroquine, amodiaquine, pyronaridine, sulfadoxine-pyrimethamine, halofantrine, or artesunate, three polymorphic markers (circumsporozoite protein, merozoite surface antigens 1 and 2) from pre-treatment and post-treatment samples were amplified by the polymerase chain reaction, and the *in vitro* response to chloroquine was determined for comparison. Of 52 paired samples, 22 (42%) were reinfections. Recrudescence occurred more frequently on or before Day 14 (22 of 30 cases, 73%). Except for one case, all reinfections were observed beyond Day 14. The phenotype determination was not sufficiently precise to distinguish between recrudescence and reinfection. Our results suggest that beyond Day 14 (and until Day 42), recrudescence and reinfection cannot be distinguished at our study site unless molecular techniques are used and that some results derived from the polymerase chain reaction need to be compared with the microscopic examination of thick blood smear to exclude gametocyte carriers without asexual parasites after treatment.

INTRODUCTION

In antimalarial chemotherapy, drug resistance is defined as the "ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject."[1] Based on this definition and a 28-day follow-up, parasitologic responses are graded as sensitive (S) or resistant (RI, RII, and RIII). The definitions of RI-RII-RIII have been modified and adapted to several variants of *in vivo* tests that differ in the duration and frequency of follow-up of asymptomatic or symptomatic patients (7 to 14 days).[2,3] Several components of the definition need to be demonstrated to establish resistance: positive blood smear during a regular follow-up, administration of the drug under supervision, measurement of plasmatic drug levels, and persistence of the same parasite population as before treatment. The first two criteria may be satisfied with ease at any health care center. The demonstration of an adequate blood level of drugs requires the high-performance liquid chromatography. The fourth component is the key element that must be demonstrated before concluding that the persistence or reappearance of parasites after therapy is due to the same strain that survived in the patient despite correct therapy.

In an endemic area where malaria transmission is intense and perennial, the reappearance (or persistence) of asexual erythrocytic stages of *Plasmodium falciparum* after a correct therapeutic dose may be attributed to either recrudescence of drug-resistant parasites that were already present before treatment or a new infection. The *in vivo* response is influenced by the parasite's resistance profile and the host immune system, which is in turn age-dependent. As part of an effort to define the epidemiology of drug-resistant parasites and evaluate the therapeutic efficacy of antimalarial drugs, it is important to distinguish between recrudescence and reinfection. In the present study, the genotypes of clinical isolates obtained before treatment and those of parasites that

reappeared or persisted in the peripheral blood at various times following correct and complete treatment were compared with the aim to differentiate between recrudescence and reinfection. In addition, the phenotype of pre-treatment and post-treatment isolates was compared in some paired samples by *in vitro* drug sensitivity assays.

PATIENTS, MATERIALS, AND METHODS

Patients. The study was part of randomized clinical trials conducted at the Nlongkak Catholic missionary dispensary in Yaoundé, Cameroon between 1994 and 1998.[4,5] The following inclusion criteria were used for enrollment: age $\geq$ 5 years old, fever at consultation (or history of fever within the past 24 hr), monoinfection with *P. falciparum,* parasite density > 5,000 asexual parasites/μl of blood (to allow the performance of *in vitro* drug assays), easy access to the dispensary for daily monitoring, and no recent history of self-medication with antimalarial drugs, as confirmed by a negative Saker-Solomons urine test result.[6] Patients with signs and symptoms of severe and complicated malaria, as defined by the World Health Organization (WHO),[7] severe anemia (hemoglobin < 5.0 g/dL), or moderate and severe malnutrition were excluded. Informed consent was obtained from either the adult patients themselves or a guardian accompanying the sick children. The study was approved by the Cameroonian National Ethics Committee and the Cameroonian Ministry of Public Health.

The patients were treated with standard oral doses of chloroquine (25 mg/kg of body weight in three divided daily doses), amodiaquine (30 mg/kg of body weight in three divided daily doses), pyronaridine (32 mg/kg in four divided doses over a three-day period), sulfadoxine-pyrimethamine (a single dose of 1,500 mg of sulfadoxine and 75 mg of pyrimethamine in adults, 1 tablet/20 kg of body weight in children), or artesunate (a total of 600 mg over a five-day period in adults) under supervision. For those responding with a therapeutic failure, quinine or halofantrine was ad-

ministered. The patients were followed for at least 14 days according to the test of therapeutic efficacy, developed by WHO in 1996.[8]

Genotyping of *P. falciparum*. Parasite DNA was extracted from venous blood samples as described in our earlier study.[9] The DNA fragments of three polymorphic markers, circumsporozoite protein (CSP), merozoite surface antigen-1 (MSA-1), and merozoite surface antigen-2 (MSA-2) genes, were amplified by the polymerase chain reaction using two methods. In the first method, three pairs of synthetic oligonucleotides flanking the polymorphic regions of CSP, MSA-1, and MSA-2 were used simultaneously in an amplification procedure termed "multiplex polymerase chain reaction" to estimate the degree of allelic diversity in consecutive clinical isolates obtained in Yaoundé.[10] In the second method, each of the three loci was amplified separately. This approach allowed an unambiguous counting of the number of polymerase chain reaction products yielded for each locus. The following species-specific oligonucleotide primers were designed from published sequences of the complete gene sequences: CSP-1, 5'-AAATTACAATTCATGATGAGAAAA TTAGC-3' (forward primer), CSP-2, 5'-GATGTGTTCTTT ATCTAATTAAGGAACAAG-3' (reverse primer), MSA2-1, 5'-ATGAAGGTAATTAAAACATTGTCTATTATA-3' (forward primer), MSA2-2, 5'-TTATATGAATATGGCAAAAG ATAAAACAAG-3' (reverse primer), MSA1-1, 5'-ATGAA GATCATATTCTTTTTATGTTCATTT-3' (forward primer), and MSA1-2, 5'-TACGTCTAATTCATTTGCACGAATTT TAAG-3' (reverse primer).[11–13]

The reaction mixture consisted of approximately 200 ng of genomic DNA, 15 picomole of forward and reverse primers, buffer (50 mM KCl, 10 mM Tris, pH 8.3), 1.5 mM $MgCl_2$, 200 µM dNTP, and 1 unit of *Taq* DNA polymerase (Roche Diagnostics, Meylan, France) in a final volume of 50 µl. The PTC-100 thermal cycler (MJ Research, Watertown, MA) was programmed as follows: 94°C for 2 min for the first cycle and 30 sec in subsequent cycles, 50°C for 1 min for the first cycle and 30 sec in subsequent cycles, and 72°C for 1 min for all cycles (2 min for amplifying the entire *csp* gene), for a total of 30 cycles, followed by a 15 min extension step at 72°C. The amplified DNA fragments were resolved by electrophoresis in a 1.8% agarose gel, stained with ethidium bromide, and visualized under ultraviolet transillumination.

***In vitro* drug sensitivity assay.** The isotopic semi-microtest and microtest used in this study were described in previous studies.[14,15] The suspension (200 µl for the microtest and 700 µl for the semi-microtest) was distributed in each well of the tissue culture plates (96-well plate for the microtest and 24-well plate for the semi-microtest). The parasites were incubated at 37°C in 5% CO_2 for 42 hr. ^{3}H-hypoxanthine, 1 µCi/well (Amersham International, Plc., Buckinghamshire, United Kingdom) was added to assess parasite growth. The incorporation of ^{3}H-hypoxanthine was quantitated using a liquid scintillation counter (Wallac 1409; Pharmacia, Uppsala, Sweden). The 50% inhibitory concentration (IC_{50}), defined as the drug concentration corresponding to 50% of the uptake of ^{3}H-hypoxanthine measured in the drug-free control wells, was determined by non-linear regression analysis of logarithm of concentrations plotted against growth inhibition. The threshold IC_{50} value for *in vitro* resistance to chloroquine was estimated to be ≥ 100 nM.[15]

Data interpretation. Fifty-two paired samples of *P. falciparum*-infected erythrocytes were obtained and analyzed in this study. The recrudescent sample was included in this study if the patient had a positive blood smear, without fever, during the follow-up period on Days 7 or 14 or a second episode of acute uncomplicated malarial infection on or beyond Day 7 and within 100 days after the first (Day 0) infection. Blood samples from patients requiring alternative treatment on or before Day 3 were excluded. These excluded patients satisfied one of the four criteria of an early treatment failure: positive smear and signs and symptoms of severe malaria on Days 1, 2, or 3; positive smear (parasite density > Day 0 density) and fever on Day 2; positive smear and fever on Day 3; and positive smear on Day 3 (parasite density ≥ 25 % of pre-treatment density).[8] For some paired samples, there were additional samples from patients with multiple recrudescences. Further recrudescent samples of the patients were included for comparison until Day 300 from the initial drug therapy.

Based on the definitions of RI, RII, and RIII, paired samples were classified into three groups depending on the day when recrudescent parasites were obtained: Group 1, between Day 7 and Day 14; Group 2, between Day 15 and Day 28; and Group 3, beyond Day 29. Recrudescence was defined as the presence of identical amplification products in pre-treatment and post-treatment samples. Type A recrudescence consisted of either single alleles at all three loci, suggesting monoclonal infections, or multiple alleles at 1, 2, or 3 loci, suggesting multiclonal infections. Type B recrudescence was defined as a multiclonal infection before treatment, indicated by two or more bands in at least one locus, and the persistence of a subset of the alleles (multiclonal or monoclonal) after treatment. Type B recrudescence is likely to be due to the selection of drug-resistant populations after drug therapy and the missing band after treatment probably corresponds to the sensitive parasites. Type C recrudescence was defined as the presence of identical bands (monoclonal or multiclonal) in the pre-treatment and post-treatment samples, with an additional band appearing after treatment. The additional allele in type C recrudescence may suggest one of the following possibilities: 1) multiplication and/or selection of the minority of parasite populations present before treatment and undetected by the polymerase chain reaction, 2) appearance of secondary or tertiary broods with delayed or asynchronous development, as compared with the primary brood detected on Day 0, 3) additional parasite populations inoculated by the same or another mosquito few days after the initial infective bite, or 4) gametocyte formation, maturation, and release in the peripheral circulation.[16] Type C recrudescence may also be due to a mixture of type A recrudescence and reinfection, but the probability of obtaining amplification products of same sizes at three loci is slight in an area of intense transmission. Reinfection was defined as the presence of different alleles at all three loci. When different alleles were observed at two loci, with an allele at one locus (usually CSP) which appeared to have an identical or similar size, the post-treatment sample was classified as reinfection.

TABLE 1

Recrudescence and reinfection occurring within 100 days after antimalarial drug therapy

Days after treatment	Recrudescence* no. of cases			Reinfection†
	Type A	Type B	Type C	
Group 1 (Days 7–14)				
Chloroquine	6	9	3	1
Sulfadoxine-pyrimethamine	1	3	0	0
Group 2 (Days 15–28)	-			
Chloroquine	2	2	0	2
Sulfadoxine-pyrimethamine	0	0	0	1
Amodiaquine	0	0	0	1
Halofantrine	0	0	0	1
Artesunate	1	0	0	0
Group 3 (> Day 28)‡				
Chloroquine	0	0	0	3
Sulfadoxine-pyrimethamine	0	0	0	3
Amodiaquine	0	1	0	4
Pyronaridine	0	2	0	6

* Type A refers to the presence of identical bands (same size and same number). Type B refers to the presence of multiple bands at day 0 and the same bands minus 1 or more bands post-treatment. Type C refers to the presence of additional bands not seen before treatment.

† Number of cases presenting a new infection.

‡ In Group 3, reinfection occurred between Day 52 and Day 78 (chloroquine), between Day 55 and Day 80 (sulfadoxine-pyrimethamine), between Day 38 and Day 99 (amodiaquine), and between Day 36 and Day 63 (pyronaridine). Recrudescence occurred on Day 35 (amodiaquine), Day 35 (pyronaridine), and Day 42 (pyronaridine).

RESULTS

In our preliminary study, consecutive pre-treatment samples were analyzed for allelic differences at the three loci by a multiplex polymerase chain reaction. The amplification products yielded different alleles at the three loci, indicating the wide diversity of *P. falciparum* isolates circulating in Yaoundé among symptomatic patients. The overlapping band sizes obtained from multiclonal infections did not allow a precise analysis of the frequency of each allele in this preliminary approach.

Of 52 paired samples obtained during the clinical trials conducted in Yaoundé, 22 (42%) were reinfections. The other samples were recrudescence, with either identical parasite populations (type A) (n = 10; 19%), selection of parasite populations (type B) (n = 17; 33%), or persistence of initial populations and appearance of other populations (type C) (n = 3; 6%) (Table 1). Recrudescence tended to occur more frequently on or before Day 14 (22 of 30 recrudescent cases, 73%), while reinfections were observed almost exclusively beyond Day 14 (21 of 22 reinfections, 95%). The only case (no. 1055) of reinfection on Day 14 occurred after chloroquine treatment. The patient had a negative blood smear on Day 7 but a positive smear on Day 14. The pre-treatment isolate was resistant *in vitro* (IC_{50} = 253 nM), while the post-treatment isolate was at the borderline (IC_{50} = 106 nM).

The characteristics of the patients and the corresponding isolates obtained at three or more time points, including pre-treatment isolate, are summarized in Table 2. The evolution of parasite genotype within a short time interval after chloroquine treatment was observed in five patients with recrudescence before Day 15. These patients were not treated with an alternative drug until Day 14 or 15 because they were asymptomatic between Day 3 and Day 13. Three of these isolates (no. 157, 1032, and 3009) analyzed at an interval of 1–4 days presented an identical recrudescent ge-

notype. One isolate (no. 156) had type B recrudescence and gametocytes on Day 7 but, on Day 15, had an identical (type A recrudescence) genotypic pattern as that of Day 0. Another isolate (no. 182) had different type B recrudescent genotypes on Day 7 and Day 14. Patients who were treated with amodiaquine, pyronaridine, or sulfadoxine-pyrimethamine and who had several malarial episodes at an interval exceeding seven weeks were infected with new parasite populations. In one of these patients (no. 1062), the sample corresponding to an adequate clinical response with no fever and no detectable asexual parasites on Day 258 (after the third malarial attack on Day 244) but with gametocytes yielded a type B recrudescent genotype. A similar phenomenon was also observed in two other patients treated with sulfadoxine-pyrimethamine. None of these three gametocyte carriers required further treatment.

The phenotype of pre-treatment and post-treatment paired samples was compared if the parasitemia of the post-treatment infection was ≥ 0.1%. Chloroquine was chosen as a phenotypic marker for this study. The most significant change in the phenotype occurred when the initially chloroquine-sensitive isolate was characterized to be chloroquine-resistant, or vice versa, at the time of recrudescence or reinfection (Table 3). Such a shift in chloroquine sensitivity/resistance occurred in nine (including one borderline, 106 nM) of 12 reinfecting isolates as well as in three of 11 recrudescent parasites. Eight of 9 recrudescent isolates after chloroquine treatment were resistant *in vitro* to chloroquine.

DISCUSSION

For several decades after the official formulation of the definition of antimalarial drug resistance in 1973, there was no adequate laboratory tool to demonstrate whether a given parasite strain was able to survive and multiply despite correct administration of the drug. To resolve this technical limitation, malaria researchers were usually required to conduct *in vivo* tests of resistance for 28 days in an area where there is no malaria transmission. Otherwise, the reappearance of parasites cannot be attributed to recrudescence or reinfection with certitude. This limitation of *in vivo* tests represented a major disadvantage in areas where malaria transmission occurs throughout the year in the entire country, as in most areas in sub-Saharan Africa.

In the late 1970s and during the 1980s, *in vitro* assay techniques were adapted from the *in vitro* culture method developed by Trager and Jensen to measure drug sensitivity/resistance of *P. falciparum* isolates.[14,17,18] However, *in vitro* and *in vivo* responses are not always concordant because host immunity may intervene in *in vivo* tests but not in *in vitro* assays and threshold levels of resistance for *in vitro* tests are arbitrary and may not correlate with *in vivo* response.[19] Furthermore, our results on the comparison of the level of *in vitro* chloroquine sensitivity/resistance of pre-treatment and post-treatment isolates were not decisive in distinguishing recrudescence from reinfection. The primary underlying reason for the poor performance of the comparison of phenotype may be related to the presence of multiple *P. falciparum* populations before treatment that may undergo selection or modify the proportion of different parasite populations after treatment. Drug treatment may kill the drug-

TABLE 2
Multiple episodes of recrudescence and reinfections occurring in the same patients

Patient no.	Age (yr)	Day of infection	Treatment	Parasitemia/μl	Genotype*
156	14	0	Chloroquine	394,000	
		7	—	6	Recrudescence B
		15	Halofantrine	3,290	Recrudescence A
157	11	0	Chloroquine	455,000	
		7	—	4,750	Recrudescence B
		11	Halofantrine		Recrudescence B
182	7	0	Chloroquine	579,000	
		7	—	784	Recrudescence B1
		14	Halofantrine	2,740	Recrudescence B2
1032	23	0	Chloroquine	13,600	
		13	—		Recrudescence C
		14	Halofantrine	4,400	Recrudescence C
3009	11	0	Chloroquine	8,180	
		7	—	408	Recrudescence B
		10	Halofantrine	3,010	Recrudescence B
1001	17	0	Amodiaquine	55,400	
		99	Amodiaquine	540	Reinfection 1
		234	Amodiaquine	2,640	Reinfection 2
3006	10	0	Amodiaquine	103,000	
		52	Amodiaquine	4,000	Reinfection 1
		293	Amodiaquine	240	Reinfection 2
3031	11	0	Amodiaquine	86,200	
		35	Halofantrine	720	Recrudescence B
		86	Amodiaquine	28,300	Reinfection
151	45	0	Pyronaridine	98,800	
		35	Amodiaquine	25,200	Recrudescence B
		211	Amodiaquine	200	Reinfection
1062	22	0	SP†	102,000	
		62	Amodiaquine	180	Reinfection 1
		244	SP†	46,200	Reinfection 2
		258	—	0	Recrudescence B

* Recrudescence Type A, identical bands; Type B, selection; Type C, appearance of new bands. Reinfection 1 and 2 refer to two different new infections. Patient no. 1062 (Day 258) had no detectable asexual parasites but carried gametocytes. The presence of gametocytes is not an evidence of recrudescence; but unless microscopic examination is conducted, the polymerase chain reaction alone may lead to a wrong conclusion.
† SP = sulfadoxine-pyrimethamine.

sensitive populations but select resistant parasites, which may persist or reappear in the peripheral blood several days or weeks after the treatment has been completed. This *in vivo* phenomenon has been reproduced experimentally by cloning or maintaining a clinical isolate in continuous *in vitro* culture.[20–23] Another reason is the minimal requirement of 0.1% parasitemia to perform the *in vitro* assay at the time of recrudescence. Even if this requirement is fulfilled, *in vitro* assays performed with recrudescent isolates obtained several days to few weeks after treatment may not be able to reproduce the results obtained with the initial isolates due to the presence of antimalarial drugs concentrated within infected erythrocytes. The residual drug in the blood sample tends to lower the IC_{50} value. Moreover, reinfection cannot be deduced on the sole basis of the chloroquine sensitivity/resistance pattern since there is a probability of approximately 50% to be infected with sensitive or resistant parasites, based on the epidemiology of drug-resistant *P. falciparum* in Yaoundé.[15,19]

These technical limitations of *in vivo* and *in vitro* tests of drug resistance can be overcome by the polymerase chain reaction. Some investigators have applied nested polymerase chain reaction, hybridization of amplification products with labeled probes, single-strand conformational polymorphism, and DNA sequencing to genotype field isolates of *P. falciparum*.[24–29] Our study showed that even a single 30-cycle round of polymerase chain reaction to amplify three inde-

pendent polymorphic markers provides sufficient data to distinguish recrudescence from reinfection. The genotypic markers can also be amplified in a single reaction tube by multiplex polymerase chain reaction.[10]

The randomized clinical study from which paired samples were obtained for this study included 131 patients treated with chloroquine and followed until at least Day 14, 81 with pyronaridine, 59 with amodiaquine, and 66 with sulfadoxine-pyrimethamine. Recrudescence occurred most frequently after treatment with chloroquine, which is still the first-line drug in Cameroon. This result was expected from our regular *in vitro* monitoring of drug resistance which showed that about 50% of the clinical isolates obtained at the same dispensary in Yaoundé are resistant to chloroquine since 1994.[15,19] Amodiaquine has been known to be a highly effective alternative first-line drug in Cameroon, as confirmed by our *in vitro* results.[30] Pyronaridine is also highly active *in vitro* against Cameroonian isolates and effective in clearing parasitemia in patients.[4,5,31] Sulfadoxine-pyrimethamine is a second-line drug in Cameroon and has been shown to have a cure rate of 89%.[32] Thus, the pattern of recrudescent profiles observed in our study closely follows the epidemiology of antimalarial drug resistance in Yaoundé.

Halofantrine and artesunate are new drugs that have been introduced in Cameroon several years ago. Halofantrine has been shown to be effective in a clinical trial conducted in Yaoundé, even with a single cure of 25 mg/kg over a 12-hr

TABLE 3

Comparison of the *in vitro* chloroquine response between pre-treatment and post-treatment isolates

Isolate no.*	Treatment	Recrudescence day	Genotype†	Chloroquine IC$_{50}$ (nM)	
				Pre-treatment	Post-treatment
157	Chloroquine	11	Recrudescence B	159	213
143	Chloroquine	14	Recrudescence A	151	154
CQ9	Chloroquine	14	Recrudescence A	117	550
182	Chloroquine	14	Recrudescence B	24.4	205
1019	Chloroquine	14	Recrudescence B	287	290
175	Chloroquine	14	Recrudescence C	102	107
156	Chloroquine	15	Recrudescence A	163	328
CQ11	Chloroquine	15	Recrudescence B	57.4	436
147	Chloroquine	22	Recrudescence A	278	69
180	Chloroquine	23	Reinfection	45.6	245
CQ17	Chloroquine	21	Reinfection	81.1	130
1055	Chloroquine	14	Reinfection	253	106
1060	Amodiaquine	28	Reinfection	39.4	336
1104	Amodiaquine	42	Reinfection	55.2	48.1
133	Pyronaridine	42	Recrudescence B	58.9	10.1
7	Pyronaridine	63	Reinfection	94.0	288
113	Pyronaridine	50	Reinfection	310	13.0
161	Pyronaridine	51	Reinfection	474	47.8
185	Pyronaridine	36	Reinfection	391	241
1014	Sulfadoxine-pyrimethamine	28	Reinfection	282	139
3063	Sulfadoxine-pyrimethamine	80	Reinfection	184	51.1
106	Halofantrine	22	Reinfection	355	37.5
HAR	Artesunate	20	Recrudescence A	186	316

* In addition to chloroquine, paired 50% inhibitory concentration (IC$_{50}$) values of the drugs used for treatment were determined for the following isolates: no. 1060, monodesethylamodiaquine (active metabolite of amodiaquine) IC$_{50}$ values = 16.4 nM vs 54.4 nM; no. 1104, monodesethylamodiaquine IC$_{50}$ values = 19.0 nM vs 9.02 nM; no. 133, pyronaridine IC$_{50}$ values = 3.03 nM vs 8.40 nM; no. 113, pyronaridine IC$_{50}$ values = 3.50 nM vs 1.33 nM; no. 161, pyronaridine IC$_{50}$ values = 7.93 nM vs 5.37 nM; no. 1014, pyrimethamine IC$_{50}$ values = 2,160 nM vs 281 nM; and no. 3063, pyrimethamine IC$_{50}$ values = 291 nM vs 236 nM. The threshold IC$_{50}$ values for *in vitro* resistance to chloroquine, monodesethylamodiaquine, and pyrimethamine were arbitrarily set at ≥ 100 nM, ≥ 60 nm, and ≥ 100 nM, respectively. The threshold value for pyronaridine is undetermined.
† Recrudescence Type: A = identical bands; Type B = selection; Type C = appearance of new bands.

period.[33] In the present study, only one of 56 patients requiring halofantrine for treatment failure with other drugs, essentially chloroquine (n = 48), returned 22 days after halofantrine treatment with symptomatic malaria due to reinfection. Oral artesunate was administered for five days to one patient, who had recrudescent malaria on Day 21. The two patients were cured with five days of quinine. Reappearance of parasites after halofantrine or artesunate treatment requires the dosage of plasmatic drug level few hours or days after drug administration because recrudescence is known to occur due to inadequate drug absorption or rapid elimination. [34–37]

The results of our study do not agree with the results of some of the previous studies. In the study of Babiker and others,[25] six Sudanese isolates obtained from patients responding with early and late RI chloroquine treatment failure (from Day 9 to Day 30) had identical MSA-1 and MSA-2 alleles, leading the investigators to conclude that RI chloroquine resistance is due to recrudescence. Using the same markers, a similar observation was made on 12 recrudescent isolates obtained 14–21 days after chloroquine or amodiaquine therapy in Papua New Guinea.[27] None of the parasites was characterized as reinfection in that study. In Thailand, recrudescence accounted for 38% of post-treatment reappearance of parasitemia up to Day 62 in nonpregnant patients, and new infections were detected as early as 21 days post-treatment.[38] However, most Thai patients were treated with quinine, mefloquine, and/or artemisinin derivatives. The long elimination half-life of mefloquine and the rapid elimination of quinine and artemisinin derivatives are expected to prolong and shorten the recrudescence period, respectively. Moreover, in Thailand and Sudan, malaria transmission

is seasonal and/or hypoendemic, suggesting a relatively lower risk of reinfection as compared with the tropical rain forest region in Africa. These differences preclude a direct comparison of results with those of our study. In Cameroonian patients residing in Yaoundé, reinfections were clearly present between Days 15 and 28 after chloroquine, amodiaquine, or sulfadoxine-pyrimethamine treatment. Similar observations were made in other African countries, such as Tanzania and Gabon, described as holoendemic areas.[29, 39]

While the definition of resistance is sufficiently precise from the operational viewpoint, the time-period of the grading system that allows a distinction between late and early RI seems to be arbitrary and possibly variable depending on the phamacokinetics of antimalarial drugs, risk of exposure to new infections, and degree of acquired immunity. Despite these shortcomings, Day 14 as the arbitrary cut-off point for true recrudescence after chloroquine therapy may be acceptable for practical reasons in Yaoundé at present. However, between Day 14 and Day 28, recrudescence and reinfection cannot be distinguished by clinical and parasitologic response to chloroquine, and we need to resort to molecular techniques to genotype the isolates. Other standard antimalarial drugs and new drugs are still generally effective in Cameroon, which means that isolates from any patient who returns with symptomatic malaria after treatment need to be genotyped and compared with the pre-treatment isolate as part of the surveillance program of antimalarial drug resistance and test of therapeutic efficacy.

Catholic missionary dispensary for precious aid in recruiting patients.

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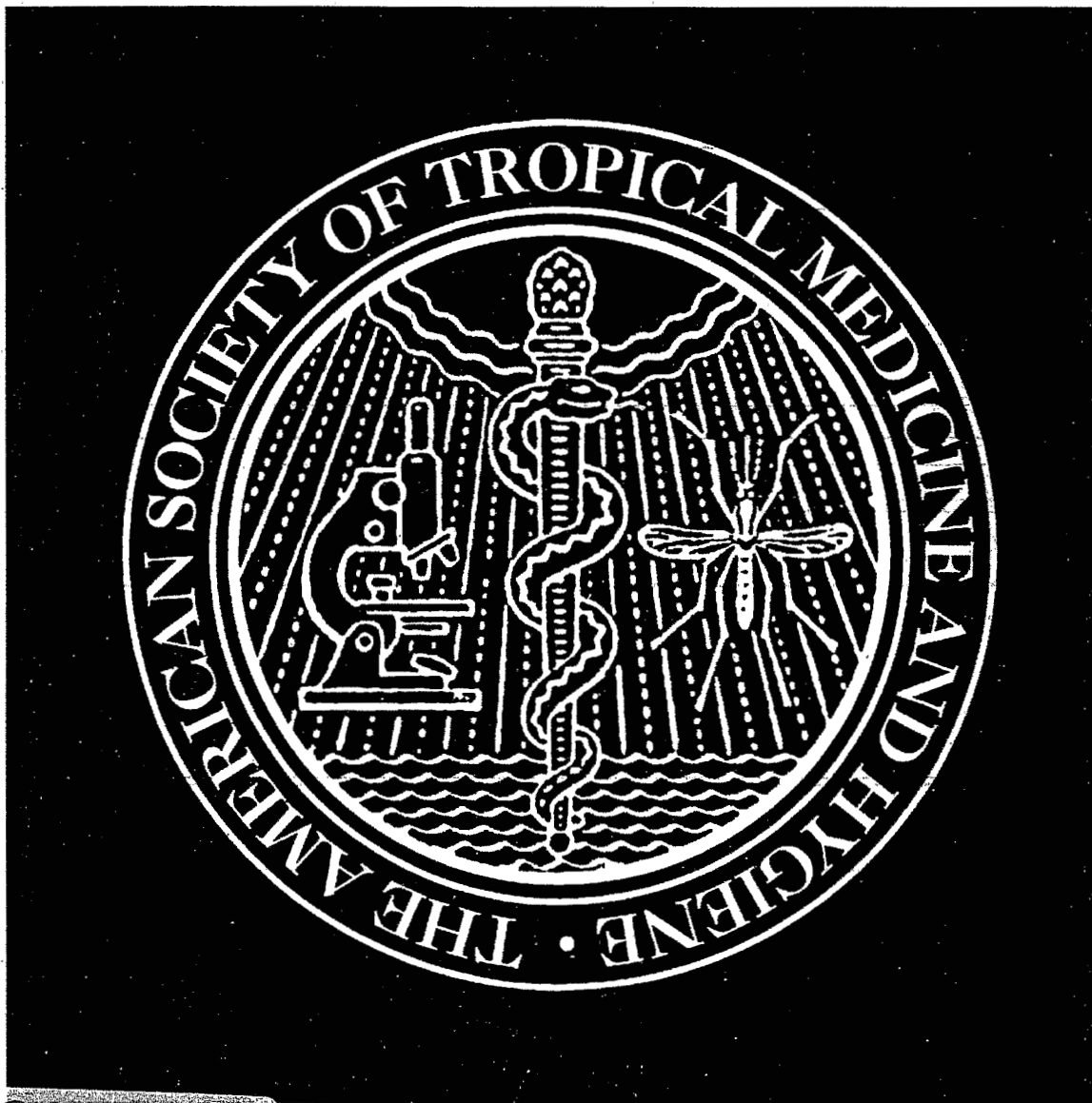
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