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Risk associated with asymptomatic parasitaemia occurring post-antimalarial treatment

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Summary

OBJECTIVE Parasites may recur asymptomatically after initial clearance by antimalarial treatment. Current guidelines recommend treatment only when patients develop symptoms or at the end of follow-up. We wanted to assess prospectively the probability of becoming symptomatic and the risks of this practice.

METHODS We analysed data collected in 13 trials of uncomplicated paediatric malaria conducted in eight sub-Saharan African countries. These studies followed all cases of post-treatment asymptomatic parasitaemia until they developed symptoms or to the end of the 28-day follow-up period, at which time parasite genotypes were compared to pre-treatment isolates to distinguish between recrudescences and new infections.

RESULTS There were 425 asymptomatic recurrences after 2576 treatments with either chloroquine, sulfadoxine/pyrimethamine or amodiaquine, of which 225 occurred by day 14 and 200 between day 15 and day 28. By day 28, 42% developed fever (median time to fever = 5 days) and 30% remained parasitaemic but afebrile, while 23% cleared their parasites (outcome unknown in 4%). Young age, parasitaemia ≥500 parasites/μl; onset of parasitaemia after day 14, and treatment with amodiaquine were the main variables associated with higher risk of developing fever.

CONCLUSION In areas of moderate to intense transmission, asymptomatic recurrences of malaria after treatment carry a substantial risk of becoming ill within a few days and should be treated as discovered. Young children are at higher risk. The higher risk carried by cases occurring in the second half of follow-up may be explained by falling residual drug levels.

keywords malaria, malaria treatment, asymptomatic parasitaemia

Introduction

Asymptomatic parasitaemia may occur after initial clearance of parasites after antimalarial treatment. The clinical relevance of such episodes is unclear and until recently they were considered as treatment failures requiring rescue treatment only when accompanied by fever (WHO 1996). While the latest WHO protocol (WHO 2003) now considers these episodes as (late) parasitological failures (LPF), it recommends treatment only when they become symptomatic, or, if they remain asymptomatic, at the end of follow-up.

In order to assess if this practice is justified, and if not offering rescue treatment immediately unduly exposes study participants to a potential risk of disease progression, we assessed the occurrence and outcome of post-treatment recurrent asymptomatic parasitaemia in a large collection of studies (13 trials enrolling 2576 children with uncomplicated falciparum malaria in eight African countries during 2001–2004, comprising a total of 24 treatment arms with chloroquine (CQ), sulfadoxine/pyrimethamine (SP), or amodiaquine (AQ); reported in Guthmann *et al.* 2006).

Methods

Asymptomatic recurrent parasitaemia is defined based on blood smear results as a case of initial clearance of parasites by treatment with subsequent (i.e. any time before day 28) recurrence of parasites in the absence of fever. The study population were children aged 5–59 months with acute uncomplicated falciparum malaria

treated with either CQ, SP or AQ in 13 non-comparative trials done in eight African countries during 2001–2004 (the methods and results of these studies are reported in Guthmann *et al.* 2006). Asymptomatic recurrent parasitaemias occurring post-treatment were recorded but not treated and followed until the end of follow-up (day 28) and treated as they developed symptoms or on day 28 if still parasitaemic according to the 2003 WHO protocol (WHO 2003).

We observed the evolution of these cases for as long as they were on study, and classified children based on whether they (i) developed fever [late treatment failures (LTF)], (ii) remained parasitaemic and asymptomatic up to the end of follow-up on day 28 [late parasitological failures (LPF)], or (iii) cleared parasites 'spontaneously' [adequate clinical and parasitological response (ACPR)].

In all cases occurring on days 15–28 (hereinafter 'post-d14'), blood spots on filter-paper were taken either when they had become febrile or at the end of follow-up. Parasite DNA extracted was compared to the pre-treatment sample by polymerase chain reaction (PCR) with regards to the merozoite surface protein msp1 and msp2, and recurrences were classified as new or recrudescent infections. Recurrences occurring before or on day 14 (hereinafter, 'by d14') were assumed to be recrudescences and thus not genotyped.

We performed univariate Kaplan–Meier survival analyses of the risk of developing fever stratified by treatment, sex, age, day and level of parasitaemia at recurrence and type of infection (new or recurrent). All comparisons were done using the log-rank test. Three groups were considered: (i) all 425 patients with asymptomatic recurrent parasites; (ii) the 180 patients who became LTF; (iii) the 153 who became LTF and could be classed as either new or recurrent infections by PCR.

Multivariate analyses were conducted by descending manual modelling using a likelihood ratio test to verify the significance of loosing information in the model as a consequence of the deletion of a variable. A Cox proportional hazard ratio model was used to study risk ('hazard') of fever. The proportional hazard assumptions of the Cox model were verified for groups (ii) and (iii) above but not (i). For this group (all 425 patients) we used a logistic model in which, however, the time under observation could not be used because it was redundant with the risk of fever (significant Hosmer and Lemeshow goodness-of-fit test.) Data were analysed using STATA 8.2 (Stata Corp., College Station, TX, USA) and SAS System 9.1.3 (SAS Institute, Cary, NC, USA).

Results

The summary results are in Table 1. Of the 2576 patients included in these studies, 593 received CQ, 989 SP and 994 AQ (Guthmann *et al.* 2006). The median 28-day risk of failure were 81% for CQ, 25% for SP and 17% for AQ. In this population, we detected 425 episodes of asymptomatic parasitaemia following initial clearance after treatment with CQ (n = 141, 24% of the patients enrolled), SP (n = 170, 17%) or AQ (n = 114, 11%). The occurrence of asymptomatic parasitaemia paralleled success rates and was lowest after AQ and highest for CQ. Of these 425 cases, 180 (42%) developed symptoms within the 28-day follow-up; 52 cases (37% of asymptomatic parasitaemias) with CQ, 65 (38%) with SP and 63 (55%) with AQ.

Overall, 225 episodes (53%) occurred by d14 and were followed for a median of 14 days [interquartile range (IQR): 7–14], and 200 (47%) occurred post-d14 and were followed for a median of 7 days (IQR: 4–7). Parasite counts were significantly higher (Mann–Whitney, P = 0.01) in the post-d14 episodes [geometric mean, 95% confidence intervals: = 781 (95% CI 568–1073) post-d14 *vs.* 276 (209–363) by d14] (Table 2).

By day 28, 17 patients (4.0%) were lost to follow-up or excluded, 99 (23.3%) had cleared their parasitaemia (ACPR), 129 (30.3%) remained positive but afebrile (LPF) and 180 (42.4%) developed fever within the 28-day follow-up (LTF). Of the latter, nine developed fever by day 14 and were considered a recrudescence without PCR genotyping, and 171 developed fever post-d14 and were genotyped at the time of reappearance of symptoms or on day 28 if LPF. PCR was resolved for 144 cases (76

Table 1 Treatment outcome and occurrence of asymptomatic parasitaemia and symptoms by drug

Drugs	Enrolled	D28 risk of failure, median (range)	Asymptomatic recurrence, *n* (%)	Became febrile by day 28, *n* (%)	Time to fever (days), median (IQR)
CQ	593	81 (24–87)	141 (24)	52 (37)	5 (2–7)
SP	989	25 (7–41)	170 (17)	65 (38)	7 (3–8)
AQ	994	17 (5–28)	114 (11)	63 (55)	3 (2–5)
Total	2576		425 (16)	180 (42)	5 (2–7)

Table 2 Asymptomatic parasitaemias by the time of recurrence (crude outcomes prior to PCR genotyping)

				Outcome by day 28, *n* (%)			
Recurrent parasitaemia occurring	*n* (%)	Parasite counts geometric mean (95% CI)	Follow-up (days), median (IQR)	Cleared parasites (ACPR)	Parasites no fever (LPF)	Parasites and fever (LTF)	Not evaluable
By day 14	225 (53)	276 (209–363)	14 (7–14)	62 (28)	52 (23)	96 (46)	15 (7)
Post day 14	200 (47)	781 (568–1073)	7 (4–7)	37 (18)	77 (33)	84 (42)	2 (1)
All	425 (100)	450 (363–557)	7 (5–14)	99 (23.3)	129 (30.3)	180 (42.4)	17 (4)

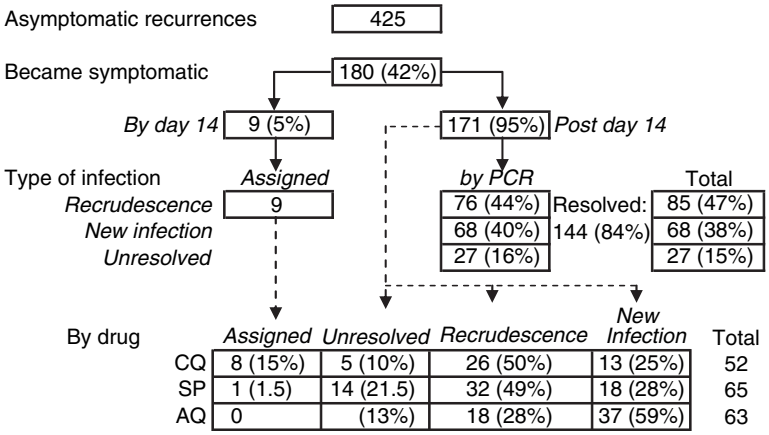

Figure 1 Genotyping of isolates at the time of fever.

recrudescent and 68 new infections) and unresolved for 27 (eight missing sample, three with no DNA, 16 not interpretable). Therefore, in total we had 153 cases which were ascribed to either a recrudescence (n = 85) or a new infection (n = 68) (Figure 1).

Overall, 50% of fevers occurred within 5 days (Table 1), 84% within 7 days and 90% within 10 days of detection of parasites (Figure 2a). Time to fever by drug, time of appearance and level of parasitaemia at detection are presented in Figure 2b–d. The risk of fever varied widely across study sites (from 7% in Chad to 74% in Kuito, Angola), and even within the same country (10–68% in Sierra Leone) (data not shown).

The results of the univariate Kaplan–Meier analysis are in Table 3. For all the 425 patients with recurrent parasites the risk of developing fever and thus becoming a LTF was significantly associated with age, treatment, level of parasitaemia at detection and type of infection (all $P < 0.001$) and just reaching significance levels for the day of discovery of the asymptomatic parasitaemia ($P = 0.05$). Considering the 180 patients who became LTF, risk of fever was significantly associated with sex (shorter in male), treatment, days of recurrent asymptomatic parasitaemia (shorter if post-day 15), level of parasitaemia (shorter if >500 parasites/μl) and type of infection (shorter if a new infection). There was no association with age. This remains true when restricting the model to the 153 patients who could be assigned to a new or reinfection.

In the logistic model used for all 425 patients the independent variables significantly associated with risk of LTF were age, treatment and level of parasitaemia at detection (Table 4). In this model without time under observation the there was no evidence of lack of fit (Hosmer and Lemeshow goodness of fit $P = 0.31$). Here the risk of fever was significantly higher (i) with AQ than both SP and CQ ($P = 0.007$, not shown); (ii) for parasitaemias ≥500/μl; and (iii) for ages >24 months than 6–12 month olds. We found no interaction between any variables.

In the Cox model (Table 5) which could be applied to the 180 patients who developed fever and the 153 who were also assigned to a new or recrudescent infection, treatment, day and level of parasitaemia remained associated with risk of fever. The difference in parasitaemia between new infections and recrudescences was statistically non-significant when they were asymptomatic when first detected [geometric mean (95% CI) 799.4 (460.5–1387.8) *vs.* 648.9 (395.7–1064.1), $P = 0.56$] and when

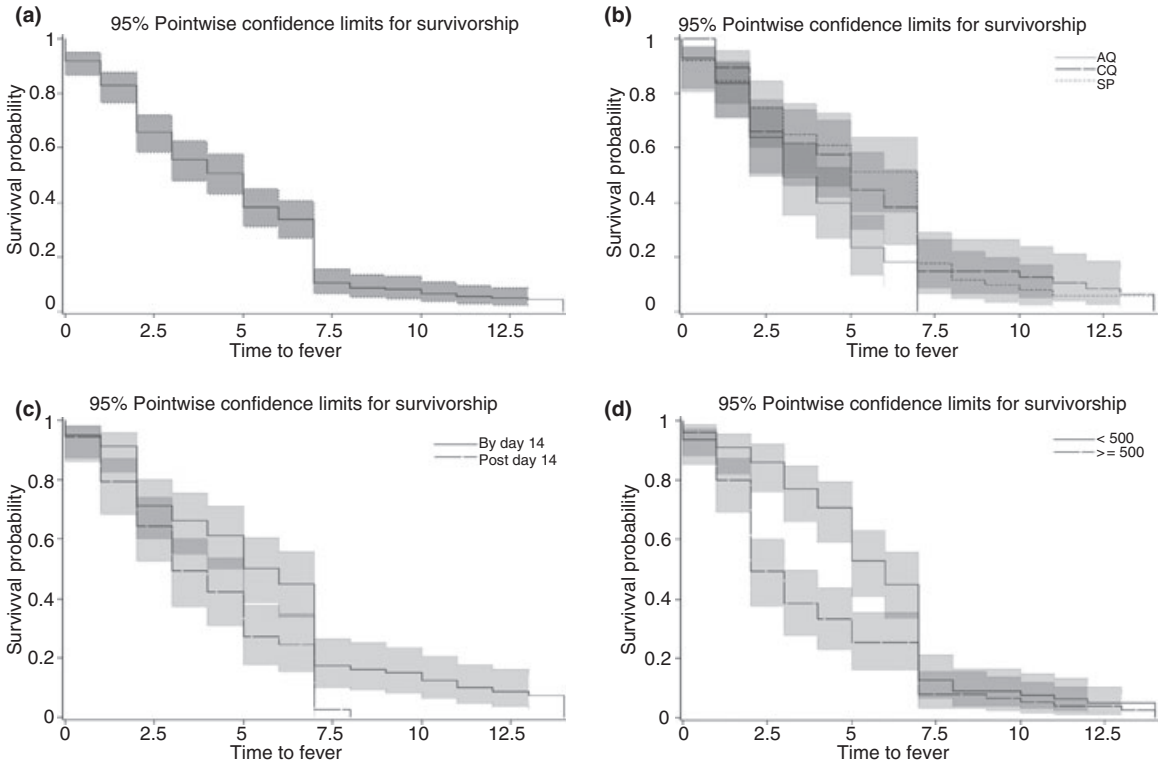

Figure 2 Time to fever (a) overall, (b) by drug, (c) by time of appearance of asymptomatic parasitaemia and (d) by level of parasitaemia on occurrence.

they became symptomatic [geometric mean (95% CI) 27161.1 (18153.4–40638.4) *vs.* 21785.1 (15077.6–31476.4), $P = 0.34$] (Table 6).

Discussion

Case management and treatment guidelines

Our analysis is based on a large database of non-comparative studies of paediatric malaria treated with monotherapies of variable efficacies, conducted in areas of moderate to intense malaria transmission. These data allow three main sets of conclusions. In these settings, reappearance of parasites after initial clearance carries a considerable risk for the patient of becoming ill within a few days (in 50% of cases within 5 days.) Thus, we conclude that the risk of disease progression justifies treating parasitaemia occurring during follow-up as detected.

WHO (2005) guidelines now recognize the risk of persistent parasitaemia post-treatment (patients who never clear parasites but become asymptomatic, as opposed to these cases in which parasites were cleared initially but reappear later during follow-up not accompanied by symptoms) and state that they should be considered as failures and 'be treated with drugs or combinations that are more effective than those administered on day 0, in order to avoid delayed manifestation of clinical signs and symptoms' The data presented here call for also asymptomatic parasitological recurrences to be treated as detected.

Weekly follow-up visits as per the WHO protocol appear to be an effective means of detecting asymptomatic parasitaemias, but ensuring optimal attendance to follow-up visits and minimizing losses to follow-up are crucial to capture these cases early. After discounting the 14 exclusions, in these studies the risk for a patient of having neither parasites nor symptoms at the end of the 4-week follow-up (ACPR) was 0.24; of having persisting parasites but no symptoms (LPF), 0.31; and of having parasites and developing symptoms (LTF), 0.44. Comparisons with other datasets are difficult, as the outcome of asymptomatic parasitaemia is not systematically investigated. In a trial with SP in Tanzania (Mutabingwa *et al.* 2001), of the 140 evaluable patients who cleared symptoms but not parasites, these risks were: ACPR = 0.19, LPF = 0.15 and LTF = 0.66. For comparison in survey studies, the risk of

Table 3 Risk of fever in based on Kaplan–Meier estimates for all the 425 patients with recurrent parasitaemia, the 180 cases who became febrile (LTF) and the 153 of these who could be assigned to a new or recrudescent infection

	425 patients with recurrent asymptomatic parasitaemia								180 patients who became LTF							153 patients who became LTF and were assigned to new or recrudescent infection						
Variable	*n*	Failed	Median	Lower 95% CI	Upper 95% CI	Mean	SD	*P*-value	*n*	Median	Lower 95% CI	Upper 95% CI	Mean	SD	*P*-value	*n*	Median	Lower 95% CI	Upper 95% CI	Mean	SD	*P*-value
Gender																						
Female	212	92	14	11	.	10	0.4	0.8814	92	5	4	7	5	0.4	0.03	76	5	4	7	5	0.4	0.035
Male	213	88	.	12	.	10	0.4		88	3	2	5	4	0.4		77	4	3	5	4	0.4	
Age																						
>=6 to 12<= months	78	45	7	6	14	8	0.6	<0.0001	45	5	3	6	5	0.5	0.87	37	5	3	6	5	0.6	0.98
>12 to 24<= months	133	67	13	7	.	9	0.5		67	3	2	5	4	0.5		56	4	3	5	5	0.5	
>24 to 36<= months	114	44	.	10	.	8	0.3		44	5	3	7	5	0.4		38	5	3	7	5	0.4	
>36 to 42<= months	26	8	.	9	.	8	0.6		8	3.5	1	7	4	1.1		8	3.5	1	7	4	1.1	
>42 to 59<= months	74	16				12	0.5		16	5	3	7	6	1.0		13	5	2	7	5	1.0	
Treatment																						
AQ	114	63	7	5	.	5	0.2	<0.0001	63	3	2	5	4	0.3	0.0007	55	3	3	5	4	0.3	0.001
CQ	141	52				11	0.4		52	5	4	7	6	0.5		47	5	3	7	5	0.5	
SP	170	65	.	14	.	10	0.4		65	5	3	7	5	0.5		51	7	4	7	5	0.5	
Day of recurrent parasitaemia																						
By day 14	226	97	.	14	.	10	0.3	0.05	97	5	4	7	5	0.4	0.0003	80	5.5	5	7	6	0.4	0.0003
Post day 14	199	83	8	7	8	6	0.2		83	3	3	5	4	0.3		73	3	3	5	4	0.3	
Level of parasitaemia at detection																						
<500/μl	239	89				11	0.3	<0.0001	89	6	5	7	6	0.4	<0.0001	77	6	5	7	6	0.4	0.0009
>500/μl	186	91	10	7	.	9	0.4		91	2	2	3	4	0.3		75	2	2	3	4	0.4	
Type of infection																						
New infection	116	68	7	5	9	8	0.5	<0.0001	68	3	3	5	4	0.4	0.03	68	3	3	5	4	0.4	0.001
Recrudescence	152	85	8	7	12	9	0.4		85	5	5	7	5	0.4		85	5	5	7	5	0.4	
Not genotyped	41	27	6	3	14	7	0.9		27	3	1	6	4	0.8								

Table 4 Logistic regression model of risk of fever

	Risk of fever			
	425 patients with recurrent asymptomatic parasitaemia			
Parameter	Estimate	95% Confidence limits		*P*-value
Treatments				
SP	1			
AQ	1.87	1.13	3.09	0.01
CQ	0.91	0.56	1.47	0.70
Level of parasitaemia at detection				
<500	1			
≥500	1.52	1.01	2.30	0.05
Age				
6–≤12 months	1			
>12–≤24 months	0.80	0.45	1.41	0.43
>24–≤36 months	0.50	0.27	0.90	0.02
>36–≤42 months	0.30	0.12	0.80	0.02
>42–≤59 months	0.22	0.11	0.45	<0.0001
Hosmer and Lemeshow goodness-of-fit test		$P = 0.31$		

Table 5 Cox proportional hazard ratio model of time to fever

	Time to fever 180 patients who became LTF				Time to fever 153 patients who became LTF and were assigned to new or recrudescent infection			
Parameter	Hazard ratio	95% Hazard ratio confidence limits		*P*-value	Hazard ratio	95% hazard ratio confidence limits		*P*-value
Treatment								
SP	1				1			
AQ	1.60	1.11	2.30	0.01	1.69	1.14	2.51	0.01
CQ	1.06	0.72	1.55	0.78	1.22	0.80	1.89	0.36
Day of recurrent parasitaemia								
By day 14	1			0.05	1			0.02
Post day 14	1.40	0.99	1.97		1.57	1.07	2.29	
Level of parasitaemia the day of reappearance								
<500	1			0.003	1			0.02
>=500	1.57	1.16	2.12		1.47	1.07	2.04	

episodes of asymptomatic parasitaemia to be found later with no parasites and no symptoms was 0.05 within 30 days in Gabon (Missinou *et al.* 2003) and 0.11 within 1 year in Uganda Njama-Meya *et al.* 2004). In our study, we may have underestimated the risk of clinical progression to a LTF because of the short duration of follow-up with the 28-day study and because we cannot rule out the use of antimalarials in the cases subsequently found with no parasites. We believe that the occurrence of these 'spontaneous conversions' does not justify complacency in case management.

The risk of LTF after initial clearance varied with these mono-therapies (hence related parasite susceptibility at the study site.) With more effective treatments, like the currently recommended combinations (WHO 2006), one would expect fewer such cases. Here, on aggregate all drugs tested had failure rates >10%, a level at which the WHO now recommends considering an alternative treatment.

Risk factors for LTF after an asymptomatic recurrence

Both the univariate analysis and the logistic model concur in pointing out treatment with AQ, parasitaemia ≥500/μl and detected post-d14 as carrying a greater risk of developing fever and LTF. When considering all patients, young age was at higher risk (with an apparent linear trend

Table 6 Type of infection (as per PCR genotyping) in patients who became symptomatic by time of occurrence of asymptomatic parasitaemia (n = 153)

	Asymptomatic recurrence		
Outcome	By day 14	Post day 14	All
Recrudescences			
By day 14	9	0	9
Post day 14	45	31	76
All	54	31	85
New infections			
Post day 14	26	42	68

in this age-range 6–59 months), while age was unimportant for those who did become LTF. This shows that immunity builds up relatively early in life in these areas of moderate to intense transmission, and that, for those who do become symptomatic, the pre-patent period is largely determined by the treatment given and not by age. Of the three monotherapies used, patients on AQ were more likely to become symptomatic and to do so sooner than CQ and SP. The level of parasitaemia at which new and recrudescent infections are detected and become symptomatic is similar, but new infections carry a higher risk of progressing to symptomatic disease (in the univariate analysis).

These observations are intriguing and elicit a number of hypotheses with respect to parasite dynamics, parasite/host interaction and drug disposition and effects. To this effect we could not identify in the literature similar studies documenting prospectively the pre-patent period of malaria infections for comparison. Further studies will be needed to substantiate possible explanations.

Drug pharmacokinetics and pharmacodynamics

Declining drug levels with time explain why recurrent parasitaemias became symptomatic sooner when they occurred in the second part of follow-up (post-d14). Drug pharmacokinetics and pharmacodynamics may also help account for and when they were due to a new infection. There were differences between drugs. Time to fever was significantly shorter (i) for asymptomatic parasitaemias occurring post-d14 when the treatment was AQ or SP but not CQ; and (ii) for new infections when the treatment was SP but not AQ or CQ. In addition, time to fever was also longer with SP and CQ than AQ, but this should be considered with caution as these were not direct comparisons.

An antimalarial drug's post-treatment effect is likely to depend upon the time during which plasma levels remain above inhibitory concentrations (which vary with the levels of parasite susceptibility in the different areas). This time is related to the drug's residence time in the organism but also to the shape of the concentration-time curve. Despite decades of use, there are still uncertainties over the disposition of antimalarial drugs. It is outside the scope of this article to discuss these differences, but it appears from these data that plasma concentrations will fall below inhibitory levels earlier for AQ than SP.

Higher parasite replication rates occur in the latter part of follow-up after plasma levels had fallen below the effective concentration necessary to inhibit parasite growth (White & Pongtavornpinyo 2003). Unlike pre-existing parasites, the parasites sustaining a new infection had not been exposed to the drug at inhibitory levels, which could provide an alternative explanation to the observation that new infections manifest themselves sooner than recrudescent infections. A limitation of this study is the absence of drug levels measured in particular at the time of the asymptomatic recurrence and fever. While generated with single-agent treatments, these data are relevant to the current use of these drugs (notably AQ and SP) in combination as they are the longer-lived component of artemisinin-based combinations (ACTs).

Conclusions

Post-treatment asymptomatic parasitaemia is associated with a high risk of developing symptoms within 5–10 days; whether due to a recrudescent or new infection, treatment should not be postponed. The risk is highest early in life and decreases as children grow older. Patients with parasitaemias occurring post-d14 and ≥500/μl are at higher risk. Differences between drugs are likely due to their pharmacokinetic characteristics; amodiaquine carries a higher risk.

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Disclaimer

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