

DISTINGUISHING RECRUDESCENCES FROM NEW INFECTIONS IN ANTIMALARIAL CLINICAL TRIALS: MAJOR IMPACT OF INTERPRETATION OF GENOTYPING RESULTS ON ESTIMATES OF DRUG EFFICACY

MADÉLINE SLATER, MOSES KIGGUNDU, CHRIS DOKOMAJILAR, MOSES R. KAMYA, NATHAN BAKYAITA,
AMBROSE TALISUNA, PHILIP J. ROSENTHAL, AND GRANT DORSEY*

Department of Medicine, San Francisco General Hospital, University of California, San Francisco, California; Makerere University
Medical School, Kampala, Uganda; Ugandan Ministry of Health, Kampala, Uganda

Abstract. The use of molecular genotyping to distinguish recrudescence from new infections has become common in antimalarial clinical trials. However, methods used to interpret genotyping results have not been standardized. We analyzed data from 3,000 patients enrolled in clinical trials at seven sites in Uganda. Late treatment failure requiring genotyping occurred in 51% of the patients. Among samples successfully genotyped, 21% were definitive new infections (no recrudescence present on day of failure), 35% were definitive recrudescences (only recrudescence present), and 44% were mixed (new and recrudescence present). The probability of having a mixed genotyping result increased as transmission intensity increased. At the highest transmission site, the estimated risk of treatment failure increased from 34% to 84% for chloroquine plus sulfadoxine-pyrimethamine, from 18% to 45% for amodiaquine plus sulfadoxine-pyrimethamine, and from 12% to 57% for amodiaquine plus artesunate, depending on whether mixed genotyping results were classified as new infections or recrudescences, respectively. The method used to classify treatment outcomes can have a major impact on estimates of drug efficacy, especially in areas of high transmission intensity.

INTRODUCTION

Resistance to antimalarial drugs is now a major problem in Africa.¹ After several decades of relying on chloroquine (CQ), resistance to this drug has reached unacceptable levels, leading to increases in the incidence of disease, progression from uncomplicated to complicated forms, and increased mortality.² A few African countries changed first-line therapy for uncomplicated malaria from CQ to sulfadoxine-pyrimethamine (SP) in the 1990s; however, resistance to SP has spread rapidly, limiting its utility as a single agent.³ More recently, combination antimalarial therapy has been widely advocated for use in Africa, particularly combination therapy including artemisinin compounds.⁴ Indeed, in the last few years several African countries have chosen artemisinin combination therapy as first-line treatment despite limited studies of their efficacy in Africa.⁵

Policy decisions about antimalarial therapy rely largely on the results of clinical drug efficacy studies. As recommended therapy for malaria has markedly changed in Africa over the last few years, so have recommendations about how antimalarial drug efficacy studies should be performed. It is now clear that studies limiting follow-up to ≤ 14 days significantly underestimate the true risk of treatment failure,^{6,7} and follow-up for at least 28 days after therapy is now generally recommended.⁸ However, longer follow-up in areas of Africa highly endemic for malaria requires the use of molecular genotyping to distinguish recrudescence from new infection to accurately estimate the true risk of treatment failure. Although the principle behind molecular genotyping is straightforward, methods used to interpret genotyping results have not been standardized. This is especially important in areas of Africa highly endemic for malaria, where complex infections with multiple parasite strains are common.

In this study, we applied molecular genotyping techniques to adjust the results of a large antimalarial drug efficacy trial

involving 3,000 patients at seven sites in Uganda. Patients were treated with one of three combination regimens and followed for 28 days. Genotyping patterns for paired samples were compared for all patients who failed therapy on follow-up days 4–28. We found that the method of categorization of outcomes for those with mixed genotype patterns had a profound influence on estimates of treatment efficacy, especially in areas of high malaria transmission.

METHODS

Clinical trials and study sites. All clinical data came from randomized clinical trials performed between October 2002 and March 2004 at seven sites in Uganda as part of a national surveillance project. The clinical results of these trials have been reported separately.^{9–11} Similar study protocols were used to compare the treatment efficacies of combinations of CQ plus SP, amodiaquine (AQ) plus SP, and AQ plus artesunate (AS); the last regimen was included only at four sites. Briefly, patients presenting to health centers with a positive screening blood smear fulfilling the following selection criteria were enrolled: 1) age ≥ 6 months (no upper limit for age); 2) history of fever in the last 24 hours or an axillary temperature $\geq 37.5^\circ\text{C}$; 3) no history of serious side effects to study medications; 4) no evidence of a concomitant febrile illness; 5) provision of informed consent; 6) ability to participate in a 28-day follow-up; 7) no history of treatment with an antifolate or AQ during the previous week; 8) absence of pregnancy based on history of last menstrual period; 9) no danger signs (prostration, inability to drink, recent convulsion, persistent vomiting) or evidence of severe malaria¹²; 10) a *Plasmodium falciparum* mono-infection; and 11) a parasite density of 2,000–200,000/ μL . Patients were given directly observed therapy and followed for 28 days with their outcomes classified according to World Health Organization guidelines for areas of intense transmission as adequate clinical and parasitologic response, early treatment failure, late clinical failure, or late parasitologic failure.¹² Measures of transmission intensity at the various sites were obtained from previous studies of parasite prevalence in asymptomatic children.^{13,14} In-

* Address correspondence to Dr. Grant Dorsey, Department of Medicine, University of California, Parnassus Avenue, Box 0811, San Francisco, CA 94143. E-mail: grantd@itsa.ucsf.edu

formed consent was obtained from all patients or their parents/guardians, and the studies were reviewed and approved by the institutional review boards of the University of California, San Francisco, the University of California, Berkeley, and the Ugandan National Council for Science and Technology.

Molecular genotyping and outcome classification. Blood was collected on filter paper (No. 3; Whatman, Brentford, United Kingdom) on the day of enrollment and the day treatment failure occurred. Molecular genotyping was performed using paired samples from all patients experiencing late clinical or parasitologic failure on follow-up days 4–28. Parasite DNA was extracted with Chelex as previously described.¹⁵ The polymorphic block 3 region of the merozoite surface protein-2 gene (*msp-2*) was amplified by a nested polymerase chain reaction (PCR). First-round PCR primers corresponded to conserved sequences flanking this region.¹⁶ Second round PCR primers were then used to amplify the IC3D7 and FC27 allelic families of *msp-2*.¹⁷ For controls, genomic DNA from HB3 and 3D7 laboratory strains was isolated by standard techniques. Nested PCR products were analyzed by electrophoresis using 2% agarose gels. Paired samples from the same patient were run on adjacent lanes. If there was no amplification for any allelic family, the PCR was repeated with 2.5 times the quantity of template DNA. If no amplification was detected after this second reaction, genotyping was classified as unsuccessful. Gel images were digitized and molecular weights were assigned to bands using GelCompar II software (Applied Maths, Austin, TX).

Treatment outcomes were assessed by comparing genotyping patterns on the day of treatment failure with genotyping patterns on the day of enrollment. Each band assigned a molecular weight was considered an individual strain. Strains were considered the same if molecular weights were within 10 basepairs. Definitive recrudescence (true treatment failure) was defined as all early treatment failures (genotyping not performed) and any late treatment failure where all strains present on the day of failure were present on the day of enrollment. Definitive new infections were defined as any late treatment failures where none of the strains present on the day of failure were present on the day of enrollment. Mixed results were defined as late treatment failures where the day of failure sample contained some strains present on the day of

enrollment and some strains not present on the day of enrollment (Figure 1).

Statistical methods. To estimate the relative population diversity of strains at the different sites, the frequency distribution of all strains detected from pretreatment samples were compared. Strains were categorized into 20-basepair ranges (i.e., 201–220 basepairs, 221–240 basepairs. etc.), considering inter-gel variations in size of known laboratory strains (HB3 and 3D7). Relative strain diversity was assessed based on the cumulative probability of two independent pretreatment strains being in the same 20-basepair range. Cumulative risks of definitive new infection, definitive recrudescence, and mixed genotyping results over the 28-day follow-up period were estimated using the Kaplan-Meier product limit formula (survival analysis) with censoring of mutually exclusive events for each outcome group. Independent risk factors for each genotyping outcome category were estimated using a Cox proportional hazards model. To allow for direct comparability between the three genotyping outcome categories (definitive new infection, definitive recrudescence, and mixed result), the same model containing the following predictor variables was used: 1) patient age, 2) baseline temperature, 3) pretreatment parasite density, 4) treatment group, and 5) level of endemicity.

RESULTS

Study site characteristics and genotyping results. A total of 3,131 patients were enrolled at the seven study sites, of which 3,000 (96%) completed 28-day follow-up and were included in this analysis. Late clinical or parasitologic treatment failure occurred in 51% of all patients completing the studies, ranging from 29% to 72% at the seven sites (Table 1). Genotyping was successful in 1,516 (98.4%) of 1,540 samples tested. Genotyping results were classified into three categories: 1) definitive new infections, 2) definitive recrudescence, and 3) mixed results (Figure 1). Genotyping results classified as definitive new infections occurred in 11% of all patients enrolled in the studies, ranging from 8% to 16% at the seven sites. Genotyping results classified as definitive recrudescence occurred in 18% of all patients enrolled in the studies, ranging from 12% to 36% at the seven sites. Genotyping results classified as mixed (both recrudescence and new strains present)

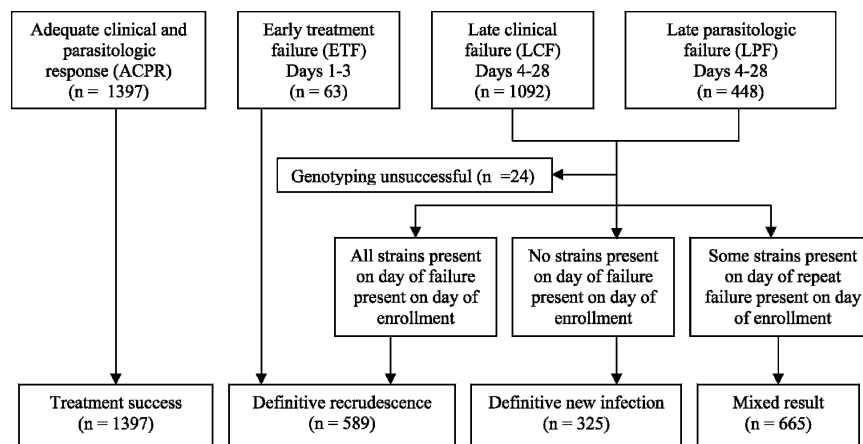


FIGURE 1. Schematic of outcomes classification systems used in antimalarial drug efficacy trials. Upper boxes: World Health Organization clinical classification system unadjusted by genotyping. Lower boxes: treatment outcomes adjusted by genotyping.

TABLE 1
Study site characteristics and genotyping results in Uganda

	Study site						
	Kampala	Jinja	Kanungu	Kyenjojo	Mubende	Apac	Tororo
Levels of transmission intensity*	mesoendemic	mesoendemic	hyperendemic	hyperendemic	hyperendemic	holoendemic	holoendemic
Treatment arms†	CQ + SP AQ + SP AQ + AS	CQ + SP AQ + SP AQ + AS	CQ + SP AQ + SP	CQ + SP AQ + SP	CQ + SP AQ + SP	CQ + SP AQ + SP AQ + AS	CQ + SP AQ + SP AQ + AS
Median age in years (range)	4.3 (0.5–10)	3.8 (0.5–65)	1.9 (0.5–65)	4.4 (0.5–52)	1.8 (0.5–65)	1.8 (0.5–47)	1.3 (0.5–56)
Number with treatment outcomes	384	514	357	349	351	530	515
Number genotyped‡ (%)	111 (29)	177 (34)	230 (64)	181 (52)	200 (57)	271 (51)	370 (72)
Genotyping results§							
Definitive new infection (%)	33 (9)	39 (8)	31 (9)	37 (11)	38 (11)	67 (13)	80 (16)
Definitive recrudescence (%)	56 (15)	78 (15)	129 (36)	61 (17)	64 (18)	61 (12)	77 (15)
Mixed result (%)	19 (5)	55 (11)	69 (19)	78 (22)	95 (27)	139 (26)	210 (41)
Unsuccessful	3 (1)	5 (1)	1 (0.3)	5 (1)	3 (1)	4 (1)	3 (1)
Complexity of infection¶ (SD)	2.5 (1.4)	3.2 (1.7)	2.8 (1.5)	3.4 (1.7)	3.3 (1.6)	4.9 (1.7)	4.6 (2.1)
Strain diversity#	5.4%	4.9%	4.8%	4.8%	4.2%	5.0%	5.0%

* Mesoendemic = parasite prevalence < 30%; hyperendemic = parasite prevalence 30–70%; holoendemic = parasite prevalence > 70%.

† CQ = chloroquine; SP = sulfadoxine-pyrimethamine; AQ = amodiaquine; AS = artesunate.

‡ All late clinical and parasitologic failures genotyped.

§ Definitive new infection = none of the strains present on the day of failure present on the day of enrollment; Definitive recrudescence = all strains present on the day of failure present on the day of enrollment; Mixed result = day of failure sample contains some strains present on the day of enrollment and some strains not present on the day of diagnosis.

¶ Mean number of unique strains present on day of enrollment.

Cumulative probability that two independent pre-treatment strains were the same.

occurred in 22% of all patients enrolled in the study, ranging from 5% to 41% at the seven sites, with the probability of a having a mixed genotyping result increased as measures of transmission intensity increased (Table 1).

The mean complexity of infection (average number of strains) in pretreatment samples ranged from 2.5 to 4.6, with the general trend of increasing complexity as measures of transmission intensity increased (Table 1). The cumulative probability of two individual strains from our population of pretreatment samples being the same was low (< 5%) and consistent across the seven sites (range = 4.2–5.4%) (Table 1).

Characteristics of different genotyping outcome categories. The cumulative risks for each treatment group over the 28-day follow-up period and risk factors for each of the genotyping outcome categories are presented in Figure 2 and Table 2, respectively. Definitive new infections were first detected 12 days after treatment was initiated and primarily occurred between follow-up days 21 and 28 (Figure 2). The risk of definitive new infection decreased in older patients and was increased in patients with higher pre-treatment parasite densities. The risk of definitive new infection was higher in the AQ plus AS group compared with the CQ plus SP and AQ plus SP treatment groups, which is likely due to the post-treatment prophylactic effect of SP, which has a very long half-life. As expected, definitive new infections were more likely to occur in holoendemic and hyperendemic sites compared with mesoendemic sites (Table 2). Definitive recrudescences occurred throughout the follow-up period, although they primarily occurred between follow-up days 21 and 28 in the AQ plus SP and AQ plus AS groups (Figure 2). Younger age (a surrogate marker of acquired immunity), higher baseline temperature, and higher pretreatment parasite densities were all independent risk factors for definitive recrudescence. The risk of definitive recrudescence was significantly different between the three treatment groups, being highest in the CQ plus SP treatment group and lowest in the AQ plus AS treat-

ment group, which is consistent with previous findings from Uganda.¹⁸ Definitive recrudescences were more likely to occur in hyperendemic sites compared with mesoendemic sites (Table 2). Samples with mixed genotyping results had characteristics of both new infections and recrudescences. Mixed results occurred almost exclusive during follow-up days 14–28 and were much more common in areas of higher transmission intensity, which is consistent with new infections. The risk of mixed results was highest in the CQ plus SP group and lowest in the AQ plus SP group, which is consistent with both new infections and recrudescences.

Impact of method of classifying genotyping results on estimates of drug efficacy. Estimates of drug efficacy were compared using the two most common systems for classifying genotyping outcomes. First, late treatment failures were defined as recrudescences only if all parasite strains present on the day of failure were present when therapy was initiated (mixed results all classified as new infections). Second, late treatment failures were defined as recrudescences if any parasite strain present on the day of failure was present when therapy was initiated (mixed results all classified as recrudescences). For the CQ plus SP treatment group, the risk of treatment failure adjusted by genotyping at the seven sites ranged from 22% to 64% (average = 39%) if mixed genotyping results were classified as new infections and from 50% to 84% (average = 68%) if mixed genotyping results were classified as recrudescences (Table 3). For the AQ plus SP treatment group, the risk of treatment failure ranged from 10% to 33% (average = 16%) if mixed genotyping results were classified as new infections and from 11% to 46% (average = 30%) if mixed genotyping results were classified as recrudescence (Table 3). For the AQ plus AS treatment group, the risk of treatment failure ranged from 3% to 12% (average = 7%) if mixed genotyping results were classified as new infections and from 6% to 57% (average = 25%) if mixed genotyping results were classified as recrudescence (Table 3). Differences in estimates of treatment failure using

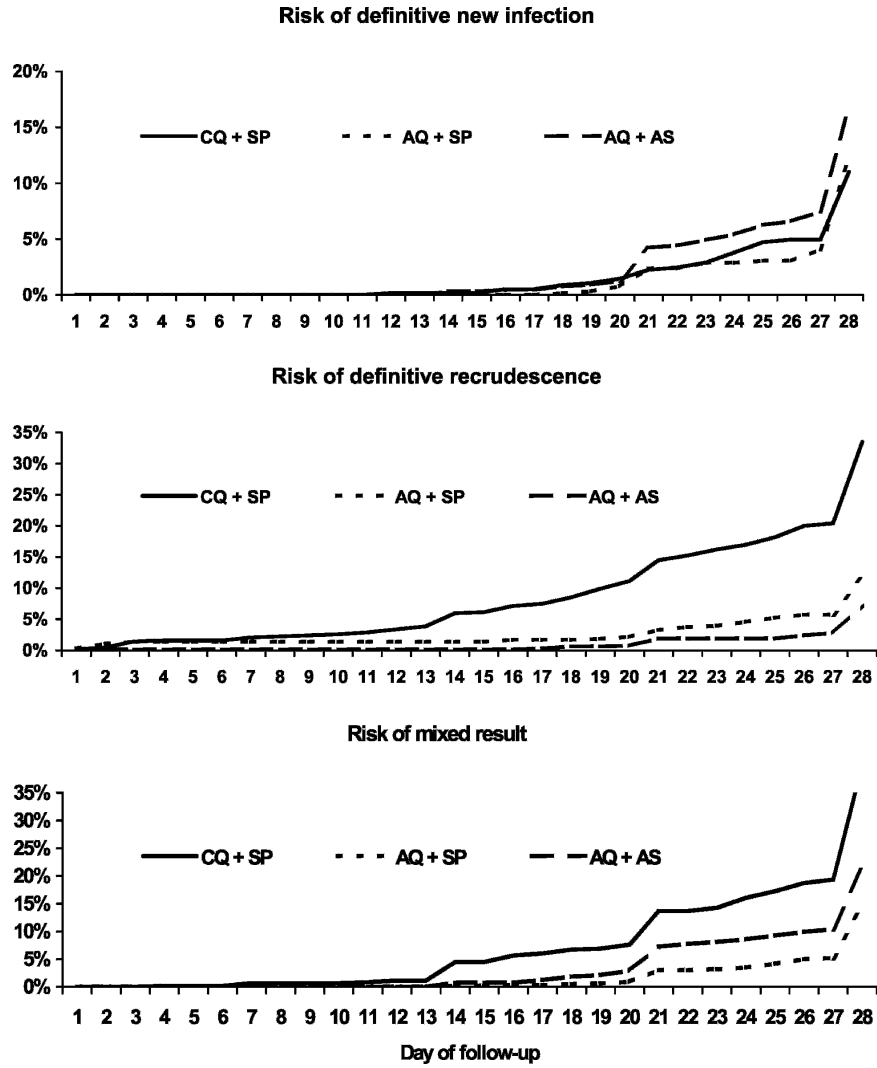


FIGURE 2. Cumulative risk of different outcomes based on genotyping results stratified by treatment group. Log-rank test for equality of survivor functions: $P = 0.01$ (definitive new infections), $P < 0.001$ (definitive recrudescence and mixed results). CQ = chloroquine; SP = sulfadoxine-pyrimethanine; AQ = amodiaquine; AS = artesunate. Only data from the four sites with three-arm studies were included to allow for direct comparability across treatment groups.

the two classification systems increased as transmission intensity increased for all three treatment groups. At the lowest transmission intensity site (Kampala), the risk of treatment failure increased by 10%, 1%, and 3% for CQ plus SP, AQ

plus SP, and AQ plus AS, respectively. In contrast, at the highest transmission intensity site (Tororo), the risk of treatment failure increased by 50%, 27%, and 42% for CQ plus SP, AQ plus SP, and AQ plus AS, respectively (Table 3).

TABLE 2
Risk factors for different outcomes based on genotyping results*

Risk factor	Definitive new infection		Definitive recrudescence		Mixed result	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Age (per five-year increase)	0.83 (0.74–0.93)	0.002	0.71 (0.65–0.79)	< 0.001	0.64 (0.57–0.73)	< 0.001
Baseline temperature (per 1°C increase)	1.02 (0.92–1.13)	0.71	1.11 (1.03–1.19)	0.008	0.99 (0.92–1.06)	0.76
Pre-treatment parasite density (per 1n increase)	1.14 (1.04–1.26)	0.004	1.18 (1.10–1.26)	< 0.001	1.06 (0.99–1.13)	0.10
AQ + SP vs. CQ + SP	0.94 (0.72–1.23)	0.65	0.32 (0.27–0.38)	< 0.001	0.33 (0.28–0.40)	< 0.001
AQ + AS vs. CQ + SP	1.47 (1.08–2.00)	0.01	0.18 (0.13–0.25)	< 0.001	0.53 (0.43–0.65)	< 0.001
AQ + AS vs. AQ + SP	1.57 (1.17–2.09)	0.002	0.57 (0.40–0.81)	0.002	1.59 (1.25–2.02)	< 0.001
Hyperendemic vs. mesoendemic	2.19 (1.58–3.04)	< 0.001	1.73 (1.41–2.13)	< 0.001	3.59 (2.73–4.71)	< 0.001
Holoendemic vs. mesoendemic	1.93 (1.42–2.62)	< 0.001	0.87 (0.68–1.11)	0.26	3.42 (2.62–4.46)	< 0.001

* HR = hazard ratio; CI = confidence interval. For definitions of abbreviations and other terms, see Table 1.

TABLE 3
Impact of method of classifying mixed genotyping results on estimates of drug efficacy*

Treatment group	Study site	Risk of treatment failure if mixed result = new infection	Risk of treatment failure if mixed result = recrudescence	Risk difference (95% CI)	P
CQ + SP	Kampala	40%	50%	10% (-3-22%)	0.15
	Jinja	40%	59%	19% (7-30%)	0.001
	Kanungu	64%	81%	17% (6-26%)	0.002
	Kyenjojo	39%	63%	24% (13-36%)	< 0.001
	Mubende	37%	67%	30% (19-40%)	< 0.001
	Apac	22%	57%	35% (25-45%)	< 0.001
	Tororo	34%	84%	50% (40-61%)	< 0.001
AQ + SP	Kampala	10%	11%	1% (-7-9%)	0.69
	Jinja	13%	19%	6% (-1-15%)	0.09
	Kanungu	33%	46%	13% (3-24%)	0.01
	Kyenjojo	15%	29%	14% (5-22%)	0.002
	Mubende	13%	34%	21% (12-30%)	< 0.001
	Apac	7%	24%	17% (9-24%)	< 0.001
	Tororo	18%	45%	27% (17-37%)	< 0.001
AQ + AS	Kampala	3%	6%	3% (-3-8%)	0.36
	Jinja	4%	9%	5% (0-10%)	0.06
	Apac	10%	35%	25% (17-34%)	< 0.001
	Tororo	12%	57%	42% (35-55%)	< 0.001

* For definitions of abbreviations, see Tables 1 and 2.

DISCUSSION

The use of molecular genotyping to distinguish recrudescence from new infections has become increasingly common in antimalarial drug efficacy studies, but methods of categorizing outcomes based on genotyping results are not standardized. In this study, we evaluated molecular genotyping results from samples collected as part of a large national drug efficacy study comparing three different combination therapies at seven Ugandan sites of varying transmission intensity. We found that as the level of transmission intensity increased, an increasing proportion of treatment outcomes were late treatment failures containing both parasite strains present in pretreatment samples and new strains. Whether these mixed results were classified as recrudescences (true treatment failures) or new infections had a profound impact on estimates of drug resistance, especially in areas with the highest transmission intensity.

The use of molecular genotyping offers a potentially powerful tool to improve the accuracy of estimates of treatment failure due to antimalarial drug resistance. A number of polymorphic genes have been used for genotyping *P. falciparum* isolates, but most recent applications of genotyping in clinical trials have relied on genes encoding *msp-1* and *msp-2* and the glutamine-rich protein (*glurp*).¹⁹⁻²² Some researchers use multiple gene targets to minimize the probability of two independent infections having the same genotyping pattern. This technique has been especially useful in low transmission areas where infections containing multiple strains are uncommon and strain diversity may be low.²³ However, in high transmission areas where multiple infections are common, it is frequently impossible to assign multilocus genotypes to individual parasites.²³ In fact, the use of multiple versus a single genetic marker of strain diversity can only increase the proportion of outcomes that are classified as having mixed genotyping results when taking into account the results of multiple genetic loci simultaneously. Other investigators have suggested that in areas of high transmission, evaluation of recrudescence is best achieved by detailed analysis of a single,

highly polymorphic locus.²⁴ In this study, we chose to use the *msp-2* gene alone due to its high degree of diversity in our populations. Other studies from Africa have consistently shown *msp-2* to have a higher degree of diversity compared with *msp-1* or *glurp*.²⁵⁻²⁸ However, our estimates of strain diversity may have underestimated the true probability of two infections having the same genotype due to clustering of parasite strains in time and space, as well as the frequency of infections containing multiple strains. Another inherent limitation of genotyping is the assumption that parasite strains detected in pretreatment samples reflect the complete population of infecting parasites. It is possible that strains circulating at low parasite density are not detected in pretreatment samples but could emerge to cause treatment failure, leading to the misclassification of a recrudescence as a new infection.

The interpretation of genotyping results is quite simple in two circumstances, when isolates collected on the day of treatment failure are either identical or completely different from those seen at the time of initiation of treatment. More complexity in interpretation comes with mixed results, when both persistent and new isolates are seen. Indeed, prior studies have not consistently categorized outcomes with such mixed results, and this inconsistency makes it difficult to compare results from different studies.¹⁹⁻²² One potential limitation of genotyping is the possibility that post-treatment samples truly do contain both recrudescence parasites and newly infecting strains (mixed results). This could occur when recurrent disease is due to recrudescence parasites at the same time newly acquired parasites are emerging. Alternatively, recurrent disease could be due to newly acquired parasites at the same time asexual parasites or gametocytes from a prior infection are slow to be cleared. We took a practical approach to this problem by examining the impact of classifying mixed results at the two ends of the spectrum. This is a useful exercise because several recent antimalarial clinical trials using genotyping to adjust results have either 1) classified outcomes as recrudescence only if all parasite strains present on the day of repeat therapy were present when therapy was initiated (mixed results = new infection)^{20,21} or 2) classified outcomes

as recrudescence if any parasite strain present on the day of repeat therapy was present when therapy was initiated (mixed results = recrudescence).^{19,22} We clearly show that the method used to classify mixed results has a profound impact on both estimates of treatment failure due to drug resistance and conclusions about comparative efficacy.

In many antimalarial drug efficacy studies, it is assumed all treatment failures that occur during the first 14 days of follow-up are due to recrudescences, and only treatment failures occurring 14 or more days following therapy are genotyped to distinguish recrudescence from new infections. However, in areas highly endemic for malaria this assumption may not be valid because successive waves of parasites may be emerging from the liver on any day following therapy. In this study, we chose to genotype parasites for all failures occurring after day 3. We found that among 140 treatment failures that occurred prior to day 14, 3 (2%) were due to definitive new infections (with the earliest occurring on day 12), 23 (16%) had mixed genotypes, and 114 (81%) were due to definitive recrudescences, which supports the assumption that new infections are very unlikely to occur prior to day 14.

An important question that remains is how best to classify mixed genotyping results. In a previous study from Kampala, we used a statistical modeling approach to show that mixed genotyping results were more likely to represent new infections than recrudescences.²⁷ A similar conclusion was made in a study from Gabon where time to reappearance of parasites after therapy was similar when post-treatment samples contained only new strains and when post-treatment samples contained both new and recrudescence strains.²⁹ A practical conclusion based on these studies might be that mixed genotyping results are best classified as new infections as opposed to recrudescence. However, using such a simplistic approach will likely lead to a significant degree of misclassification, especially in high transmission areas. We suggest three approaches to the interpretation of genotyping results from antimalarial drug efficacy studies in areas highly endemic for malaria. First, for comparative trials, emphasis should be placed on outcomes that are unadjusted for genotyping. Such analyses are obviously limited in their ability to estimate the true risk of failure due to drug resistance, but they provide an unbiased comparison of the need for repeat therapy due to either recrudescence or new infection. Second, for the reporting of results adjusted by genotyping, it remains reasonable to classify all mixed results as recrudescences or as new infections. However, it is critical that the method used to classify genotyping results is clearly described, and caution should be taken in comparing results from studies using different methodologies and from regions with different malaria endemicities. This simple approach may be particularly useful for monitoring trends in drug resistance at individual sites. Third, when possible, more complex methods may be used to classify mixed genotyping results as either a recrudescence or new infection on an individual basis. We are currently developing a statistical model for estimating the probability of recrudescence based on characteristics of the host, drug, and parasite for each patient with mixed genotyping results. Such an approach could be useful for improving the accuracy of estimates of drug efficacy and to study associations between exposure variables (e.g., molecular polymorphisms) and drug resistance.

In summary, the use of molecular genotyping to adjust re-

sults in antimalarial clinical trials is a powerful tool that has become widely adopted. However, there is a need for standardization of the methods used to generate and interpret results. The development of a standardized approach to genotyping could allow for better comparison of results from different studies and thereby improve drug policy decision-making and our understanding of the epidemiology of antimalarial drug resistance.

Received November 19, 2004. Accepted for publication March 29, 2005.

Acknowledgments: This work would not have been possible without the establishment of the sentinel site surveillance system and continued support by the East African Network for Monitoring Antimalarial Treatment. We thank the clinical study team of Joy Bossa, Nelson Budaka, Oswald Byaruhanga, Moses Musinguzi, Isaac Kigozi, Felix Jurua, Richard Allen, Fred Kizito, and Grace Musimenta for their participation in the study. We also thank Sam Nsoby, Regina Nakafero, and Dan Kyabayinze for training the laboratory staff and providing laboratory quality control; Adoke Yeka, Kristin Banek, John Patrick Mpindi, and Sarah Staedke for their work on the clinical studies; Sara Kibirango and Kenneth Mwebaze for administrative support; Nuhu Kibampawo, Joshua Sekitoleko and Marx Dongo for logistical support; and all of the district health care workers and study participants for their cooperation.

Financial support: This study was supported by the Centers for Disease Control and Prevention/Association of Schools of Public Health cooperative agreement "Malaria Surveillance and Control in Uganda" (SA3569 and S1932-21/21), the Fogarty International Center/National Institutes of Health (TW00007, TW01506, and AI43301), and the Department for International Development.

Authors' addresses: Madeline Slater, Chris Dokomajilar, Philip J. Rosenthal, and Grant Dorsey, Department of Medicine, University of California, Parnassus Avenue, Box 0811, San Francisco, CA 94143, Telephone: 415-648-4680, Fax: 415-648-8425, E-mails: mslater@itsa.ucsf.edu, cdoko@itsa.ucsf.edu, rosntnl@itsa.ucsf.edu, and grantd@itsa.ucsf.edu. Moses Kiggundu and Moses R. Kanya, Makerere University Medical School, PO Box 7072, Kampala, Uganda, Telephone: 256-41-541-188, Fax: 256-41-540-524, E-mails: mosesk_33@hotmail.com and mkanya@infocom.co.ug. Nathan Bakyaite and Ambrose Talisuna, Ministry of Health/UMSP, PO Box 7475, Kampala, Uganda, Telephone: 256-41-231-563 or 2315-69, Fax: 256-41-540-524, E-mails: natsbach@hotmail.com and acesd@afsat.com.

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