MAJOR ARTICLE

Plasmodium vivax Recurrence Following Falciparum and Mixed Species Malaria: Risk Factors and Effect of Antimalarial Kinetics

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(See editorial commentary by Baird, on pages 621–623.)

Background. *Plasmodium vivax* malaria commonly follows treatment of falciparum malaria in regions of co-endemicity. This is an important cause of preventable morbidity.

Methods. We examined the factors contributing to the risk of recurrence of *P. vivax* infection after treatment of acute falciparum malaria in a series of clinical trials conducted on the Thai-Myanmar border from 1991 through 2005.

Results. Overall, 10,549 patients (4960 children aged <15 years and 5589 adults) were treated for falciparum malaria; of these patients, 9385 (89.0%) had *Plasmodium falciparum* monoinfection and 1164 (11.0%) had mixed *P. falciparum/P. vivax* infections according to microscopic examinations performed at screening. The cumulative proportion of patients with *P. falciparum* infection recurrence by day 63 was 21.5% (95% confidence interval [CI], 20.3%–22.8%), and the cumulative proportion with *P. vivax* infection recurrence was 31.5% (95% CI, 30.1%–33.0%). Significant risk factors for *P. vivax* infection recurrence were mixed infection at enrollment, male sex, younger age, lower hematocrit, higher asexual *P. falciparum* parasite density ($P < .001$ for all factors), and *P. falciparum* gametocytemia at enrollment ($P = .001$). By day 63, the cumulative risk of vivax malaria after *P. falciparum* monoinfection was 51.1% (95% CI, 46.1%–56.2%) after treatment with rapidly eliminated drugs ($t_{1/2}$ <1 day), 35.3% (95% CI, 31.8%–39.0%) after treatment with intermediate half-life drugs ($t_{1/2}$ 1–7 days), and 19.6% (95% CI, 18.1%–21.3%) after treatment with slowly eliminated drugs ($t_{1/2} > 7$ days) ($P < .001$, by test for trend). Artemisinin-based combinations containing mefloquine or piperaquine, compared with the artemether-lumefantrine and artesunate-atovaquone-proguanil combinations, were associated with a 3.6-fold to 4.2-fold lower adjusted hazard ratio for *P. vivax* infection recurrence within 63 days after pure or mixed *P. falciparum* infections ($P < .001$, for comparisons with artesunate-mefloquine).

Conclusions. On the Thai-Myanmar border, *P. vivax* is the most common cause of parasitological failure after treatment for falciparum malaria. Slowly eliminated antimalarials reduce the risk of early *P. vivax* infection recurrence.

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In Southeast Asia, the incidence of *Plasmodium vivax* infection after treatment of falciparum malaria is substantially greater than would be expected on the basis of entomological inoculation rates [1–7]. The reasons for this are not clear. One postulate is that contemporaneous inoculation of *P. vivax* and *Plasmodium falciparum* occurs relatively frequently and that acute *P. falciparum* infection suppresses *P. vivax* parasitemia below levels detectable by light microscopy [1, 8]. According to this hypothesis, most recurrent *P. vivax* infections after

treatment of falciparum malaria are relapses that are due to simultaneously acquired hypnozoites [1, 8]. An alternative theory is that either *P. falciparum* infection or its treatment somehow precipitate blood-stage relapse from dormant, previously acquired hypnozoites [8].

Whatever the underlying mechanism, *P. vivax* infection recurrence after falciparum malaria carries significant morbidity, impairs clinical and hematological recovery [3, 9], and worsens the socioeconomic burden of malaria [10]. Because asexual *P. vivax* parasitemia after blood-stage treatment is frequently associated with concurrent gametocytemia [3, 9, 11], it is also likely to have an important role in sustaining transmission of *P. vivax* [12]. The efficacy of antimalarial treatment for preventing *P. vivax* infection recurrence is therefore an important consideration for malaria control strategies.

We have used pooled data from a large series of clinical trials conducted at Shoklo Malaria Research Unit on the Thai-Myanmar border between 1991 and 2005 to establish the effect of demographic and clinical factors as well as antimalarial elimination kinetics on the risk of *P. vivax* infection recurrence after *P. falciparum* or mixed *P. vivax*/*P. falciparum* malaria.

METHODS

Study Sites

The studies included in this analysis were performed from 1991 through 2005 at camps for displaced persons of the Karen ethnic minority and border clinics that served mainly Karen and Burmese migrant workers along the northwestern border of Thailand. In the mid-1990s, the local annual incidence of malaria was approximately 1 episode per person-year, 53% of which were due to *P. vivax,* 37% of which were due to *P. falciparum,* and 10% of which were due to mixed infection (determined according to the results of examination with light microscopy) [13]. Virtually all *P. falciparum* infections and ~90% of *P. vivax* infections were symptomatic [13]. Standard treatment of uncomplicated falciparum malaria was mefloquine monotherapy (25 mg base/kg total dose) from 1991 through 1994 and was mefloquine (25 mg base/kg) plus artesunate (12 mg/kg over 3 days) thereafter [14].

Design of the Studies

This analysis includes 24 studies that investigated 25 different antimalarial treatment regimens. None included routine administration of primaquine (Table 1). Sixteen of the studies were randomized controlled trials of different treatments for uncomplicated falciparum malaria with or without concomitant *P. vivax* infection; the remainder were single-arm clinical trials conducted to assess drug efficacy or safety. None included children who weighed <5 kg or pregnant women. Two studies restricted recruitment to children ≤15 years of age, and 1 study restricted recruitment to children <5 years of age (Table 1).

Patients with severe disease according to World Health Organization criteria [15] were excluded, although the studies of intravenous quinine plus mefloquine and of the 5-day and 7-day courses of artesunate in combination with mefloquine included patients with uncomplicated hyperparasitemia (>4% parasitised red blood cells) (Table 1). Follow-up was standardized for all studies and lasted 28 days (6 studies; 1398 patients), 42 days (11 studies; 5354 patients), or 63 days (7 studies; 3797 patients). Patients were seen every day until they were afebrile and had experienced parasite clearance and were then seen weekly thereafter. In the event of illness that occurred between these visits, patients were asked to return to the clinic for treatment. Fully informed consent was obtained before enrollment in all of the studies. The studies were approved by the ethics committees of the Faculty of Tropical Medicine, Mahidol University, and Oxford University (OXTREC).

Study Data

Basic demographic and clinical details were recorded at enrollment, including age, sex, parasitemia, temperature, and in most cases, hematocrit and white blood cell (WBC) count. Symptoms, temperature, and parasite count were assessed at follow-up visits. Diagnosis of *Plasmodium* infection and subsequent species identification were established by examination of Giemsa-stained thick and thin blood films. Parasitemia was reported as the number of asexual parasites per 500 WBCs or per 1000 red blood cells and subsequently converted to a count per microliter using the patient's WBC count or hematocrit, if available. Population means or assumed values of 8300 WBCs/μL and 35%, respectively, were used when necessary. Asexual parasite densities in mixed infection were given as a summed total in the majority of studies and were given separately for both species in a minority. For this analysis, we used the summed total.

Patients were censored and deemed to have experienced treatment failure if there were signs of early treatment failure due to either malaria parasite species [16], if asexual *P. falciparum* or *P. vivax* parasitemia persisted beyond 7 days, or if either species reappeared in the circulation up to 63 days after initial clearance. Patients who did not experience failure were censored on the date of their last negative blood smear result.

Statistical Analysis

The primary outcome for this analysis was recurrence of *P. vivax* infection up to 63 days after treatment for *P. falciparum* or mixed *P. falciparum*/*P. vivax* infection. Potential risk factors examined were species of infection at enrollment (*P. falciparum* or mixed infection), age, sex, initial $\log_e$ parasite density, baseline hematocrit, and *P. falciparum* gametocytemia at enrollment (yes or no). We compared nonparametric continuous data using the Kruskal-Wallis test, unpaired proportions using the χ^2 test, and paired proportions using McNemar's test. The impact of antimalarial drugs was assessed in 2 separate comparisons. First, we

Table 1. Details of Treatment Regimens and Characteristics of Patients

Code	Total treatment dose (total regimen duration, total number of doses)	Year(s) studied	$t_{½}$	No. of patients	Male sex, no. (%) of patients	Age, median years (90% range)	Parasitemia, median parasites/μL (90% range)
AAP	Artesunate 12 mg/kg (3 days, 3 doses) + atovaquone 45 mg/kg (3 days, 3 doses) + proguanil 24 mg/kg (3 days, 3 doses)	1998–2000	Int	526	353 (67)	20 (7–41)	4408 (176–86,219)
AM7	Artemether 12 mg/kg (7 days, 7 doses)	1993–1996	Short	206	114 (55)	15 (2–33)	4850 (273–73,853)
AP	Atovaquone 45 mg/kg (3 days, 3 doses) + proguanil 24 mg/kg (3 days, 3 doses)	1998–2000	Int	528	354 (67)	20 (7–43)	3841 (142–66,870)
AS3	Artesunate 12 mg/kg (3 days, 3 doses)	1992–1994	Short	5	3 (60)	14 (1–25)	105,278 (4428–151,926)
AS5	Artesunate 12 mg/kg (5 days, 5 doses)	1992–1995	Short	153	86 (56)	5 (1–25)	13,842 (424–430,713)
AS7	Artesunate 12 mg/kg (7 days, 7 doses)	1992–1996	Short	452	245 (54)	10 (2–29)	6972 (331–149,142)
AS7T7	Artesunate 12 mg/kg (7 days, 7 doses) + tetracycline 112 mg/kg (7 days, 7 doses)	1993–1995	Short	20	12 (60)	14 (9–39)	9396 (1065–205,230)
COA4	Artemether 6.8 mg/kg (3 days, 4 doses) + lumefantrine 48 mg/kg (3 days, 4 doses)	1995–1997	Int	387	265 (68)	21 (9–41)	4529 (278–88,957)
COA6a	Artemether 10.2 mg/kg (60 h, 6 doses) + lumefantrine 72 mg/kg (96 h, 6 doses)	1996–1998 2000–2002	Int	1115	757 (68)	20 (7–45)	6414 (489–88,297)
COA6b	Artemether 10.2 mg/kg (96 h, 6 doses) + lumefantrine 72 mg/kg (96 h, 6 doses)	1996–1997	Int	87	62 (71)	22 (11–41)	5460 (1023–78,561)
DP+	DHA 6.3 mg/kg (3 days, 4 doses) + piperaquine 51.3 mg/kg (3 days, 4 doses) + either artesunate 400 mg (3 days, 4 doses) or extra DHA to achieve total dose of 12 mg/kg (3 days, 4 doses)	2002–2003	Long	174	125 (72)	20 (6–45)	16,830 (415–105,630)
DP3	DHA 6.3 mg/kg (3 days, 3 doses) + piperaquine 51.3 mg/kg (3 days, 3 doses)	2003–2004	Long	170	104 (61)	21 (6–43)	11,304 (496–75,360)
DP4	DHA 6.3 mg/kg (3 days, 4 doses) + piperaquine 51.3 mg/kg (3 days, 4 doses)	2002–2004	Long	340	216 (64)	22 (7–44)	13,816 (802–94,878)
M25	Mefloquine 25 mg/kg (1–2 days, 1–2 doses)	1991–1994	Long	949	543 (57)	14 (4–38)	3818 (213–36,754)
MA	Artesunate 10 mg/kg (1 day, 3 doses) + mefloquine 15 mg/kg (1 day, 1 dose)	1991	Long	323	190 (59)	15 (3–38)	3486 (249–23,652)
MAM1	Artemether 4–10 mg/kg (1 day, 2–3 doses) + mefloquine 25 mg/kg (1 day, 1 dose)	1992	Long	19	10 (53)	20 (11–50)	6739 (253–228,592)
MAM3	Artemether 12 mg/kg (3 days, 3 doses) + mefloquine 25 mg/kg (1 day>, 1 dose)	1993–1994	Long	180	86 (48)	16 (5–42)	5299 (326–78,442)
MAS1	Artesunate 4 mg/kg (1 day, 1 dose) + mefloquine 25 mg/kg (1 day, 1 dose)	1992	Long	152	94 (62)	16 (4–35)	4847 (315–26,892)
MAS3	Artesunate 12 mg/kg (3 days, 3 doses) + mefloquine 25 mg/kg (1–2 days in 1–2 doses)	1992–2005	Long	4106	2,533 (62)	14 (5–39)	7300 (349–93,085)
MAS5	Artesunate 12 mg/kg (5 days, 5 doses) + mefloquine 25 mg/kg (1 day, 1 dose)	1992–1995	Long	57	29 (51)	6 (2–23)	326,874 (14,472–707,962)
MAS7	Artesunate 12 mg/kg (7 days, 7 doses) + mefloquine 25 mg/kg (1 day, 1 dose)	1993–1995	Long	139	82 (59)	7 (3–12)	270,957 (162,778–597,555)
MASF	Artesunate 12 mg/kg (3 days, 3 doses) + mefloquine 25 mg/kg (3 days, 3 doses) in fixed combination	2004–2005	Long	247	170 (69)	20 (6–45)	14,469 (342–92,547)
MQIV	Quinine 40 mg/kg (1 day, 3 doses) + mefloquine 25 mg/kg (1 day, 1 dose)	1993	Long	31	18 (58)	9 (4–29)	309,177 (150,850–562,186)
Q7	Quinine 210 mg/kg (7 days, 7 doses)	1992–1993	Short	28	16 (57)	5 (2–8)	3819 (130–26,158)
Q7T7	Quinine 210 mg/kg (7 days, 7 doses) + tetracycline 112 mg/kg (7 days, 7 doses)	1992–1994	Short	155	97 (63)	15 (9–34)	4284 (294–79,409)
Total		1991–2005		10,549	6,564 (62)	15 (5–40)	6586 (328–101,284)

NOTE. DHA, dihydroartemisinin; int, intermediate; $t_{½}$, elimination half-life category.

examined outcomes for all antimalarial drugs or combinations grouped by their terminal elimination half-lives ($t_{1/2}$) (Table 1; short was defined as $t_{1/2} < 1$ day, intermediate was defined as $t_{1/2} > 1$ day and < 1 week, and long was defined as $t_{1/2} > 1$ week). Second, we compared outcomes between individual artemisinin combination therapies. The Kaplan–Meier function and log-

rank test were used for univariable analyses. Multivariable analyses were done using the Cox proportional hazards model with gamma frailty to account for heterogeneity of results between studies [17] (examined using the Wald test for significance of interaction terms in preliminary models). Fulfillment of the proportional hazards assumption was assessed using log-log plots for each of the model covariables. All analyses were done using Stata software, version 10.1 (Stata Corporation).

RESULTS

From 1991 through 2005, 10,549 patients (4960 children aged <15 years and 5589 adults) were treated for falciparum malaria, of whom 9385 (89.0%) had *P. falciparum* monoinfections and 1164 (11.0%) had mixed infections. Overall, 2925 patients (27.7%) had recurrence of parasitaemia, 1570 (53.7%) with monoinfection due to *P. vivax* alone, 1269 (43.4%) with monoinfection due to *P. falciparum* alone, and 86 (2.9%) with mixed infections. The median time to recurrence was 28 days for those with *P. falciparum* monoinfection, 35 days for those with *P. vivax* monoinfection, and 33 days for those with mixed infection ($P < .001$ for overall difference). The number and characteristics of individuals receiving each of the treatment regimens are shown in Table 1. According to Kaplan–Meier analyses, the cumulative proportion of patients experiencing treatment failure due to any species by day 63 was 45.6% (95% confidence interval [CI], 44.1%–47.0%), the proportion experiencing treatment failure due to *P. falciparum* infection (either monoinfection or mixed infection) was 21.5% (95% CI, 20.3%–22.8%) and due to *P. vivax* (either monoinfection or mixed infection) was 31.5% (95% CI, 30.1%–33.0%). Overall, 3.5% (36 of 1024) of recurrences with asexual *P. falciparum* infection were associated with patent *P. falciparum* gametocytemia. Gametocyte data for recurrences of *P. vivax* infection were not available.

Hematocrit data were available for 90.7% of patients (9565 of 10,549) at enrollment and 58.9% of patients (1724 of 2925) at the time of treatment failure. In total, 14.5% of patients (1382 of 9565) were anemic (hematocrit <30%) at enrollment to the studies. Of those who did not have parasitological failure, 13.5% of patients (925 of 6869) were anemic at baseline, compared with 4.0% of patients (192 of 4755) at the last follow-up visit ($P < .001$). The corresponding figures at baseline and at the time of recurrence were 14.2% of patients (169 of 1189) versus 11.3% of patients (78 of 692) for those who experienced treatment failure due to *P. falciparum* ($P = .1$) and 18.7% of patients (296 of 1586) versus 7.2% of patients (78 of 1091) for those who experienced treatment failure due to *P. vivax* ($P < .001$). Patients who had recurrent *P. falciparum* monoinfection, *P. vivax* monoinfection, or mixed infection were anemic at the time of failure in 11.9% (75 of 633), 7.3% (75 of 1032), and 5.1% (3 of 59) of cases, respectively ($P = .004$ for overall difference).

Symptomatology data were available at the time of parasitological failure for 68.3% of study participants (1997 of 2925). Recurrences with *P. falciparum* monoinfection, *P. vivax* monoinfection, and mixed infections were associated with symptoms in 65.5% (537 of 820), 44.3% (495 of 1118), and 71.2% (42 of 59) of cases, respectively ($P < .001$ for overall difference). At the time of recurrence, the proportion of patients who were febrile (temperature >37.5°C) or had a history of fever within the last 24 h was 51.7% (455 of 880) for those with *P. falciparum* monoinfections, 33.6% (386 of 1,148) for those with *P. vivax* monoinfections, and 61.4% (35 of 57) for those with mixed infections ($P < .001$ for overall difference).

Of patients who had recurrent *P. falciparum* monoinfection, *P. vivax* monoinfection, or mixed infection, 41.2% (523 of 1269), 30.5% (479 of 1570) and 58.1% (50 of 86), respectively, presented outside of routine weekly follow-up and therefore presumably of their own volition ($P < .001$ for overall difference). *P. vivax* infection recurrences after treatment with short, intermediate, and long half-life combinations were symptomatic in 58.3% (158 of 271), 42.7% (230 of 539), and 40.6% (149 of 367) of cases, respectively ($P < .001$ for overall difference).

Risk Factors for Recurrence of *P. vivax* Infection

The cumulative risk of *P. vivax* infection recurrence by day 63 after *P. falciparum* monoinfection was 29.4% (95% CI, 27.9%–30.9%), and the risk after mixed infection was 49.3% (95% CI, 44.3%–54.5%); adjusted hazard ratio (AHR), 2.47; 95% CI, 2.15–2.85; $P < .001$ (Tables 2 and 3). Univariable analyses showed a statistically significant increase in the risk of *P. vivax* infection recurrence after pure *P. falciparum* infection with decreasing age, low hematocrit (<30%), increasing $\log_e$ asexual parasite density, and presence of *P. falciparum* gametocytemia (Table 2). Male patients were significantly more likely to have recurrent *P. vivax* infection after both monoinfection due to *P. falciparum* and mixed infections (Tables 2 and 3; AHR, 1.27; 95% CI, 1.14–1.41; $P < .001$).

Effect of Antimalarial Drugs on Risk of Recurrence of *P. vivax* Infection

The median times to *P. vivax* infection recurrence after treatment with short, intermediate, and long half-life regimens were 28, 29, and 49 days, respectively ($P < .001$ for overall difference; Figure 1). Treatment with slowly eliminated antimalarials was associated with a significant trend to decreasing risk of *P. vivax* infection recurrence up to 63 days after both malaria due to *P. falciparum* monoinfection and malaria due to mixed infection ($P < .001$ for trend in both cases; Figure 2). The cumulative proportion of patients treated with a rapidly eliminated antimalarial who had a recurrence of *P. vivax* infection after pure falciparum malaria was 53.8% (95% CI, 48.5%–59.3%),

Table 2. Baseline Risk Factors for *Plasmodium vivax* Recurrence, by Initial Species Isolated

Variable	*Plasmodium falciparum* infection				Mixed *P. falciparum*/*P. vivax* infection			
	No. of cases	Treatment failure[a]	95% CI	*P*	No. of cases	Treatment failure, %[a]	95% CI	*P*
Age group								
<5 years	802	39.4	33.7–45.6	<.001[b]	204	56.2	43.1–70.1	.6[b]
5–15 years	3347	35.4	32.7–38.3		607	52.9	45.9–60.1	
>15 years	5236	24.6	22.8–26.5		353	41.4	33.7–50.1	
Sex								
Male	5925	30.3	28.4–32.3	.03	639	53.7	47.0–60.7	.02
Female	3460	27.8	25.5–30.3		525	43.7	36.7–51.5	
Hematocrit, %								
<30	1252	38.6	34.4–43.1	<.001	130	47.2	35.1–61.2	.4
⩾30	7318	27.9	26.3–29.6		865	50.4	44.9–56.2	
Log_e parasitemia								
<25th centile (~1400 parasites/μL)	2454	23.3	20.7–26.2	<.001[b]	158	39.3	28.2–53.0	.005[b]
25th–50th centile (1400–6600 parasites/μL)	2223	26.8	23.7–30.1		391	50.0	41.1–59.6	
50th – 75th centile (6600–35,900 parasites/μL)	2184	29.4	26.4–32.5		429	40.1	32.0–49.3	
>75th centile (>35,900 parasites/μL)	2524	36.9	33.9–40.0		186	69.4	59.5–78.8	
P. falciparum gametocytemia at enrollment								
No	8847	28.7	27.2–30.3	<.001	1116	48.3	43.2–53.6	.008
Yes	437	41.4	34.4–49.2		43	69.1	48.5–87.4	
Total	9385	29.4	27.9–30.9		1164	49.3	44.3–54.5	

NOTE. CI, confidence interval.

[a] Kaplan-Meier cumulative failure estimates (%) at day 63.

[b] Log-rank test for trend.

compared with 21.1% (95% CI, 19.5%–22.9%) among those treated with slowly eliminated regimens ($P < .001$). All patients with mixed-species infections who were treated with a rapidly eliminated antimalarial had a recurrent infection within 49 days of follow-up. The adjusted hazard ratios for *P. vivax* infection recurrence after either *P. falciparum* infection or mixed infection for patients receiving long or intermediate half-life regimens were 0.43 (95% CI, 0.29–0.63; $P < .001$) and 0.12 (95% CI, 0.08–0.18; $P < .001$), respectively, when compared with those receiving rapidly eliminated antimalarials (Table 3).

The median times to *P. vivax* infection recurrence after artesunate-atovaquone-proguanil, artemether-lumefantrine, artesunate-mefloquine, dihydroartemisinin-piperaquine, and artemether-mefloquine treatment were 28, 29, 49, 49, and 56 days, respectively ($P < .001$ for overall difference). Of the artemisinin combination therapies, those regimens containing mefloquine or piperaquine appeared to be equally effective at preventing *P. vivax* infection recurrence in both univariable and multivariable analyses (Figure 3 and Table 3). The shorter-acting combinations, artemether-lumefantrine and artesunate-atovaquone-proguanil, were associated with 3.6-fold and 4.2-fold increases in risk of *P. vivax* infection recurrence, respectively, when compared with artesunate-mefloquine treatment ($P <.001$ in both cases) (Table 3).

DISCUSSION

In a large series of clinical trials conducted on the Thai-Myanmar border, *P. vivax* infection accounted for substantially more malaria recurrences within 63 days of treatment for falciparum or mixed malaria than did *P. falciparum* infection. Because *P. vivax* is more frequently associated with gametocytemia [3, 9, 11] and is more transmissible at low parasite densities [18], the most commonly transmitted parasite after treatment for falciparum malaria, paradoxically, was not *P. falciparum,* but *P. vivax.*

Statistically significant baseline risk factors for *P. vivax* infection recurrence after acute falciparum malaria included initial mixed-species infection, male sex, younger age, higher total asexual parasitemia, lower hematocrit, and the presence of *P. falciparum* gametocytemia. Slowly eliminated antimalarial regimens, such as those containing mefloquine or piperaquine, were associated with a markedly lower risk of *P. vivax* infection recurrence than were rapidly eliminated drugs.

High asexual *P. falciparum* parasitemia is a well-recognized risk factor for subsequent *P. falciparum* recrudescence [19–23]. In the present analysis, we have shown that it also increases the risk of *P. vivax* infection recurrence. One potential explanation for this phenomenon is that higher *P. falciparum* density, lower hematocrit, and younger age are proxy markers of malaria

Table 3. Multivariable Cox Proportional Hazards Models Showing the Effect of Baseline Factors and Antimalarial Drugs on Risk of *Plasmodium vivax* Recurrence

	Recurrence with *P. vivax*		
	AHR	95% CI	*P*
All drugs			
Drug half-life			
Short ($t_{1/2}$ < 1 day)	1	…	…
Intermediate ($t_{1/2}$ 1–7 days)	.43	.29–.63	<.001
Long ($t_{1/2}$ > 7 days)	.12	.08–.18	<.001
Species at enrollment			
Pure *P. falciparum*	1	…	…
Mixed *P. falciparum/P. vivax*	2.47	2.15–2.85	<.001
Age, per year increase	.98	.97–.98	<.001
Sex			
Female	1	…	…
Male	1.27	1.14–1.41	<.001
Hct, per percentage point increase	.98	.97–.99	<.001
Log_e parasite density, per log_e order	1.09	1.07–1.12	<.001
P. falciparum gametocytemia			
No	1	…	…
Yes	1.38	1.14–1.69	.001
Artemisinin combination therapies[a]			
Artesunate + mefloquine combinations	1	…	…
DHA + piperaquine combinations	1.12	.79–1.58	.5
Artemether + mefloquine combinations	.80	.42–1.51	.5
Artemether + lumefantrine	3.57	2.91–4.37	<.001
Artesunate + atovaquone + proguanil	4.20	2.79–6.31	<.001

NOTE. CI, confidence interval; DHA, dihydroartemisinin; Hct, hematocrit; AHR, adjusted hazard ratio.

[a] Model also includes species at enrollment, age, sex, hematocrit, log_e parasite density, and *P. falciparum* gametocytemia at enrollment.

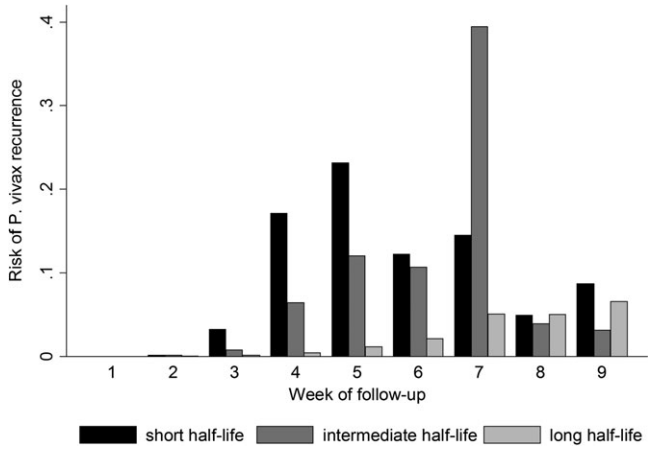

Figure 1. Risk of *Plasmodium vivax* recurrence after *Plasmodium falciparum* monoinfection or mixed *P. vivax/P. falciparum* malaria by week of follow-up and antimalarial half-life.

naivety and hence poor immunity to both *P. falciparum* and *P. vivax* infections. If this is true, relapses due to *P. vivax* hypnozoites acquired at or around the same time as the index *P. falciparum* infection would have a greater chance of reaching patency. Simultaneous or near simultaneous infection due to *P. falciparum* and *P. vivax* is probably relatively common. Mason et al [24] showed that 10.5% of patients treated for *P. vivax* malaria in Bangkok subsequently had a recurrence of *P. falciparum* infection within 28 days. Because *P. falciparum* does not have a dormant form, and because there is no local malaria transmission in Bangkok, these parasites are most likely to have been acquired at the same time as the *P. vivax* infections.

An alternative, but potentially complimentary, hypothesis is that high parasitemia and low hematocrit are indicators of greater disease severity and hence of pathophysiological and immunological derangement, a consequence of which may be stimulation of *P. vivax* infection relapse and/or failure to suppress growth of recurrent blood stage infection. This mechanism would be equally plausible regardless of whether the relapsing *P. vivax* hypnozoites had been acquired at the same time or prior to the index *P. falciparum* infection. Because the excess risk of *P. vivax* infection recurrence is seen even after slowly eliminated therapies, these putative factors would either have to be long-lasting or induce a prolonged stream rather than a single pulse of relapsing merozoites from the liver.

Highly sensitive polymerase chain reaction–based assays typically reveal a much higher prevalence of concurrent mixed-species infection than does examination with light microscopy [5, 25–28]. This suggests that a sizeable proportion of patients with microscopically confirmed *P. falciparum* monoinfection in regions of co-endemicity actually have subpatent *P. vivax* parasitemia. In our study, patients presenting with falciparum gametocytemia were at 1.38 times the risk of early recurrence with *P. vivax* infection, compared with the risk among patients without gametocytemia. The presence of gametocytes is more likely in patients with chronic, asymptomatic infections and may therefore be suggestive of multiple previous exposures to both *Plasmodium* species and thus a greater risk of subpatent vivax infection at enrollment.

Our pooled meta-analysis included a large number of individuals who were treated with multiple different antimalarial regimens. The individual trials were conducted in similar physical environments, which helped to ensure the comparability of their results. Nevertheless, several sources of inter-study heterogeneity remain. Some of these could be partially addressed in multivariable models by controlling for differences in the age structure and median parasite density of study participants. Other known and unknown sources of heterogeneity, such as differences in dosing schedules for individual regimens and temporal differences in local malaria incidence, could not be controlled for. By using Cox

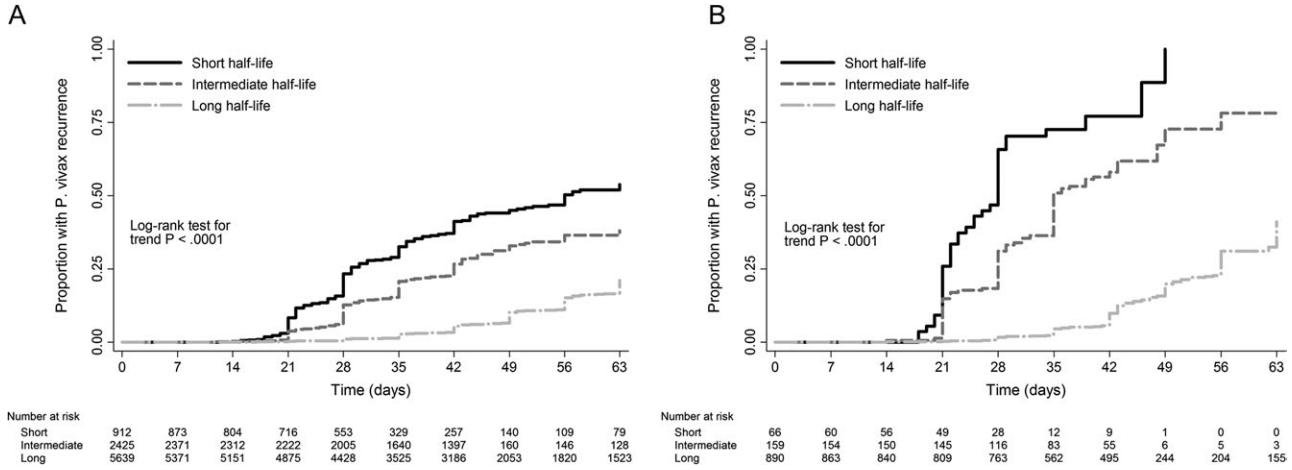

Figure 2. Kaplan–Meier failure estimates for the cumulative risk of *Plasmodium vivax* recurrence after *Plasmodium falciparum* infection (*A*) and following mixed *P. falciparum/P. vivax* infection (*B*) by antimalarial half-life.

models with gamma frailty, we have presented an averaged effect of specific regimens across the different studies [17].

The long-term benefits of prolonged post-exposure prophylaxis against recurrent parasitemia have yet to be determined. With the exception of the antifolate drugs, antimalarial compounds active against *P. falciparum* have excellent efficacy against the blood stages of *P. vivax*, and thus, the drug regimens included in this analysis should have cleared initial subpatent *P. vivax* infections [29]. The risk of *P. vivax* reinfection in this region is low (<5% during a 42-day period) [13, 30]. One can therefore assume that most of the observed *P. vivax* infection recurrences were relapses. Hypnozoites have the potential to seed multiple relapses, and it is not known whether prevention of just one of these by use of a slowly eliminated antimalarial will reduce the total number of relapses or simply delay the occurrence of the next relapse. If the former is true, the total morbidity from a given vivax infection could be reduced, and total gametocyte carriage and, hence, transmissibility would also be expected to decrease. A greater period of post-exposure prophylaxis against recurrence of infection due to any *Plasmodium* species should also facilitate fuller hematological and clinical recovery [3, 9].

These speculative benefits must be weighed against potential disadvantages. Drugs with long terminal elimination half-lives will be present in the bloodstream at subtherapeutic concentrations for longer than rapidly eliminated drugs and will

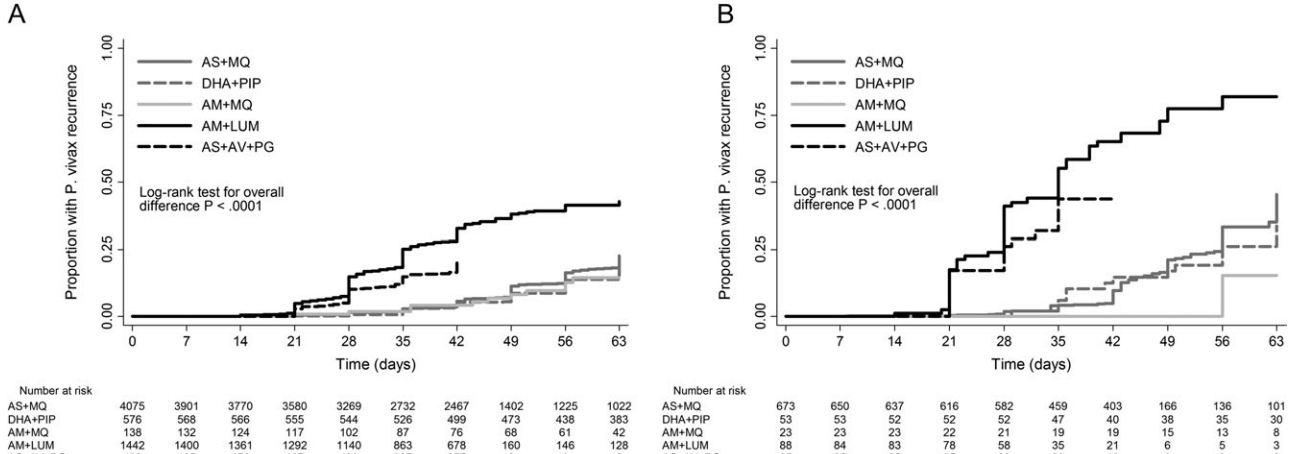

Figure 3. Kaplan–Meier failure estimates for the cumulative risk of *Plasmodium vivax* recurrence after *Plasmodium falciparum* infection (*A*) and following mixed *P. falciparum/P. vivax* infection (*B*) for artemisinin combination therapies. AS+MQ, artesunate plus mefloquine; DHA + PIP, dihydroartemisinin plus piperaquine; AM+MQ, artemether plus mefloquine; AM+LUM, artemether plus lumefantrine; AS+AV+PG, artesunate plus atovaquone plus proguanil.

therefore provide a more powerful force for the spread of drug-resistant parasites [12, 31, 32]. The combination of mefloquine and artesunate has been used for the treatment of *P. falciparum* malaria along the northwestern border of Thailand both in trials and in routine practice since 1994. Recent studies have revealed an increase in the prevalence of *PvMDR1* gene amplification in local *P. vivax* isolates, a polymorphism associated with reduced susceptibility to mefloquine [33]. Although post-hoc exploratory analyses (not presented) show that the risk of *P. vivax* infection recurrence after mefloquine-artesunate therapy has increased slightly with time, it is unclear whether this is due to emerging mefloquine tolerance or variation in background endemicity.

In this series of clinical trials, *P. vivax* was the most common cause of parasitological failure and was almost certainly the most frequently transmitted parasite after *P. falciparum* infection and mixed infection. The risk of *P. vivax* infection recurrence in the 9 weeks after initial falciparum malaria or mixed malaria is inversely correlated with antimalarial half-life. Slowly eliminated regimens should facilitate full clinical recovery and, if used on a large scale, may reduce transmission of both *P. falciparum* and *P. vivax*. Although additional work is required to establish the risk and deleterious effects of *P. vivax* infection recurrence in other regions, our study suggests that there is a coherent argument for the safe provision of a sterilizing course of antirelapse therapy (currently, 14 days of primaquine) for all patients with malaria in regions of co-endemicity.

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