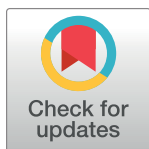


RESEARCH ARTICLE

The risk of *Plasmodium vivax* parasitaemia after *P. falciparum* malaria: An individual patient data meta-analysis from the WorldWide Antimalarial Resistance Network

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Resistance Network (WWARN.org). Requests for access will be reviewed by a Data Access Committee to ensure that use of data protects the interests of the participants and researchers according to the terms of ethics approval and principles of equitable data sharing. Requests can be submitted by email to malariaDAC@iddo.org via the Data Access Form available at WWARN.org/ [accessing-data](https://www.wwarn.org/submitting-data). The WWARN is registered with the Registry of Research Data Repositories (re3data.org).

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Abbreviations: AA, artesunate-amodiaquine; ACT, artemisinin-based combination therapy; AHR, adjusted hazard ratio; AL, artemether-lumefantrine; AM, artesunate-mefloquine; DP, dihydroartemisinin-piperazine; G6PD, glucose-6-phosphate dehydrogenase; Hb, haemoglobin; HR, hazard ratio; IQR, interquartile range; MAP, Malaria Atlas Project; PNG, Papua New Guinea;

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Abstract

Background

There is a high risk of *Plasmodium vivax* parasitaemia following treatment of falciparum malaria. Our study aimed to quantify this risk and the associated determinants using an individual patient data meta-analysis in order to identify populations in which a policy of universal radical cure, combining artemisinin-based combination therapy (ACT) with a hypnozoitocidal antimalarial drug, would be beneficial.

Methods and findings

A systematic review of Medline, Embase, Web of Science, and the Cochrane Database of Systematic Reviews identified efficacy studies of uncomplicated falciparum malaria treated with ACT that were undertaken in regions coendemic for *P. vivax* between 1 January 1960 and 5 January 2018. Data from eligible studies were pooled using standardised methodology. The risk of *P. vivax* parasitaemia at days 42 and 63 and associated risk factors were investigated by multivariable Cox regression analyses. Study quality was assessed using a tool developed by the Joanna Briggs Institute. The study was registered in the International Prospective Register of Systematic Reviews (PROSPERO: CRD42018097400). In total, 42 studies enrolling 15,341 patients were included in the analysis, including 30 randomised controlled trials and 12 cohort studies. Overall, 14,146 (92.2%) patients had *P. falciparum* mono-infection and 1,195 (7.8%) mixed infection with *P. falciparum* and *P. vivax*. The median age was 17.0 years (interquartile range [IQR] = 9.0–29.0 years; range = 0–80 years), with 1,584 (10.3%) patients younger than 5 years. 2,711 (17.7%) patients were treated with artemether-lumefantrine (AL, 13 studies), 651 (4.2%) with artesunate-amodiaquine (AA, 6 studies), 7,340 (47.8%) with artesunate-mefloquine (AM, 25 studies), and 4,639 (30.2%) with dihydroartemisinin-piperazine (DP, 16 studies). 14,537 patients (94.8%) were enrolled from the Asia-Pacific region, 684 (4.5%) from the Americas, and 120 (0.8%) from Africa. At day 42, the cumulative risk of vivax parasitaemia following treatment of *P. falciparum* was

PROSPERO, International Prospective Register of Systematic Reviews; WHO, World Health Organization; WWARN, WorldWide Antimalarial Resistance Network.

31.1% (95% CI 28.9–33.4) after AL, 14.1% (95% CI 10.8–18.3) after AA, 7.4% (95% CI 6.7–8.1) after AM, and 4.5% (95% CI 3.9–5.3) after DP. By day 63, the risks had risen to 39.9% (95% CI 36.6–43.3), 42.4% (95% CI 34.7–51.2), 22.8% (95% CI 21.2–24.4), and 12.8% (95% CI 11.4–14.5), respectively. In multivariable analyses, the highest rate of *P. vivax* parasitaemia over 42 days of follow-up was in patients residing in areas of short relapse periodicity (adjusted hazard ratio [AHR] = 6.2, 95% CI 2.0–19.5; $p = 0.002$); patients treated with AL (AHR = 6.2, 95% CI 4.6–8.5; $p < 0.001$), AA (AHR = 2.3, 95% CI 1.4–3.7; $p = 0.001$), or AM (AHR = 1.4, 95% CI 1.0–1.9; $p = 0.028$) compared with DP; and patients who did not clear their initial parasitaemia within 2 days (AHR = 1.8, 95% CI 1.4–2.3; $p < 0.001$). The analysis was limited by heterogeneity between study populations and lack of data from very low transmission settings. Study quality was high.

Conclusions

In this meta-analysis, we found a high risk of *P. vivax* parasitaemia after treatment of *P. falciparum* malaria that varied significantly between studies. These *P. vivax* infections are likely attributable to relapses that could be prevented with radical cure including a hypnozoitocidal agent; however, the benefits of such a novel strategy will vary considerably between geographical areas.

Author summary

Why was this study done?

- *Plasmodium vivax* is the most geographically widespread human malaria species; outside of sub-Saharan Africa, it almost invariably coexists with *P. falciparum*.
- A recent systematic review of 153 studies across 21 countries revealed that within 63 days of being treated for *P. falciparum* mono-infection, more than 15% of patients treated with an artemisinin-based combination therapy (ACT) had an episode of *P. vivax* malaria, far greater than expected for patients in similar locations who do not have acute malaria.
- We hypothesised that patients presenting with *P. falciparum* in coendemic locations are at high risk of carrying *P. vivax* dormant liver stages (hypnozoites) and would therefore potentially benefit from presumptive radical cure.

What did the researchers do and find?

- We undertook an individual patient data meta-analysis to define the risk of vivax parasitaemia (blood stream infection) after treatment of *P. falciparum* malaria in different coendemic environments and to explore the factors underlying these risks.
- In total, 42 studies enrolling 15,341 patients were included in the analysis. By day 63, the risk of *P. vivax* ranged from 12.8% following dihydroartemisinin-piperazine (DP) to 42.4% following artesunate-amodiaquine (AA).

- The highest rate of *P. vivax* malaria was in patients residing in areas with high risk of relapses and those who were slow to clear their initial parasitaemia.

What do these findings mean?

- There is a high risk of vivax malaria after treatment of falciparum infection after all ACTs, although the risk varies substantially between locations.
- The correlation between the risk of *P. vivax* and the initial clearance of *P. falciparum* raises the possibility that the host response to acute malaria may be triggering the reactivation of *P. vivax* dormant liver stages.
- Universal radical cure with treatment to kill the liver stages may be warranted for reducing *P. vivax*, but the benefits will vary considerably between geographical locations, and further prospective clinical efficacy studies will be needed to determine the risks and benefits of such a strategy.

Introduction

Malaria continues to exert a huge global health burden, with the latest estimates suggesting there are more than 228 million cases per year, associated with 405,000 deaths [1]. In 2015, the World Health Assembly set a target to reduce malaria prevalence by 90% by 2030 [2]. Although the burden of malaria has fallen in many regions, global estimates of malaria cases have plateaued and even risen in some areas over the past 5 years [1]. There has also been a relative rise in the proportion of malaria due to *Plasmodium vivax* outside of sub-Saharan Africa [3]. *P. vivax* is more difficult to eliminate than *P. falciparum* because it can form dormant liver stages (hypnozoites) that can reactivate weeks to months after the initial infection. Treatment of both the blood and liver stages is referred to as radical cure. If the proposed ambitious malaria elimination targets are to be achieved, innovative strategies are needed to provide safe and effective radical cure to the high proportion of individuals harbouring occult as well as circulating *P. vivax* parasites [4].

Where *P. vivax* and *P. falciparum* are coendemic (Asia, the Horn of Africa, and the Americas), reports have documented a high risk of *P. vivax* parasitaemia following treatment of *P. falciparum* infection [5, 6]. A recent meta-analysis of *P. falciparum* clinical trials revealed that within 63 days, 24% of patients presenting with *P. falciparum* had a recurrence with *P. vivax*, and almost 70% of all parasitological recurrences were due to *P. vivax* [7]. The high risk of *P. vivax* has been hypothesised to be due to reactivation of the dormant liver stages of parasites, although the mechanisms underlying this are not well understood. In areas where the risk of *P. vivax* parasitaemia is high, broadening the indication for hypnozoitocidal treatment to include patients presenting with uncomplicated malaria due to either *P. vivax* or *P. falciparum* (universal radical cure) has potential to reduce the subsequent risk of vivax parasitaemia and its ongoing transmission [8].

Radical cure with 8-aminoquinoline compounds can reduce the risk of *P. vivax* relapse significantly; however, both primaquine and tafenoquine can cause haemolysis in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency [9]. Thus, the risk versus benefit

of a policy of universal radical cure will depend upon quantifying the inherent risk of *P. vivax* recurrence and associated cofactors in different endemic settings, an approach that is more amenable to an individual patient data meta-analysis than our previous study-level systematic review [7].

The aim of this individual patient data meta-analysis was to define the risk of vivax parasitaemia after falciparum infection in different coendemic environments and to explore the factors underlying these risks in order to identify populations in which a policy of universal radical cure, with blood schizontocidal treatment plus either primaquine or tafenoquine, would be most beneficial.

Methods

Search strategy and selection criteria

A previous systematic review was used to identify suitable studies [7]. In brief, Medline, Embase, Web of Science, and the Cochrane Database of Systematic Reviews were searched for prospective studies published between 1 January 1960 and 5 January 2018 in any language that included supervised treatment of patients with uncomplicated *P. falciparum* infection (including mono-infection and mixed *P. falciparum* and *P. vivax* infection) located in areas coendemic for *P. falciparum* and *P. vivax* (S1 Box). Countries were considered coendemic if indigenous *P. falciparum* and *P. vivax* cases were reported or suspected in 2016 [10].

In the original systematic review, studies were included if they explicitly reported the presence or absence of recurrent parasitaemia. Studies were excluded if patients were followed less than 28 days, if patients were only followed passively, or if the full text manuscript was unavailable. Because primaquine and tafenoquine are contraindicated in pregnant women and not a clinical priority in patients presenting with severe malaria, studies only enrolling these patients were also excluded from the analysis. Identification of studies and extraction of data were undertaken by 2 independent authors, with discrepancies resolved by discussion with a third author.

To improve the generalisability of the results, the inclusion criteria for this individual patient data meta-analysis were restricted further to include only studies in which patients were treated with 1 of 4 widely used ACTs: artemether-lumefantrine (AL), artesunate-amodiaquine (AA), artesunate-mefloquine (AM), or dihydroartemisinin-piperaquine (DP). Furthermore, because it was not always apparent whether recurrent *P. vivax* was systematically documented during follow-up, studies were only included if at least 1 episode of *P. vivax* parasitaemia during follow-up was reported in the manuscript. Additional study-level exclusions were if studies only included travellers or soldiers or only patients with hyperparasitaemic infection, because these either reflected malaria acquired from diverse locations or a biased population of patients attending a clinic in a malaria-endemic setting. Investigators of eligible studies were invited to participate in this analysis and share their study's individual patient data with the WorldWide Antimalarial Resistance Network (WWARN) repository. Shared data were anonymised and standardised using the WWARN Data Management and Statistical Analysis Plans [11].

In addition to the systematic review, the pre-existing WWARN repository was searched for studies of uncomplicated *P. falciparum* mono-infection or mixed infection in which at least 1 patient was recorded as having *P. vivax* parasitaemia during study follow-up. This was undertaken to identify unpublished studies and published studies that did not report *P. vivax* parasitaemia during follow-up in the manuscript. Patient data from eligible studies were collated together with those from the systematic review for analysis.

Data collection and definitions

Demographic and clinical data including age, sex, weight, baseline species and parasite density, presence and species of parasites at follow-up, baseline gametocytaemia, baseline body temperature, and haemoglobin (Hb) or haematocrit at baseline and follow-up were included in the data set. Patient-level data were excluded if baseline parasitaemia was not available or did not include *P. falciparum* species, patients were treated with adjunctive antimalarials, or there was an enrolment violation according to supplied study data.

Haematocrit was converted to Hb using the formula $Hb = (Haematocrit - 5.62)/2.6$ [12]. The maximum value was used when multiple Hb measurements were recorded on a single day. The nadir Hb following acute *P. falciparum* malaria occurs between day 3 and 7, with most studies measuring the first follow-up Hb 7 days after starting antimalarial treatment [13]. The early decline and recovery of Hb was therefore assessed from the Hb measured between days 6 and 8 inclusive. Anaemia was defined as a Hb concentration less than 10 g/dL. A high parasite count was defined as a parasite count greater than 100,000 parasites per μL . Based on geographical location, studies were categorised into long or short *P. vivax* relapse periodicity using data from the Malaria Atlas Project (MAP) [14]. A median time to relapse of ≤ 47 days was categorised as short relapse periodicity. Subnational malaria parasite incidence was estimated from MAP models [15].

Data regarding the supervision of drug administration were extracted from publications or study protocols. Drug administration was categorised as ‘supervised’ if the entire treatment regimen was supervised, ‘partially supervised’ if only some treatment doses were supervised, and ‘not specified if data were not available. The year of enrolment was defined in the first instance from individual patient data or, if unavailable, from the median year during which patients were enrolled into the study or as 2 years prior to the year of publication. The study was registered in the International Prospective Register of Systematic Reviews (PROSPERO: CRD42018097400), and the protocol is available at www.wwarn.org/working-together/study-groups/vivax-after-falciparum-study-group (S1 PRISMA Checklist).

Outcomes

The primary outcome was the risk of *P. vivax* parasitaemia (*P. vivax* mono-infection or mixed infection) between days 7 and 42. Secondary outcomes were the risk of *P. vivax* parasitaemia between days 7 and 28 and days 7 and 63.

Statistical analysis

Statistical analyses were undertaken using Stata software, version 15.1 (Stata Corporation, College Station, TX, USA) and R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria), according to an a priori statistical analysis plan [16]. The cumulative risk of peripheral parasitaemia was calculated using Kaplan–Meier survival analyses. Patients were right-censored at their first recurrence, day of last follow-up, the day prior to a blood smear result gap > 18 days, or the day of the planned outcome assessment [11]. Following peer review, prediction intervals were calculated for the cumulative risk of *P. vivax* parasitaemia [17].

To estimate the association between ACTs with the rate of *P. vivax* parasitaemia, Cox’s proportional hazards regression was used with shared frailty for study sites and adjustment for age, sex, baseline parasitaemia, regional relapse periodicity, *P. falciparum* gametocytes, mixed infection at baseline, and Hb. The proportional hazards assumption was assessed visually using plots of observed versus expected values. Body weight and geographical location were not included because of collinearity with age and relapse periodicity, respectively. To investigate the association between the change in Hb and the rate of *P. vivax* parasitaemia, the

absolute fall in Hb between day 0 and day 7 was included in the multivariable Cox regression model.

The association between day of first parasite clearance (defined as parasite count below the microscopic limit of detection) and risk of *P. vivax* parasitaemia between days 7 and 63 was assessed by Cox's proportional hazards regression with shared frailty for study sites and adjustment for age, sex, baseline parasitaemia, regional relapse periodicity, *P. falciparum* gametocytes, mixed infection at baseline, Hb at baseline, and treatment. Patients treated with single low-dose primaquine were excluded. A subgroup analysis restricted data to patients treated with AL with a minimum of 42 days of follow-up to reduce the impact of slowly eliminated partner drugs with prolonged post-treatment prophylactic effect on outcome assessment. This subgroup was used to explore the association between the background incidence of *P. falciparum* and *P. vivax* and the risk of *P. vivax* parasitaemia. Following peer review, additional subgroup analyses were included for the other ACTs.

Bias related to individual studies was assessed using a tool developed by the Joanna Briggs Institute (S1 Text) [18] and in a sensitivity analysis, with removal of 1 study at a time and calculation of the coefficient of variation. Included and targeted studies that were not included were compared using baseline characteristics.

Ethics

All data included in this analysis were obtained in accordance with ethical approvals from the country of origin. The data are fully anonymised and cannot be traced back to identifiable individuals. This systematic review did not require separate ethical approval according to the guidelines of the Oxford Central University Research Ethics Committee.

Results

There were 153 clinical trials enrolling *P. falciparum* patients published between 1 January 1960 and 5 January 2018 and identified as part of the previous systematic review [7]. Of these, 51 (33.3%) studies enrolling 15,903 patients between 1991 and 2016 included treatment with the 4 major ACTs and were eligible for inclusion in this analysis. Individual patient data were available for 9,410 (59.2%) patients from 26 studies [19–44] (S1 Table and S2 Table). An additional 32 published and 2 unpublished studies with individual patient data in the WWARN repository were screened for inclusion, of which 16 studies were identified as also being eligible for the analysis [45–58]. In total, 42 studies enrolling 15,341 eligible patients were included in the analysis (Fig 1, S2 Table and S3 Table).

Of the studies included, 2,711 (17.7%) patients were treated with AL (13 studies), 651 (4.2%) with AA (6 studies), 7,340 (47.8%) with AM (25 studies), and 4,639 (30.2%) with DP (16 studies) (Fig 1 and S2 Table). In 2 studies, 448 patients were coadministered a single dose of primaquine. Patients were followed for 28 days in 3 studies (n = 670 patients), 42 days in 25 studies (n = 6,510 patients), 56 days in 1 study (n = 2,072 patients), 63 days in 11 studies (n = 5,653 patients), and a mixture of 28 and 63 days in 2 studies (436 patients).

The patients' baseline characteristics are presented in Table 1. Overall, 14,146 (92.2%) patients had *P. falciparum* mono-infection and 1,195 (7.8%) mixed infection with *P. falciparum* and *P. vivax* confirmed by blood film microscopy. The median age was 17.0 years (interquartile range [IQR] = 9.0–29.0 years; range = 0–80 years), with 1,584 (10.3%) patients younger than 5 years. Most patients (14,258; 92.9%) were recruited in areas with short relapse periodicity, and 82.5% (12,652) were enrolled in the Greater Mekong Subregion (S1 Fig). All 684 (4.5%) patients enrolled in the Americas were treated with AM or DP, whereas in Africa, all patients (120; 0.8%) were treated with AL. The 14,537 (94.8%) patients enrolled in Asia were

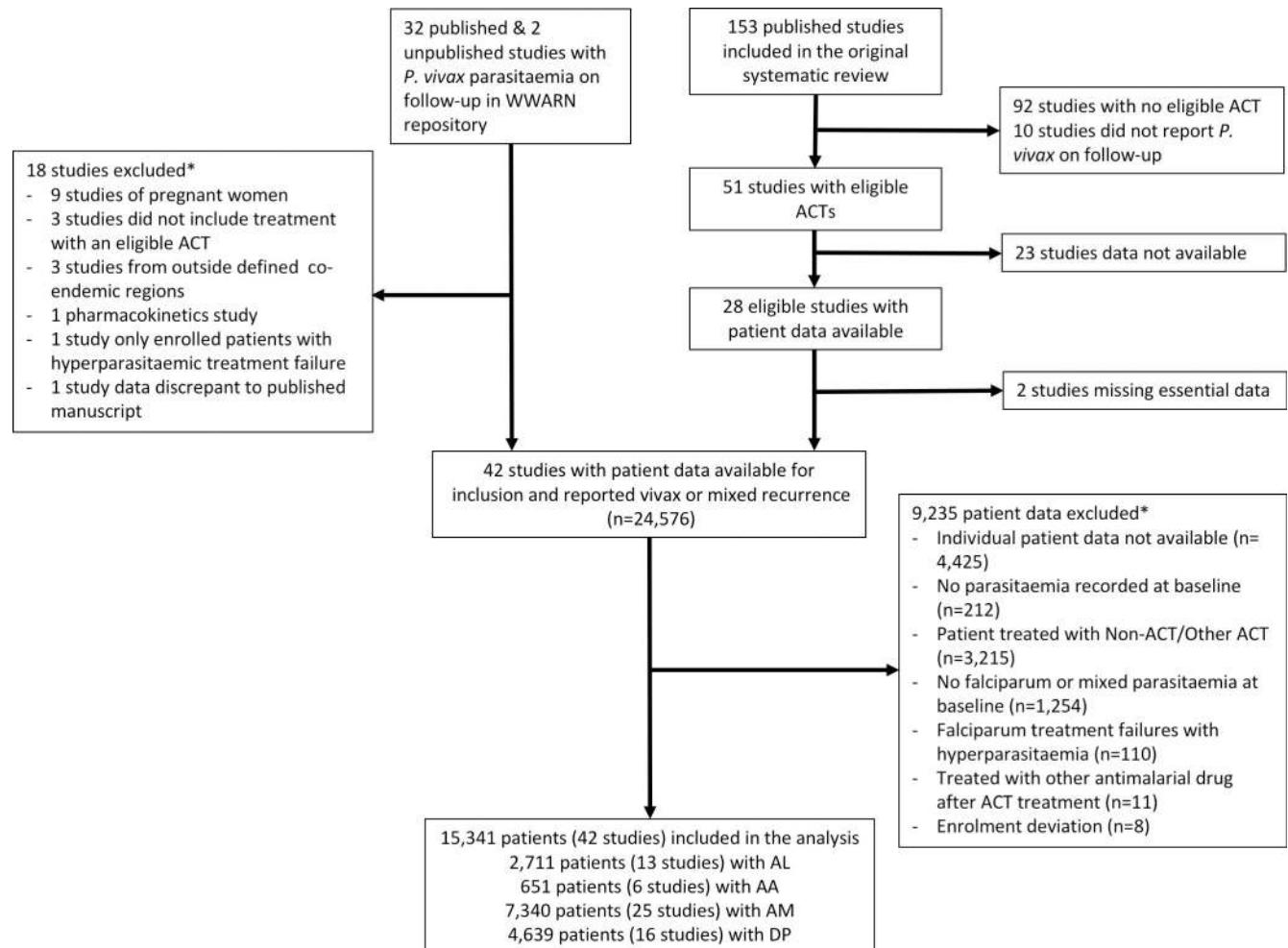


Fig 1. Study flowchart. AA, artesunate-amodiaquine; ACT, artemisinin-based combination therapy; AL, artemether-lumefantrine; AM, artesunate-mefloquine; DP, dihydroartemisinin-piperaquine; WWARN, WorldWide Antimalarial Resistance Network.

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treated with 1 of the 4 main ACTs (Table 1). The median parasite density at baseline was 7,544/ μ L (IQR = 2,000–28,021; range = 7–479,280). Baseline Hb data were available for 12,184 (79.4%) patients, of whom 27.6% (3,358) were anaemic (Hb <10 g/dL). No issues impacting data integrity were identified during collation of individual patient data. Studies that were targeted but for which data were not available were more likely to have been conducted in Africa; these studies had a similar median age and sex distribution to those included (S4 Table and S5 Table). Study quality was generally high (S1 Text).

The overall risk of *P. vivax* parasitaemia

Recurrent parasitaemia between day 7 and 42 was recorded in 2,020 (13.2%) patients, of whom 1,047 (51.8%) had *P. vivax* mono-infection, 99 (4.9%) mixed *P. vivax* and *P. falciparum* infection, and 874 (43.3%) *P. falciparum* mono-infection. The overall cumulative risks of recurrent parasitaemia of any species were 8.1% (95% CI 7.7–8.6) by day 28, 16.8% (16.2–17.5) by day 42, and 30.5% (29.4–31.6) by day 63. The corresponding cumulative risks of *P. vivax* parasitaemia (mono-infection or mixed infection) were 3.7% (3.4–4.1), 10.2% (9.6–10.7), and 22.2%

Table 1. Demographics and baseline characteristics.

	AL, n (%) (n = 2,711)	AA, n (%) (n = 651)	AM, n (%) (n = 7,340)	DP, n (%) (n = 4,639)	Overall, n (%) (n = 15,341)
Sex*					
Female	958 (35.4%)	252 (38.8%)	3,015 (41.1%)	1,391 (30.2%)	5,616 (36.7%)
Male	1,752 (64.6%)	398 (61.2%)	4,323 (58.9%)	3,214 (69.8%)	9,687 (63.3%)
Age, years†					
Median (IQR)	18.0 (9.0–28.0)	16.0 (8.0–28.0)	14.0 (8.0–25.0)	22.0 (12.0–32.0)	17.0 (9.0–29.0)
≥15	1,596 (58.9%)	352 (54.2%)	3,535 (48.2%)	3,275 (70.8%)	8,758 (57.1%)
5 to <15	753 (27.8%)	252 (38.8%)	2,994 (40.8%)	984 (21.3%)	4,983 (32.5%)
<5	361 (13.3%)	45 (6.9%)	810 (11.0%)	368 (8.0%)	1,584 (10.3%)
Weight, kg‡					
Median (IQR)	44.0 (21.0–52.0)	38.0 (20.5–49.4)	35.9 (18.3–49.0)	42.5 (19.0–53.0)	39.0 (19.0–50.0)
5 to <15	350 (13.3%)	67 (10.3%)	1,008 (14.6%)	443 (16.6%)	1,868 (14.5%)
15 to <25	418 (15.8%)	134 (20.7%)	1,575 (22.8%)	444 (16.7%)	2,571 (20.0%)
25 to <35	237 (9.0%)	80 (12.3%)	783 (11.3%)	210 (7.9%)	1,310 (10.2%)
35 to <45	361 (13.7%)	126 (19.4%)	1,004 (14.5%)	309 (11.6%)	1,800 (14.0%)
45 to <55	833 (31.6%)	155 (23.9%)	1,693 (24.5%)	677 (25.4%)	3,358 (26.1%)
55 to <80	438 (16.6%)	86 (13.3%)	837 (12.1%)	569 (21.4%)	1,930 (15.0%)
≥80	2 (0.1%)	0 (0.0%)	9 (0.1%)	12 (0.5%)	23 (0.2%)
Relapse periodicity					
Long	120 (4.4%)	202 (31.0%)	499 (6.8%)	262 (5.6%)	1,083 (7.1%)
Short	2,591 (95.6%)	449 (69.0%)	6,841 (93.2%)	4,377 (94.4%)	14,258 (92.9%)
Geographical region					
Asia-Pacific	2,591 (95.6%)	651 (100.0%)	6,918 (94.3%)	4,377 (94.4%)	14,537 (94.8%)
The Americas	0 (0.0%)	0 (0.0%)	422 (5.7%)	262 (5.6%)	684 (4.5%)
Africa	120 (4.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	120 (0.8%)
Treatment supervision					
Not specified	67 (2.5%)	202 (31.0%)	397 (5.4%)	0 (0.0%)	666 (4.3%)
Supervised	1,843 (68.0%)	294 (45.2%)	5,987 (81.6%)	2,182 (47.0%)	10,306 (67.2%)
Partial supervised	801 (29.5%)	155 (23.8%)	956 (13.0%)	2,457 (53.0%)	4,369 (28.5%)
<i>P. falciparum</i> gametocytes present§	238/1,959 (12.1%)	93/428 (21.7%)	697/5,765 (12.1%)	495/3,568 (13.9%)	1,523/11,720 (13.0%)
Mixed infection	183/2,711 (6.8%)	42/651 (6.5%)	712/7,340 (9.7%)	258/4,639 (5.6%)	1,195/15,341 (7.8%)
Baseline parasitaemia¶					
Median (IQR)	9,120 (1,900–39,600)	8,680 (2,364–24,507)	7,213 (1,976–25,622)	7,574 (2,048–25,120)	7,544 (2,000–28,022)
≤100,000 parasites per μL	2,507 (92.5%)	638 (98.0%)	6,610 (94.2%)	4,290 (94.5%)	14,045 (94.2%)
>100,000 parasites per μL	204 (7.5%)	13 (2.0%)	404 (5.8%)	250 (5.5%)	871 (5.8%)
Hb, g/dL; mean (SD)	10.4 (2.7)	10.7 (2.4)	10.8 (2.5)	10.9 (2.7)	10.8 (2.6)
Anaemic, Hb < 10 g/dL	721/2,586 (27.9%)	212/530 (40.0%)	1,655/6,481 (25.5%)	770/2,587 (29.8%)	3,358/12,184 (27.6%)
Fever, temperature >37.5°C**	1,331/2,547 (52.3%)	332/440 (75.5%)	3,713/6,565 (56.6%)	1,099/2,221 (49.5%)	6,475/11,773 (55.0%)

*Data not available for 38 patients.

†Data not available for 16 patients.

‡Data not available for 2,481 patients.

§Data not available for 3,623 patients.

¶Parasite count data not available for 427 patients.

||Data not available for 3,159 patients.

**Data not available for 3,570 patients.

Abbreviations: AA, artesunate-amodiaquine; AL, artemether-lumefantrine; AM, artesunate-mefloquine; DP, dihydroartemisinin-piperaquine; Hb, haemoglobin; IQR, interquartile range; n, number; SD, standard deviation.

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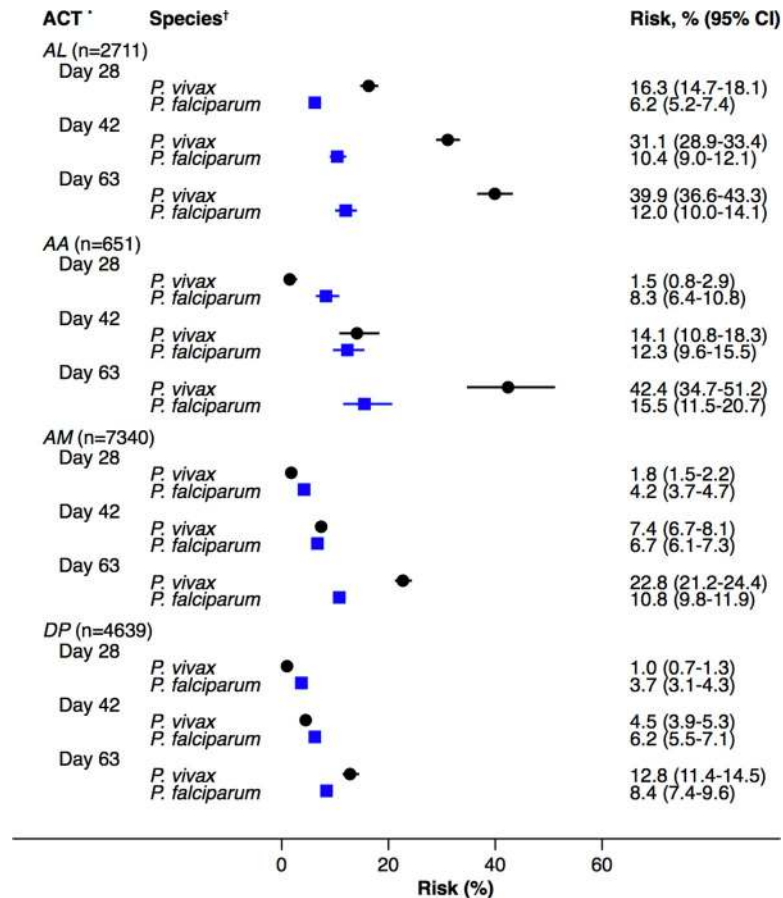


Fig 2. Cumulative risk (Kaplan–Meier analysis) of *P. vivax* parasitaemia following ACTs. * ACTs (AA, AL, AM, DP). † *P. vivax* recurrence includes recurrences with *P. vivax* monoinfection or mixed-species infection. AA, artesunate-amodiaquine; ACT, artemisinin-based combination therapy; AL, artemether-lumefantrine; AM, artesunate-mefloquine; DP, dihydroartemisinin-piperaquine.

<https://doi.org/10.1371/journal.pmed.1003393.g002>

(21.1–23.3), respectively, and for *P. falciparum* parasitaemia (monoinfection) were 4.5% (4.2–4.9), 7.3% (6.9–7.8), and 10.4% (9.8–11.2), respectively.

At day 42, the cumulative risk of *P. vivax* parasitaemia was 31.1% (95% CI 28.9–33.4; prediction interval) after AL, 14.1% (10.8–18.3) after AA, 7.4% (6.7–8.1) after AM, and 4.5% (3.9–5.3) after DP. By day 63, the risk of *P. vivax* parasitaemia had risen to 39.9% (36.6–43.3) after AL, 42.4% (34.7–51.2) after AA, 22.8% (21.2–24.4) after AM, and 12.8% (11.4–14.5) after DP (Fig 2). There was substantial heterogeneity in risk between studies (Fig 3, Fig 4, Fig 5, Fig 6 and S6 Table). Of the 39 studies in which patients were followed for at least 42 days, the risk of *P. vivax* parasitaemia was $\geq 20\%$ in 75.0% (9/12) of studies following AL, 20.0% (1/5) following AA, 21.7% (5/23) following AM, and 25.0% (4/16) following DP.

Risk factors for *P. vivax* parasitaemia

The rate of *P. vivax* parasitaemia was greatest in children. Compared with adults, children under 5 years had a hazard ratio (HR) = 4.40, 95% CI 3.66–5.29; $p < 0.001$, and children 5 to <15 years had an HR = 2.19, 1.90–2.53; $p < 0.001$. Other significant univariable baseline risk factors for *P. vivax* parasitaemia were high parasite count ($>100,000$ parasites per μL) (HR = 1.54, 1.24–1.90; $p < 0.001$), mixed *P. falciparum* and *P. vivax* infection (HR = 2.54,

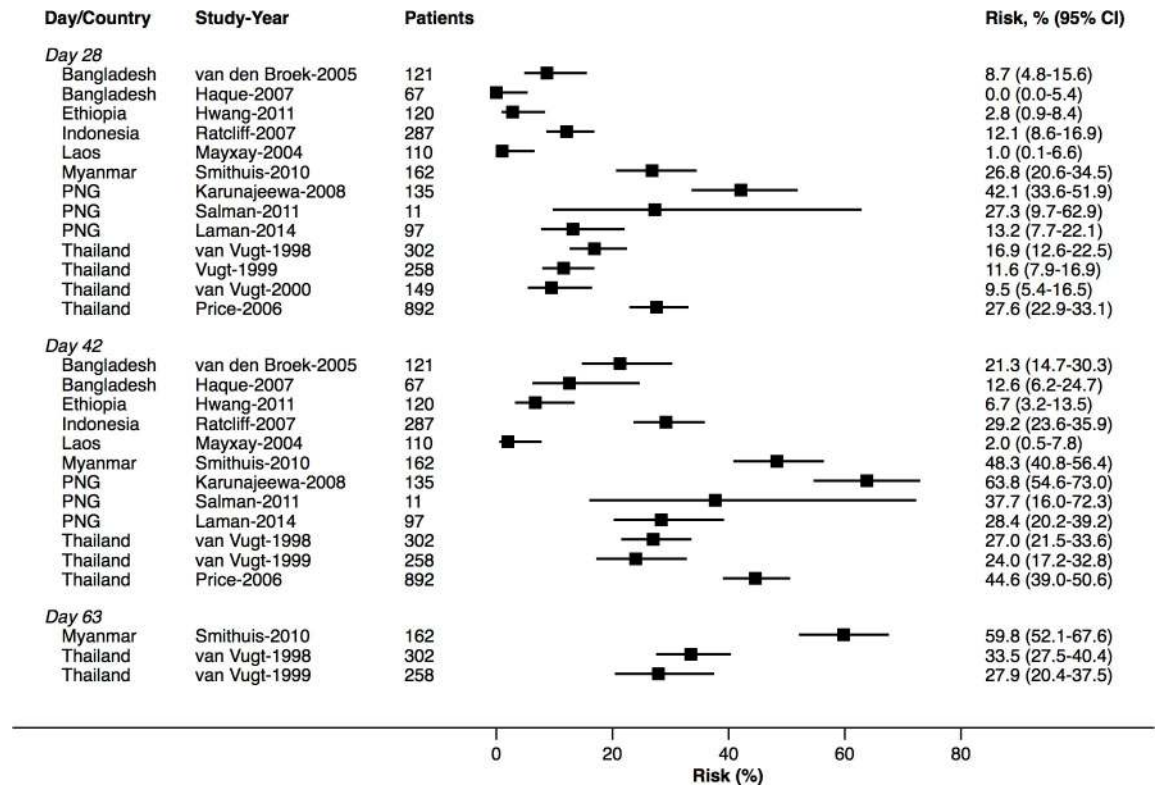


Fig 3. Cumulative risk (Kaplan–Meier analysis) of *P. vivax* parasitaemia after *P. falciparum* infection by study for AL. AL, artemether-lumefantrine.

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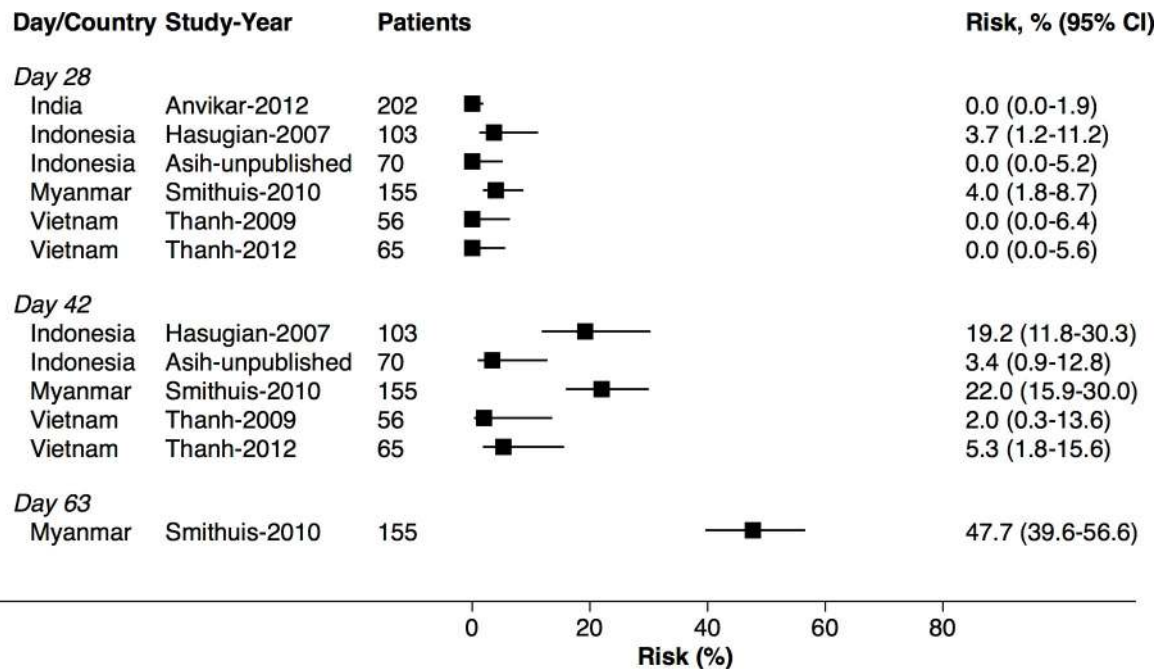


Fig 4. Cumulative risk (Kaplan–Meier analysis) of *P. vivax* parasitaemia after *P. falciparum* infection by study for AA. AA, artesunate-amodiaquine.

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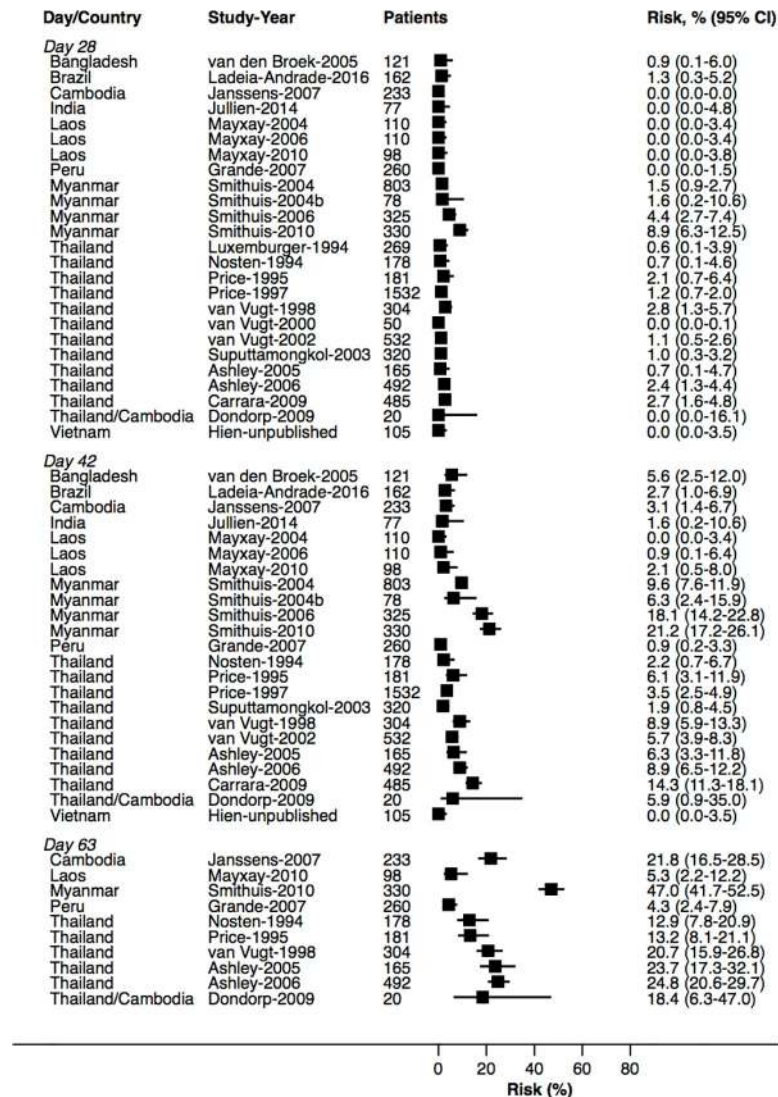


Fig 5. Cumulative risk (Kaplan–Meier analysis) of *P. vivax* parasitaemia after *P. falciparum* infection by study for AM. AM, artesunate-mefloquine.

<https://doi.org/10.1371/journal.pmed.1003393.g005>

2.14–3.01; $p < 0.001$), and *P. falciparum* gametocytaemia (HR = 1.57, 1.24–1.99; $p < 0.001$). Increasing baseline Hb was a protective factor (HR = 0.88 per 1 g/dL increase, 0.86–0.90; $p < 0.001$). Patients enrolled into studies conducted in regions of short relapse periodicity had a significantly greater rate of *P. vivax* parasitaemia than those enrolled into studies conducted in regions of long relapse periodicity (HR = 8.61, 2.34–31.65; $p = 0.001$) (Table 2).

In multivariable analysis, younger age (adjusted HR [AHR] = 3.04, 95% CI 2.39–3.87, $p < 0.001$ and AHR = 1.81, 95% CI 1.52–2.15, $p < 0.001$ comparing age <5 years and age 5 to <15 years to adults, respectively), short relapse periodicity (AHR = 6.20, 95% CI 1.98–19.47; $p = 0.002$), *P. falciparum* gametocytaemia (AHR = 1.40, 1.10–1.79; $p = 0.007$), mixed infection (AHR = 2.20, 1.79–2.70; $p < 0.001$), high parasite count (AHR = 1.59, 1.22–2.08; $p = 0.001$), male sex (AHR = 1.26, 95% CI 1.08–1.46; $p = 0.003$), and low baseline Hb (AHR = 0.94 per 1 g/dL increase, 0.90–0.97; $p < 0.001$) were independent risk factors for *P. vivax* parasitaemia (Table 2). Compared with patients treated with DP, the rate of *P. vivax* parasitaemia was

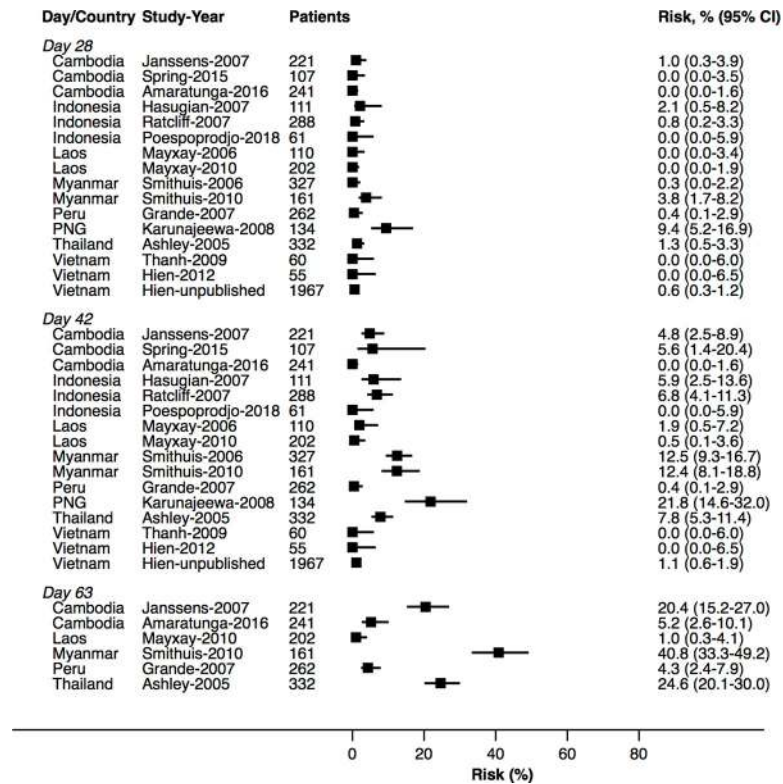


Fig 6. Cumulative risk (Kaplan–Meier analysis) of *P. vivax* parasitaemia after *P. falciparum* infection by study for DP. DP, dihydroartemisinin-piperazine.

<https://doi.org/10.1371/journal.pmed.1003393.g006>

greater after AL (AHR = 6.23, 4.55–8.53; $p < 0.001$), AA (AHR = 2.26, 1.40–3.65; $p = 0.001$), and AM (AHR = 1.40, 1.04–1.89; $p = 0.028$) (Fig 7). Removal of 1 study at a time did not reveal bias related to any individual study (S7 Table).

The mean Hb concentration at baseline was 11.8 g/dL (standard deviation [SD] 2.35) compared with 10.9 g/dL (SD 2.03) on day 7 in 6,328 patients with available data. The overall mean fall in Hb between baseline and day 7 was 0.87 g/dL (SD 1.71, range –8.8 to 11.6). The rate of *P. vivax* parasitaemia did not correlate with the absolute fall in univariable or multivariable analysis.

Association between risk of *P. vivax* parasitaemia and parasite clearance of initial *P. falciparum* infection

Overall, 46.3% (4,260/9,208) of patients were aparasitaemic by day 1 and 89.9% (8,277/9,208) by day 2; S8 Table. The risk of *P. vivax* parasitaemia between days 7 and 63 was 16.3% (95% CI: 14.7–18.1) in the patients clearing their parasitaemia on day 1, 22.6% (95% CI: 20.7–24.5) in patients clearing on day 2, and 29.2% (95% CI: 25.1–33.7) in patients clearing after this. The rate of *P. vivax* parasitaemia by day 63 was investigated in a multivariable model controlling for confounding factors. Compared with patients clearing their peripheral *P. falciparum* parasites by day 1, the rate of *P. vivax* was significantly greater in those clearing their initial parasitaemia on day 2 (AHR = 1.48, 95% CI 1.27–1.73; $p < 0.001$) and even greater in those clearing their parasitaemia thereafter (AHR = 1.82, 1.44–2.30; $p < 0.001$) (Fig 8 and S9 Table). Removal of 1 study at a time did not reveal bias related to any individual study (S10 Table).

Table 2. Risk factors for *P. vivax* parasitaemia after falciparum infection between days 7 and 42 in patients with different ACTs.

	Total Patients	Patients with <i>P. vivax</i> Parasitaemia	Risk of <i>P. vivax</i> Parasitaemia at Day 42	Univariable Analyses		Multivariable Analyses*	
				Crude HR (95% CI)	p-Value	Adjusted HR (95% CI)	p-Value
Sex							
Male	9,687	714	10.32 (9.62–11.06)	1.10 (0.97–1.24)	0.125	1.26 (1.08–1.46)	0.003
Female	5,616	426	9.94 (9.08–10.88)	Reference		Reference	
Age, per year increase	15,325	1,140		0.95 (0.95–0.96)	<0.001	–	
Age category, years							
<5	1,584	313	24.95 (22.63–27.45)	4.40 (3.66–5.29)	<0.001	3.04 (2.39–3.87)	<0.001
5 to <15	4,983	462	11.90 (10.92–12.96)	2.19 (1.90–2.53)	<0.001	1.81 (1.52–2.15)	<0.001
≥15	8,758	365	6.01 (5.44–6.64)	Reference		Reference	
Weight per kg increase	12,860	1,116		0.97 (0.96–0.97)	<0.001	–	
Relapse periodicity							
Short	14,258	1,125	10.85 (10.27–11.47)	8.61 (2.34–31.65)	0.001	6.20 (1.98–19.47)	0.002
Long	1,083	15	1.73 (1.05–2.87)	Reference		Reference	
Geographical region							
Africa	120	7	6.65 (3.23–13.46)	0.27 (0.03–2.65)	0.261	–	
The Americas	684	7	1.11 (0.53–2.31)	0.08 (0.01–0.74)	0.026	–	
Asia-Pacific	14,537	1,126	10.74 (10.16–11.35)	Reference		–	
<i>P. falciparum</i> gametocytes							
Yes	1,523	222	17.84 (15.81–20.09)	1.57 (1.24–1.99)	<0.001	1.40 (1.10–1.79)	0.007
No	10,197	575	8.12 (7.50–8.79)	Reference		Reference	
Mixed infection							
Yes	1,195	184	21.82 (19.15–24.80)	2.54 (2.14–3.01)	<0.001	2.20 (1.79–2.70)	<0.001
No	14,146	956	9.21 (8.67–9.79)	Reference		Reference	
Parasitaemia, parasites per μ L							
Every 10-times increase	14,916	1,134		1.31 (1.20–1.41)	<0.001	–	
>100,000 parasites/ μ L	871	112	17.68 (14.90–20.91)	1.54 (1.24–1.90)	<0.001	1.59 (1.22–2.08)	0.001
≤100,000 parasites/ μ L	14,045	1,022	9.93 (9.36–10.52)	Reference		Reference	
Hb every 1 g/dL increase	12,184	1,090		0.88 (0.86–0.90)	<0.001	0.94 (0.90–0.97)	<0.001
Anaemic, Hb < 10 g/dL							
Yes	3,358	482	18.55 (17.09–20.10)	1.70 (1.49–1.94)	<0.001	–	
No	8,826	608	8.94 (8.29–9.65)	Reference		–	
Drug							
AL	2,711	522	31.08 (28.89–33.40)	5.18 (4.10–6.56)	<0.001	6.23 (4.55–8.53)	<0.001
AA	651	47	14.07 (10.77–18.27)	1.75 (1.19–2.55)	0.004	2.26 (1.40–3.65)	0.001
AM	7,340	423	7.36 (6.71–8.06)	1.37 (1.09–1.72)	0.006	1.40 (1.04–1.89)	0.028
DP	4,639	148	4.50 (3.85–5.27)	Reference		Reference	

*Geographical region and weight excluded from multivariable analysis due to collinearity with relapse periodicity and age.

Abbreviations: AA, artesunate-amodiaquine; ACT, artemisinin-based combination therapy; AL, artemether-lumefantrine; AM, artesunate-mefloquine; CI, confidence interval; DP, dihydroartemisinin-piperaquine; Hb, haemoglobin; HR, hazard ratio.

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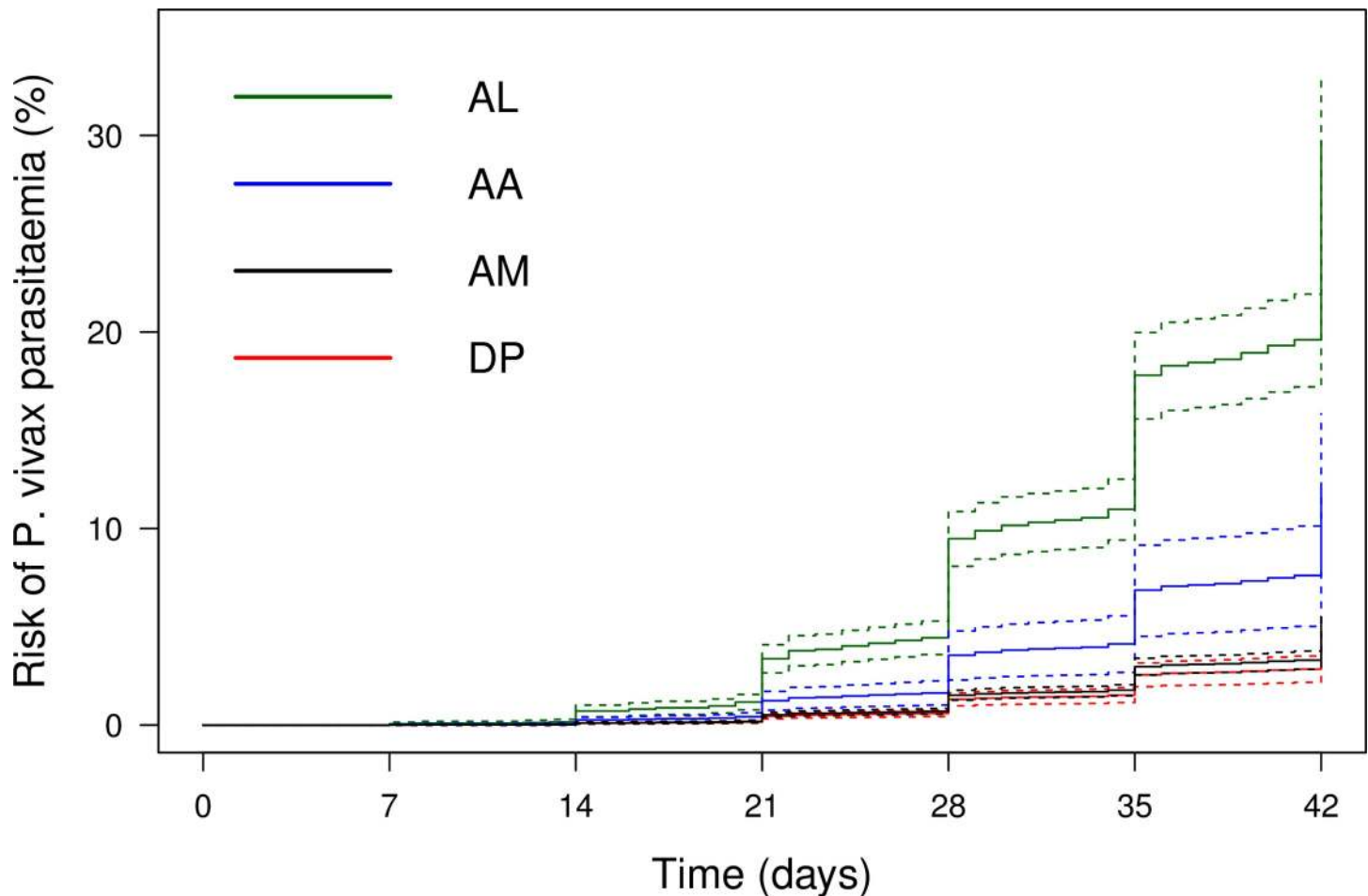


Fig 7. Risk of *P. vivax* parasitaemia following falciparum infection between day 7 and day 42 according to treatment. Dotted lines demonstrate 95% CIs. Figure adjusted for age, sex, baseline parasitaemia, regional relapse periodicity, *P. falciparum* gametocytes, mixed infection, and baseline Hb, assuming no study-site effect. AA, artesunate-amodiaquine; AL, artemether-lumefantrine; AM, artesunate-mefloquine; DP, dihydroartemisinin-piperazine; Hb, haemoglobin.

<https://doi.org/10.1371/journal.pmed.1003393.g007>

A sensitivity analysis including patients treated with single low-dose primaquine did not change these results substantially (compared with patients who cleared parasitaemia by day 1, AHR = 1.48 [95% CI 1.27–1.73] if cleared on day 2 and AHR = 1.82 [95% CI 1.44–2.29] if cleared thereafter).

Site factors associated with risk of *P. vivax* parasitaemia following treatment

The study-specific risks of *P. vivax* during follow-up are presented in Fig 3, Fig 4, Fig 5 and Fig 6. The risk of *P. vivax* parasitaemia at day 42 was greatest following treatment with AL. Twelve studies across 18 study sites and 7 countries enrolled and treated 2,562 patients with AL who were followed for 42 days or longer (S2 Fig). Nine (75%) studies enrolled children younger than 5 years. The study-specific risk of *P. vivax* at day 42 varied from 0% to 63.8%. The risk was $\geq 20\%$ in study sites from Thailand (4 study sites), Papua New Guinea (PNG) (4 study sites), Myanmar (3 study sites), and Indonesia (1 study site); 12%–26% in 3 study sites from Bangladesh; 0%–7% in 2 study sites from Ethiopia; and 2.0% in 1 study from Laos (Fig 3). The corresponding data for the other treatment arms are presented in Fig 4, Fig 5, Fig 6 and S11 Table.

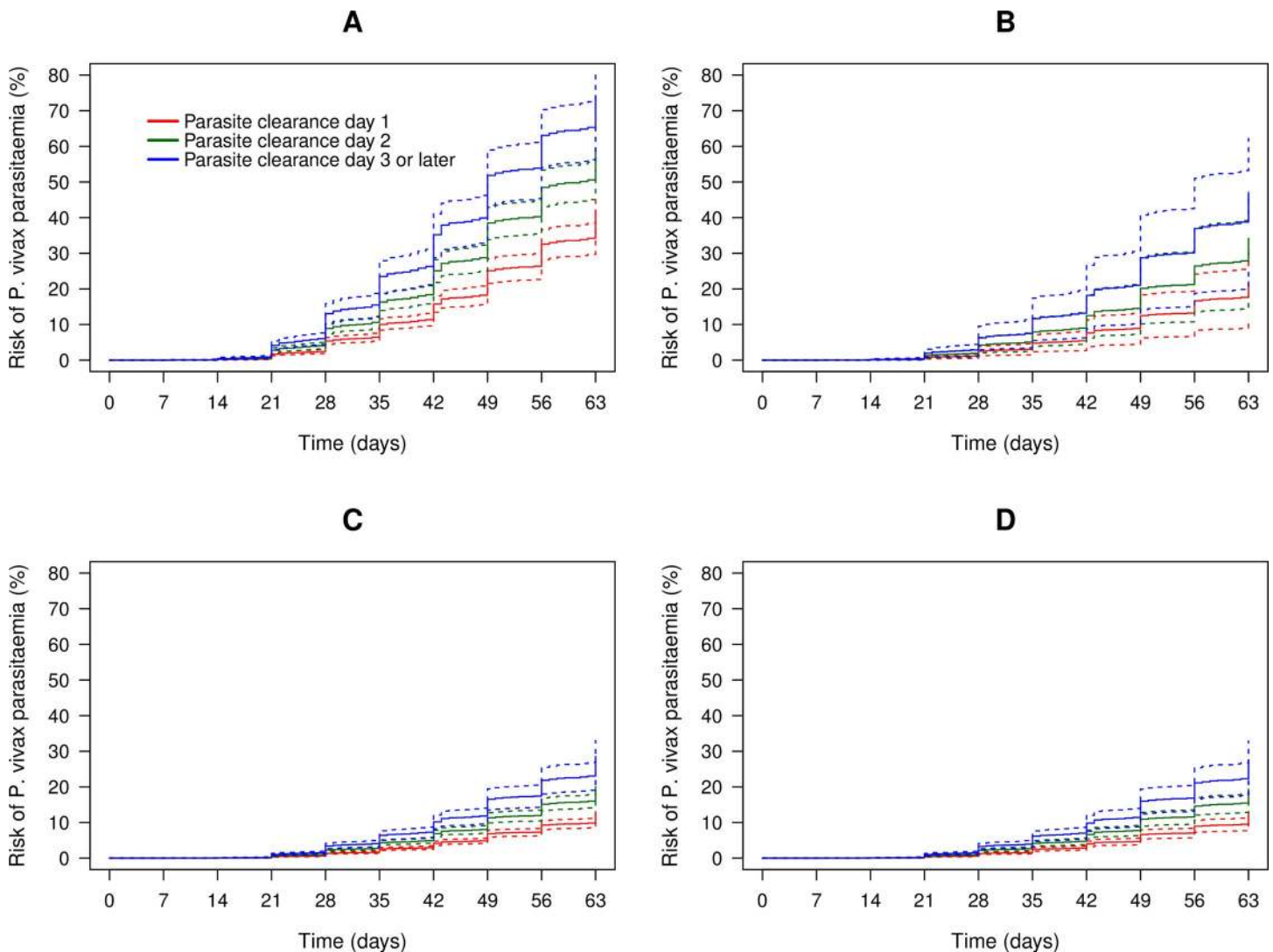


Fig 8. Risk of *P. vivax* parasitaemia following falciparum infection between days 7 and 63 according to treatment and day of parasite clearance: (A) AL, (B) AA, (C) AM, and (D) DP. Figure adjusted for age, sex, baseline parasitaemia, regional relapse periodicity, *P. falciparum* gametocytes, mixed infection at baseline, and Hb at baseline, assuming no study-site effect. AA, artesunate-amodiaquine; AL, artemether-lumefantrine; AM, artesunate-mefloquine; DP, dihydroartemisinin-piperazine; Hb, haemoglobin.

<https://doi.org/10.1371/journal.pmed.1003393.g008>

Using subnational data, the estimated incidence of *P. falciparum* at each site varied from 0.5 to 154 cases per 1,000 person-years, and the background incidence of *P. vivax* varied from 1.6 to 151 cases per 1,000 person-years. The risk of *P. vivax* following AL was correlated with the site-specific background incidence of *P. vivax* ($r_s = 0.676$, $p = 0.0029$) and incidence of *P. falciparum* ($r_s = 0.607$, $p = 0.0098$) (Fig 9). However, after controlling for confounding factors in a Cox regression model, neither of the background incidences were associated significantly with the risk of *P. vivax* (AHR = 0.99, 0.97–1.02, $p = 0.634$ and AHR = 1.00, 0.99–1.02, $p = 0.629$, respectively, for every 1 case increase per 1,000 person-years) (S12 Table). The results were similar for the other ACTs (S13 Table, S14 Table, S15 Table, S3 Fig, S4 Fig, S5 Fig). The year of enrolment was not independently associated with the risk of *P. vivax* (AHR = 0.93 per year later, 0.84–1.03; $p = 0.173$).

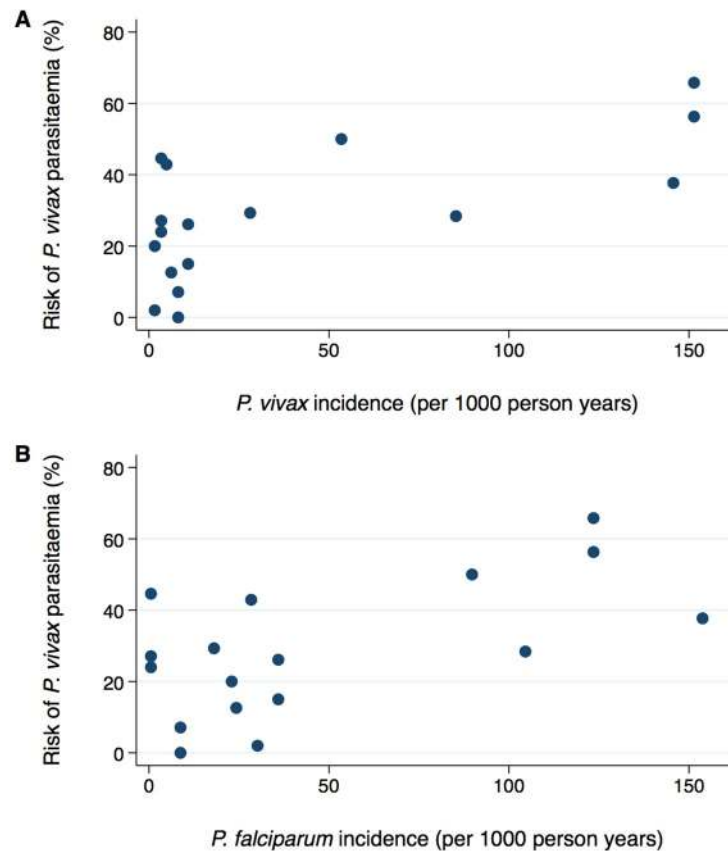


Fig 9. Risk of *P. vivax* parasitaemia at day 42 following treatment with AL according to background subnational incidence of *P. vivax* (A) and *P. falciparum* (B). $r_s = 0.676$, $p = 0.0029$ (A) and $r_s = 0.607$, $p = 0.0098$ (B). AL, artemether-lumefantrine.

<https://doi.org/10.1371/journal.pmed.1003393.g009>

Discussion

Our individual patient data meta-analysis, including more than 15,000 patients, confirms a high risk of *P. vivax* parasitaemia following treatment of *P. falciparum* with ACTs; however, there was marked heterogeneity between study populations and sites. The risk of *P. vivax* was highest in studies undertaken in regions with short relapse periodicity and in patients who were young or presented with mixed-species infections or a high baseline parasitaemia.

Overall, 10% of patients treated with an ACT had *P. vivax* recurrence by day 42, and this increased to 22% by day 63. The risk of *P. vivax* was significantly lower in patients treated with an ACT containing a slowly eliminated partner drug such as piperaquine, mefloquine, and amodiaquine [5, 7]. Lumefantrine is eliminated faster, with a half-life of 4 days, and after 16 days (4 half-lives) provides minimal post-treatment prophylaxis against *P. vivax* relapses [59]. This is reflected by the high risk of *P. vivax* parasitaemia following AL, which reached 31% by day 42.

The heterogeneity in the risk of *P. vivax* was highlighted in our recent systematic review of clinical trials undertaken in coendemic areas [7] and has been investigated further in a review of trials from the eastern border of Myanmar [60]. On the Thailand–Myanmar border, the risk of *P. vivax* parasitaemia within 42 days of treatment of falciparum malaria exceeded 20% between 2003 and 2010 and reached 85% in 1 study. However, after 2010, the risk fell to less

than 5%. The authors hypothesise that the decline in the risk of *P. vivax* was attributable to substantial improvements in malaria control and thus a decline in the proportion of patients presenting with *P. falciparum* who were coinfecting with *P. vivax*. A key determinant of *P. vivax* relapse is the number of hypnozoite parasites present in the liver, which varies with local *P. vivax* transmission intensity, immunity, and sporozoite inoculum [61]. Although the risk of *P. vivax* was correlated positively with the predicted background incidence of both *P. vivax* and *P. falciparum*, this was not apparent after adjusting for confounding factors. We observed no temporal trends indicative of a lower risk of *P. vivax* parasitaemia in more recent studies. This may relate to the relatively few studies available to compare the use of an individual ACT at a single site, lack of temporally distinct data from multiple studies in the same regions, or heterogeneity between study populations from different locations. Incidence estimates are also inexact, relying on subnational regional data that may not equate to the study site and do not account for seasonal variation of transmission [15]. There is also a risk of inclusion bias, with prospective clinical efficacy studies generally undertaken in regions with a relatively high malaria burden to ensure adequate recruitment. The ability to compare the effect of malaria prevalence between clinical efficacy studies is therefore likely to be limited compared with data from longitudinal cohorts [62].

Our individual patient data meta-analysis confirms an increased risk of recurrence in regions where the duration between *P. vivax* relapses is short [5, 7] and highlights the additional risks of young age, high baseline parasitaemia, low baseline Hb, and presenting with either mixed-species infection or *P. falciparum* gametocytes. More than 15% of patients presenting with mixed infection of *P. falciparum* and *P. vivax* had *P. vivax* parasitaemia within 42 days, supporting the World Health Organization (WHO) recommendation for the use of primaquine radical cure in these patients. However, the high risk of *P. vivax* was also apparent in those presenting with *P. falciparum* mono-infection. We could not determine whether recurrences were due to relapse or reinfection in patients continuing to reside in a malaria-endemic area. The subnational incidence estimates of *P. vivax* infection in the study sites included in our study ranged from 0.2 to 151 per 1,000 person-years [15]. If one conservatively assumes 2 weeks post-treatment prophylaxis following antimalarial treatment and the highest risk of reinfection of the included study sites, then the maximum risk of a new infection with *P. vivax* within 63 days of *P. falciparum* would be 2.0%. The risks of *P. vivax* parasitaemia in our analysis were thus at least 6- to 21-fold-greater than could be explained by reinfection alone.

It is possible that patients with mixed infections of *P. falciparum* with low-level *P. vivax* parasitaemia were misdiagnosed as *P. falciparum* mono-infections. However, all of the ACTs included in the analysis have high efficacy against *P. vivax* [63], and even if 10% of patients had had submicroscopic *P. vivax* parasitaemia at presentation, the risk of recrudescence *P. vivax* by day 63 would be no greater than 0.5%. Conversely, we may have underestimated the risk of vivax recurrence because submicroscopic relapses were not quantified and the duration of follow-up was restricted to 63 days, thus preventing detection of later relapses. In a longitudinal cohort in which asymptomatic submicroscopic infections were quantified over a 12-month period, there was a 2.4-fold-higher risk of asymptomatic vivax recurrence following asymptomatic falciparum infections [64].

Our findings are more consistent with the *P. vivax* parasitaemia during follow-up arising from activation of *P. vivax* hypnozoites present at the time of the initial presentation with *P. falciparum* [5, 65, 66]. Importantly, the initial therapeutic response, as measured by parasite clearance, was correlated with the subsequent risk of *P. vivax*. Patients taking longer to clear their *P. falciparum* parasitaemia were at almost 2-fold-greater risk of *P. vivax* than those who cleared their parasitaemia rapidly. Whilst this could reflect vulnerability of a host with low immunity to both slower parasite clearance and recurrent parasitaemia, the increased risk of *P.*

vivax was independent of the patients' age, a surrogate marker of host immunity. We believe this supports the hypothesis that *vivax* parasitaemia following *falciparum* malaria relates to a host–parasite interaction that activates hypnozoites acquired from a prior infection. Haemolysis has been postulated to trigger *P. vivax* reactivation [66, 67], although we found no correlation between the early fall in Hb concentration and the risk of subsequent *vivax* parasitaemia.

Our study has a number of limitations. The systematic review included all *P. falciparum* clinical trials between 1960 and 2018, although the analysis was restricted to patients who were treated with an ACT with studies conducted between 1991 and 2018. The analysis included 42 studies, enrolling 15,341 patients in 12 malaria-endemic countries, and the quality of the included studies suggested a low risk of systematic bias (S1 Text); however, there were significant differences in these data and those included in our previous systematic review [7]. Fourteen studies uploaded to the WWARN repository with *P. vivax* recurrences were not included as part of the 153 studies in the previous review. Ten of these were not included in the initial systematic review because the corresponding manuscripts reported neither the presence nor absence of *vivax* recurrences. An additional limitation of our current analysis was the inability to include data from 25 targeted studies. Although the patient demographics from these studies were similar to the included studies, they included a greater number of African studies, which might have allowed better generalisability of results to this region. Furthermore, the studies that were included were not equally representative of all *vivax*-endemic locations, with the majority undertaken in southeast Asia and only a single study included from India. Studies in the WWARN repository were only included into the analysis if they reported a *P. vivax* recurrence in at least 1 treatment arm. This was justified because in many *P. falciparum* studies, *P. vivax* parasitaemia may not be deemed relevant to the efficacy analysis, and data may not be recorded or shared with WWARN. Hence, it is possible that exclusion of these may have systematically biased against studies without any *P. vivax* recurrence and thus overestimated the risk of *P. vivax*. Reassuringly, only 3 out of 33 clinical trial arms in an independent systematic review reported no *P. vivax* recurrences [60].

The rapid elimination of *P. vivax* will require a greater emphasis on addressing the hidden reservoirs of the parasite, including individuals with asymptomatic carriage of blood- or liver-stage parasites. Our analysis highlights that in coendemic areas, patients presenting with *P. falciparum* may be at significant risk of carrying dormant hypnozoites. Hence, whilst current WHO guidelines only recommend treatment with a prolonged course of primaquine for patients with *P. vivax* or a *P. vivax*–mixed-species infection [68], consideration should be given to offering radical cure, with either primaquine or tafenoquine, with appropriate G6PD testing, to patients presenting with *P. falciparum* monoinfection in coendemic regions. In view of the heterogeneity in the risk of *P. vivax* and drug-induced haemolysis between locations, such a strategy should be recommended with caution. The risk factors for *P. vivax* identified in this analysis may help define populations for whom the benefit of a universal policy of radical cure has greatest benefit but should be guided by complementary prospective clinical efficacy studies, and in coendemic areas, these should include quantifying the subsequent risk of all species of *Plasmodia* following antimalarial treatment.

Supporting information

S1 PRISMA Checklist. PRISMA-IPD. PRISMA-IPD, Preferred Reporting Items for Systematic Review and Meta-Analyses of individual participant data (PDF)

S1 Box. Search strategy. (PDF)

S1 Text. Assessment of risk of bias relating to individual studies.

(PDF)

S1 Fig. Map of study-site locations. Map created using ggplot2 in R.

(TIF)

S2 Fig. Map of cumulative risk of *P. vivax* parasitaemia at day 42 following AL. Map created using ggplot2 in R. AL, artemether-lumefantrine

(TIF)

S3 Fig. Risk of *P. vivax* parasitaemia at day 42 following treatment with AA according to subnational (A) *P. vivax* incidence and (B) *P. falciparum* incidence. AA, artesunate-amodiaquine

(PDF)

S4 Fig. Risk of *P. vivax* parasitaemia at day 42 following treatment with AM according to subnational (A) *P. vivax* incidence and (B) *P. falciparum* incidence. AM, artesunate-mefloquine

(PDF)

S5 Fig. Risk of *P. vivax* parasitaemia at day 42 following treatment with DP according to subnational (A) *P. vivax* incidence and (B) *P. falciparum* incidence. DP, dihydroartemisinin-piperaquine

(PDF)

S1 Table. Reasons for studies not being included in analysis.

(PDF)

S2 Table. Studies included in analysis.

(PDF)

S3 Table. Study sites included in analysis.

(PDF)

S4 Table. Studies targeted for the analysis but not available.

(PDF)

S5 Table. Comparison of baseline characteristics from included and targeted studies.

(PDF)

S6 Table. Prediction intervals for cumulative risk of *P. vivax* parasitaemia following ACTs. ACT, artemisinin-based combination therapy

(PDF)

S7 Table. Sensitivity analysis for associations between patient characteristics and rate of *P. vivax* parasitaemia between days 7 to 42 for the general model.

(PDF)

S8 Table. Parasite clearance according to treatment.

(PDF)

S9 Table. Relationship between day of parasite clearance, patient characteristics, and rate of *P. vivax* parasitaemia between days 7 and 63.

(PDF)

S10 Table. Sensitivity analysis for associations between patient characteristics and rate of *P. vivax* parasitaemia between days 7 to 63 for the model including day of parasite clearance.

(PDF)

S11 Table. Studies with follow-up for 42 days or longer included in analysis of site factors.

(PDF)

S12 Table. Relationship between patient characteristics and study-site malaria prevalence and rate of *P. vivax* parasitaemia between days 7 and 42 in patients treated with AL. AL, artemether-lumefantrine

(PDF)

S13 Table. Relationship between patient characteristics and study-site malaria prevalence and rate of *P. vivax* parasitaemia between days 7 and 42 in patients treated with AA. AA, artesunate-amodiaquine.

(PDF)

S14 Table. Relationship between patient characteristics and study-site malaria prevalence and rate of *P. vivax* parasitaemia between days 7 and 42 in patients treated with AM. AM, artesunate-mefloquine.

(PDF)

S15 Table. Relationship between patient characteristics and study-site malaria prevalence and rate of *P. vivax* parasitaemia between days 7 and 42 in patients treated with DP. DP, dihydroartemisinin-piperaquine.

(PDF)

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References

1. World Health Organization. World Malaria Report 2019. Geneva, Switzerland: World Health Organization; 2019 [cited 2020 Aug 26]. Available from: <https://www.who.int/publications/i/item/world-malaria-report-2019>
2. World Health Organization. Global technical strategy for malaria 2016–2030. Geneva, Switzerland: World Health Organization; 2015 [cited 2020 Aug 26]. Available from: <https://www.who.int/malaria/publications/atoz/9789241564991/en/>
3. Price RN, Commons RJ, Battle KE, Thriemer K, Mendis K. *Plasmodium vivax* in the Era of the Shrinking *P. falciparum* Map. Trends Parasitol. 2020; 36(6): 560–70. <https://doi.org/10.1016/j.pt.2020.03.009> PMID: 32407682
4. White MT, Walker P, Karl S, Hetzel MW, Freeman T, Waltmann A, et al. Mathematical modelling of the impact of expanding levels of malaria control interventions on *Plasmodium vivax*. Nat Commun. 2018; 9(1): 3300. <https://doi.org/10.1038/s41467-018-05860-8> PMID: 30120250; PubMed Central PMCID: PMC6097992
5. Douglas NM, Nosten F, Ashley EA, Phaiphun L, van Vugt M, Singhasivanon P, et al. *Plasmodium vivax* recurrence following falciparum and mixed species malaria: risk factors and effect of antimalarial kinetics. Clin Infect Dis. 2011; 52(5): 612–20. <https://doi.org/10.1093/cid/ciq249> PMID: 21292666; PubMed Central PMCID: PMC3060895

6. Vitor-Silva S, Siqueira AM, de Souza Sampaio V, Guinovart C, Reyes-Lecca RC, de Melo GC, et al. Declining malaria transmission in rural Amazon: changing epidemiology and challenges to achieve elimination. *Malar J*. 2016; 15(1): 266. <https://doi.org/10.1186/s12936-016-1326-2> PMID: 27165432; PubMed Central PMCID: PMC4863332
7. Commons RJ, Simpson JA, Thriemer K, Hossain MS, Douglas NM, Humphreys GS, et al. Risk of *Plasmodium vivax* parasitaemia after *Plasmodium falciparum* infection: a systematic review and meta-analysis. *Lancet Infect Dis*. 2019; 19(1): 91–101. Epub 2018 Dec 28. [https://doi.org/10.1016/S1473-3099\(18\)30596-6](https://doi.org/10.1016/S1473-3099(18)30596-6) PMID: 30587297
8. Lacerda MVG, Bassat Q. Primaquine for all: is it time to simplify malaria treatment in co-endemic areas? *Lancet Infect Dis*. 2019; 19(1): 10–12. [https://doi.org/10.1016/S1473-3099\(18\)30612-1](https://doi.org/10.1016/S1473-3099(18)30612-1) PMID: 30587279
9. Ashley EA, Reicht J, White NJ. Primaquine: the risks and the benefits. *Malar J*. 2014; 13: 418. <https://doi.org/10.1186/1475-2875-13-418> PMID: 25363455; PubMed Central PMCID: PMC4230503
10. World Health Organization. World Malaria Report 2017. Geneva, Switzerland: World Health Organization; 2017 [cited 2020 Aug 26]. Available from: <https://www.who.int/malaria/publications/world-malaria-report-2017/report/en/>
11. WorldWide Antimalarial Resistance Network. Data management and statistical analysis plan v1.2 2012 [Internet]. 2012 [cited 2018 Nov 13]. Available from: <http://www.wwarn.org/tools-resources/clinical-data-management-and-analysis-plan>
12. Lee SJ, Stepniewska K, Anstey N, Ashley E, Barnes K, Binh TQ, et al. The relationship between the haemoglobin concentration and the haematocrit in *Plasmodium falciparum* malaria. *Malar J*. 2008; 7: 149. <https://doi.org/10.1186/1475-2875-7-149> PMID: 18673575; PubMed Central PMCID: PMC2515851
13. Price RN, Simpson JA, Nosten F, Luxemburger C, Hkirjaroen L, ter Kuile F, et al. Factors contributing to anemia after uncomplicated falciparum malaria. *Am J Trop Med Hyg*. 2001; 65(5): 614–22. <https://doi.org/10.4269/ajtmh.2001.65.614> PMID: 11716124; PubMed Central PMCID: PMC4337986
14. Battle KE, Karhunen MS, Bhatt S, Gething PW, Howes RE, Golding N, et al. Geographical variation in *Plasmodium vivax* relapse. *Malar J*. 2014; 13: 144. <https://doi.org/10.1186/1475-2875-13-144> PMID: 24731298; PubMed Central PMCID: PMC4021508
15. Battle KE, Lucas TCD, Nguyen M, Howes RE, Nandi AK, Twohig KA, et al. Mapping the global endemicity and clinical burden of *Plasmodium vivax*, 2000–17: a spatial and temporal modelling study. *Lancet*. 2019; 394(10195):332–343. [https://doi.org/10.1016/S0140-6736\(19\)31096-7](https://doi.org/10.1016/S0140-6736(19)31096-7) PMID: 31229233
16. WorldWide Antimalarial Resistance Network. Statistical Analysis Plan: WWARN Vivax after Falciparum Study Group v0.1 Oxford: WorldWide Antimalarial Resistance Network [Internet]. 2018 [cited 2019 Jul 14]. Available from: <https://www.wwarn.org/working-together/study-groups/vivax-after-falciparum-study-group>
17. Riley RD, Higgins JP, Deeks JJ. Interpretation of random effects meta-analyses. *BMJ*. 2011; 342: d549. <https://doi.org/10.1136/bmj.d549> PMID: 21310794
18. Munn Z, Moola S, Lisy K, Riitano D, Tufanaru C. Methodological guidance for systematic reviews of observational epidemiological studies reporting prevalence and cumulative incidence data. *Int J Evid Based Healthc*. 2015; 13(3): 147–53. <https://doi.org/10.1097/XEB.000000000000054> PMID: 26317388
19. van Vugt M, Brockman A, Gemperli B, Luxemburger C, Gathmann I, Royce C, et al. Randomized comparison of artemether-benflumetol and artesunate-mefloquine in treatment of multidrug-resistant falciparum malaria. *Antimicrob Agents Chemother*. 1998; 42(1): 135–9. <https://doi.org/10.1128/AAC.42.1.135> PMID: 9449273; PubMed Central PMCID: PMC105468
20. van Vugt M, Leonardi E, Phaipun L, Slight T, Thway KL, McGready R, et al. Treatment of uncomplicated multidrug-resistant falciparum malaria with artesunate-atovaquone-proguanil. *Clin Infect Dis*. 2002; 35(12): 1498–504. <https://doi.org/10.1086/344901> PMID: 12471569
21. van Vugt M, Wilairatana P, Gemperli B, Gathmann I, Phaipun L, Brockman A, et al. Efficacy of six doses of artemether-lumefantrine (benflumetol) in multidrug-resistant *Plasmodium falciparum* malaria. *Am J Trop Med Hyg*. 1999; 60(6): 936–42. <https://doi.org/10.4269/ajtmh.1999.60.936> PMID: 10403324
22. Suputtamongkol Y, Chindarat S, Silpasakorn S, Chaikachonpatd S, Lim K, Chanthapakajee K, et al. The efficacy of combined mefloquine-artesunate versus mefloquine-primaquine on subsequent development of *Plasmodium falciparum* gametocytemia. *Am J Trop Med Hyg*. 2003; 68(5): 620–3. <https://doi.org/10.4269/ajtmh.2003.68.620> PMID: 12812357
23. Mayxay M, Khanthavong M, Lindegardh N, Keola S, Barends M, Pongvongsa T, et al. Randomized comparison of chloroquine plus sulfadoxine-pyrimethamine versus artesunate plus mefloquine versus artemether-lumefantrine in the treatment of uncomplicated falciparum malaria in the Lao People's

- Democratic Republic. Clin Infect Dis. 2004; 39(8): 1139–47. <https://doi.org/10.1086/424512> PMID: [15486837](https://pubmed.ncbi.nlm.nih.gov/15486837/)
24. Smithuis F, van der Broek I, Katterman N, Kyaw MK, Brockman A, Lwin S, et al. Optimising operational use of artesunate-mefloquine: a randomised comparison of four treatment regimens. Trans R Soc Trop Med Hyg. 2004; 98(3): 182–92. [https://doi.org/10.1016/s0035-9203\(03\)00035-x](https://doi.org/10.1016/s0035-9203(03)00035-x) PMID: [15024929](https://pubmed.ncbi.nlm.nih.gov/15024929/)
 25. Ashley EA, McGready R, Hutagalung R, Phaiphun L, Slight T, Proux S, et al. A randomized, controlled study of a simple, once-daily regimen of dihydroartemisinin-piperaquine for the treatment of uncomplicated, multidrug-resistant falciparum malaria. Clin Infect Dis. 2005; 41(4): 425–32. <https://doi.org/10.1086/432011> PMID: [16028147](https://pubmed.ncbi.nlm.nih.gov/16028147/)
 26. Mayxay M, Thongpraseuth V, Khanthavong M, Lindegardh N, Barends M, Keola S, et al. An open, randomized comparison of artesunate plus mefloquine vs. dihydroartemisinin-piperaquine for the treatment of uncomplicated *Plasmodium falciparum* malaria in the Lao People's Democratic Republic (Laos). Trop Med Int Health. 2006; 11(8): 1157–65. <https://doi.org/10.1111/j.1365-3156.2006.01671.x> PMID: [16903879](https://pubmed.ncbi.nlm.nih.gov/16903879/)
 27. Grande T, Bernasconi A, Erhart A, Gamboa D, Casapia M, Delgado C, et al. A randomised controlled trial to assess the efficacy of dihydroartemisinin-piperaquine for the treatment of uncomplicated falciparum malaria in Peru. PLoS ONE. 2007; 2(10): e1101. <https://doi.org/10.1371/journal.pone.0001101> PMID: [17971864](https://pubmed.ncbi.nlm.nih.gov/17971864/); PubMed Central PMCID: PMC2040506
 28. Janssens B, van Herp M, Goubert L, Chan S, Uong S, Nong S, et al. A randomized open study to assess the efficacy and tolerability of dihydroartemisinin-piperaquine for the treatment of uncomplicated falciparum malaria in Cambodia. Trop Med Int Health. 2007; 12(2): 251–9. <https://doi.org/10.1111/j.1365-3156.2006.01786.x> PMID: [17300633](https://pubmed.ncbi.nlm.nih.gov/17300633/)
 29. Dondorp AM, Nosten F, Yi P, Das D, Phyto AP, Tarning J, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med. 2009; 361(5): 455–67. <https://doi.org/10.1056/NEJMoa0808859> PMID: [19641202](https://pubmed.ncbi.nlm.nih.gov/19641202/); PubMed Central PMCID: PMC3495232
 30. Karunajeewa HA, Mueller I, Senn M, Lin E, Law I, Gomorra PS, et al. A trial of combination antimalarial therapies in children from Papua New Guinea. N Engl J Med. 2008; 359(24): 2545–57. <https://doi.org/10.1056/NEJMoa0804915> PMID: [19064624](https://pubmed.ncbi.nlm.nih.gov/19064624/)
 31. Hwang J, Alemayehu BH, Hoos D, Melaku Z, Tekleyohannes SG, Teshi T, et al. In vivo efficacy of artemether-lumefantrine against uncomplicated *Plasmodium falciparum* malaria in Central Ethiopia. Malar J. 2011; 10: 209. <https://doi.org/10.1186/1475-2875-10-209> PMID: [21798054](https://pubmed.ncbi.nlm.nih.gov/21798054/); PubMed Central PMCID: PMC3163628
 32. Smithuis F, Kyaw MK, Phe O, Win T, Aung PP, Oo AP, et al. Effectiveness of five artemisinin combination regimens with or without primaquine in uncomplicated falciparum malaria: an open-label randomised trial. Lancet Infect Dis. 2010; 10(10): 673–81. [https://doi.org/10.1016/S1473-3099\(10\)70187-0](https://doi.org/10.1016/S1473-3099(10)70187-0) PMID: [20832366](https://pubmed.ncbi.nlm.nih.gov/20832366/); PubMed Central PMCID: PMC2947715
 33. Hien TT, Thuy-Nhien NT, Phu NH, Boni MF, Thanh NV, Nha-Ca NT, et al. In vivo susceptibility of *Plasmodium falciparum* to artesunate in Binh Phuoc Province, Vietnam. Malar J. 2012; 11: 355. <https://doi.org/10.1186/1475-2875-11-355> PMID: [23101492](https://pubmed.ncbi.nlm.nih.gov/23101492/); PubMed Central PMCID: PMC3504531
 34. Laman M, Moore BR, Benjamin JM, Yadi G, Bona C, Warrel J, et al. Artemisinin-naphthoquine versus artemether-lumefantrine for uncomplicated malaria in Papua New Guinean children: an open-label randomized trial. PLoS Med. 2014; 11(12): e1001773. <https://doi.org/10.1371/journal.pmed.1001773> PMID: [25549086](https://pubmed.ncbi.nlm.nih.gov/25549086/); PubMed Central PMCID: PMC4280121
 35. Poespoprodjo JR, Kenangalem E, Wafom J, Chandrawati F, Puspitasari AM, Ley B, et al. Therapeutic Response to Dihydroartemisinin-Piperaquine for *P. falciparum* and *P. vivax* Nine Years after Its Introduction in Southern Papua, Indonesia. Am J Trop Med Hyg. 2018; 98(3):677–82. <https://doi.org/10.4269/ajtmh.17-0662> PMID: [29345221](https://pubmed.ncbi.nlm.nih.gov/29345221/); PubMed Central PMCID: PMC5850981
 36. Smithuis F, Shahmanesh M, Kyaw MK, Savran O, Lwin S, White NJ. Comparison of chloroquine, sulfadoxine/pyrimethamine, mefloquine and mefloquine-artesunate for the treatment of falciparum malaria in Kachin State, North Myanmar. Trop Med Int Health. 2004; 9(11):1184–90. <https://doi.org/10.1111/j.1365-3156.2004.01323.x> PMID: [15548314](https://pubmed.ncbi.nlm.nih.gov/15548314/)
 37. Smithuis F, Kyaw MK, Phe O, Aye KZ, Htet L, Barends M, et al. Efficacy and effectiveness of dihydroartemisinin-piperaquine versus artesunate-mefloquine in falciparum malaria: an open-label randomised comparison. Lancet. 2006; 367(9528): 2075–85. [https://doi.org/10.1016/S0140-6736\(06\)68931-9](https://doi.org/10.1016/S0140-6736(06)68931-9) PMID: [16798391](https://pubmed.ncbi.nlm.nih.gov/16798391/)
 38. Haque R, Thriemer K, Wang Z, Sato K, Wagatsuma Y, Salam MA, et al. Therapeutic efficacy of artemether-lumefantrine for the treatment of uncomplicated *Plasmodium falciparum* malaria in Bangladesh. Am J Trop Med Hyg. 2007; 76(1): 39–41. PMID: [17255226](https://pubmed.ncbi.nlm.nih.gov/17255226/)
 39. Mayxay M, Keomany S, Khanthavong M, Souvannasing P, Stepniewska K, Khomthilath T, et al. A phase III, randomized, non-inferiority trial to assess the efficacy and safety of dihydroartemisinin-

- piperazine in comparison with artesunate-mefloquine in patients with uncomplicated *Plasmodium falciparum* malaria in southern Laos. *Am J Trop Med Hyg.* 2010; 83(6): 1221–9. <https://doi.org/10.4269/ajtmh.2010.10-0276> PMID: 21118925; PubMed Central PMCID: PMC2990035
40. Thanh NX, Trung TN, Phong NC, Quang HH, Dai B, Shanks GD, et al. The efficacy and tolerability of artemisinin-piperazine (Artequick(R)) versus artesunate-amodiaquine (Coarsucam) for the treatment of uncomplicated *Plasmodium falciparum* malaria in south-central Vietnam. *Malar J.* 2012; 11: 217. <https://doi.org/10.1186/1475-2875-11-217> PMID: 22741618; PubMed Central PMCID: PMC3411481
 41. Spring MD, Lin JT, Manning JE, Vanachayangkul P, Somethy S, Bun R, et al. Dihydroartemisinin-piperazine failure associated with a triple mutant including kelch13 C580Y in Cambodia: an observational cohort study. *Lancet Infect Dis.* 2015; 15(6): 683–91. [https://doi.org/10.1016/S1473-3099\(15\)70049-6](https://doi.org/10.1016/S1473-3099(15)70049-6) PMID: 25877962
 42. Amaratunga C, Lim P, Suon S, Sreng S, Mao S, Sopha C, et al. Dihydroartemisinin-piperazine resistance in *Plasmodium falciparum* malaria in Cambodia: a multisite prospective cohort study. *Lancet Infect Dis.* 2016; 16(3): 357–65. [https://doi.org/10.1016/S1473-3099\(15\)00487-9](https://doi.org/10.1016/S1473-3099(15)00487-9) PMID: 26774243; PubMed Central PMCID: PMC4792715
 43. Ladeia-Andrade S, de Melo GN, de Souza-Lima Rde C, Salla LC, Bastos MS, Rodrigues PT, et al. No Clinical or Molecular Evidence of *Plasmodium falciparum* Resistance to Artesunate-Mefloquine in Northwestern Brazil. *Am J Trop Med Hyg.* 2016; 95(1):148–54. <https://doi.org/10.4269/ajtmh.16-0017> PMID: 27068396; PubMed Central PMCID: PMC4944679
 44. van den Broek IV, Maung UA, Peters A, Liem L, Kamal M, Rahman M, et al. Efficacy of chloroquine + sulfadoxine—pyrimethamine, mefloquine + artesunate and artemether + lumefantrine combination therapies to treat *Plasmodium falciparum* malaria in the Chittagong Hill Tracts, Bangladesh. *Trans R Soc Trop Med Hyg.* 2005; 99(10): 727–35. <https://doi.org/10.1016/j.trstmh.2005.02.007> PMID: 16095643
 45. Nosten F, Luxemburger C, ter Kuile FO, Woodrow C, Eh JP, Chongsuphajaisiddhi T, et al. Treatment of multidrug-resistant *Plasmodium falciparum* malaria with 3-day artesunate-mefloquine combination. *J Infect Dis.* 1994; 170(4): 971–7. <https://doi.org/10.1093/infdis/170.4.971> PMID: 7930743
 46. Luxemburger C, ter Kuile FO, Nosten F, Dolan G, Bradol JH, Phaipun L, et al. Single day mefloquine-artesunate combination in the treatment of multi-drug resistant falciparum malaria. *Trans R Soc Trop Med Hyg.* 1994; 88(2): 213–7. [https://doi.org/10.1016/0035-9203\(94\)90303-4](https://doi.org/10.1016/0035-9203(94)90303-4) PMID: 8036679
 47. Price RN, Nosten F, Luxemburger C, Kham A, Brockman A, Chongsuphajaisiddhi T, et al. Artesunate versus artemether in combination with mefloquine for the treatment of multidrug-resistant falciparum malaria. *Trans R Soc Trop Med Hyg.* 1995; 89(5): 523–7. [https://doi.org/10.1016/0035-9203\(95\)90094-2](https://doi.org/10.1016/0035-9203(95)90094-2) PMID: 8560531
 48. Price RN, Nosten F, Luxemburger C, van Vugt M, Phaipun L, Chongsuphajaisiddhi T, et al. Artesunate/mefloquine treatment of multi-drug resistant falciparum malaria. *Trans R Soc Trop Med Hyg.* 1997; 91(5):574–7. [https://doi.org/10.1016/s0035-9203\(97\)90032-8](https://doi.org/10.1016/s0035-9203(97)90032-8) PMID: 9463672
 49. van Vugt M, Looareesuwan S, Wilairatana P, McGready R, Villegas L, Gathmann I, et al. Artemether-lumefantrine for the treatment of multidrug-resistant falciparum malaria. *Trans R Soc Trop Med Hyg.* 2000; 94(5): 545–8. [https://doi.org/10.1016/s0035-9203\(00\)90082-8](https://doi.org/10.1016/s0035-9203(00)90082-8) PMID: 11132386
 50. Price RN, Uhlemann AC, van Vugt M, Brockman A, Hutagalung R, Nair S, et al. Molecular and pharmacological determinants of the therapeutic response to artemether-lumefantrine in multidrug-resistant *Plasmodium falciparum* malaria. *Clin Infect Dis.* 2006; 42(11): 1570–7. <https://doi.org/10.1086/503423> PMID: 16652314; PubMed Central PMCID: PMC4337983
 51. Ashley EA, Lwin KM, McGready R, Simon WH, Phaipun L, Proux S, et al. An open label randomized comparison of mefloquine-artesunate as separate tablets vs. a new co-Trop Med Int Health. 2006; 11(11): 1653–60. <https://doi.org/10.1111/j.1365-3156.2006.01724.x> PMID: 17054744
 52. Ratcliff A, Siswantoro H, Kenangalem E, Maristela R, Wuwung RM, Laihad F, et al. Two fixed-dose artemisinin combinations for drug-resistant falciparum and vivax malaria in Papua, Indonesia: an open-label randomised comparison. *Lancet.* 2007; 369(9563): 757–65. [https://doi.org/10.1016/S0140-6736\(07\)60160-3](https://doi.org/10.1016/S0140-6736(07)60160-3) PMID: 17336652; PubMed Central PMCID: PMC2532500
 53. Hasugian AR, Purba HL, Kenangalem E, Wuwung RM, Ebsworth EP, Maristela R, et al. Dihydroartemisinin-piperazine versus artesunate-amodiaquine: superior efficacy and posttreatment prophylaxis against multidrug-resistant *Plasmodium falciparum* and *Plasmodium vivax* malaria. *Clin Infect Dis.* 2007; 44(8): 1067–74. <https://doi.org/10.1086/512677> PMID: 17366451; PubMed Central PMCID: PMC2532501
 54. Thanh NX, Trung TN, Phong NC, Thien NX, Dai B, Shanks GD, et al. Open label randomized comparison of dihydroartemisinin-piperazine and artesunate-amodiaquine for the treatment of uncomplicated *Plasmodium falciparum* malaria in central Vietnam. *Trop Med Int Health.* 2009; 14(5): 504–11. <https://doi.org/10.1111/j.1365-3156.2009.02269.x> PMID: 19320869

55. Carrara VI, Zwang J, Ashley EA, Price RN, Stepniewska K, Barends M, et al. Changes in the treatment responses to artesunate-mefloquine on the northwestern border of Thailand during 13 years of continuous deployment. *PLoS ONE*. 2009; 4(2): e4551. <https://doi.org/10.1371/journal.pone.0004551> PMID: [19234601](https://pubmed.ncbi.nlm.nih.gov/19234601/); PubMed Central PMCID: PMC2641001
56. Salman S, Page-Sharp M, Griffin S, Kose K, Siba PM, Ilett KF, et al. Population pharmacokinetics of artemether, lumefantrine, and their respective metabolites in Papua New Guinean children with uncomplicated malaria. *Antimicrob Agents Chemother*. 2011; 55(11): 5306–13. <https://doi.org/10.1128/AAC.05136-11> PMID: [21876056](https://pubmed.ncbi.nlm.nih.gov/21876056/); PubMed Central PMCID: PMC3194999
57. Anvikar AR, Sharma B, Shahi BH, Tyagi PK, Bose TK, Sharma SK, et al. Artesunate-amodiaquine fixed dose combination for the treatment of *Plasmodium falciparum* malaria in India. *Malar J*. 2012; 11: 97. <https://doi.org/10.1186/1475-2875-11-97> PMID: [22458860](https://pubmed.ncbi.nlm.nih.gov/22458860/); PubMed Central PMCID: PMC3351019
58. Jullien V, Valecha N, Srivastava B, Sharma B, Kiechel JR. Population pharmacokinetics of mefloquine, administered as a fixed-dose combination of artesunate-mefloquine in Indian patients for the treatment of acute uncomplicated *Plasmodium falciparum* malaria. *Malar J*. 2014; 13: 187. <https://doi.org/10.1186/1475-2875-13-187> PMID: [24886117](https://pubmed.ncbi.nlm.nih.gov/24886117/); PubMed Central PMCID: PMC4046089
59. Ezzet F, van Vugt M, Nosten F, Looareesuwan S, White NJ. Pharmacokinetics and pharmacodynamics of lumefantrine (benflumetol) in acute falciparum malaria. *Antimicrob Agents Chemother*. 2000; 44(3): 697–704. <https://doi.org/10.1128/aac.44.3.697-704.2000> PMID: [10681341](https://pubmed.ncbi.nlm.nih.gov/10681341/); PubMed Central PMCID: PMC89749
60. Ashley EA, Phyo AP, Carrara VI, Tun KM, Nosten F, Smithuis F, et al. *Plasmodium vivax* relapse rates following falciparum malaria reflect previous transmission intensity. *J Infect Dis*. 2019; 220(1): 100–104. <https://doi.org/10.1093/infdis/jiz052> PMID: [30698794](https://pubmed.ncbi.nlm.nih.gov/30698794/).
61. White NJ. Determinants of relapse periodicity in *Plasmodium vivax* malaria. *Malar J*. 2011; 10: 297. <https://doi.org/10.1186/1475-2875-10-297> PMID: [21989376](https://pubmed.ncbi.nlm.nih.gov/21989376/); PubMed Central PMCID: PMC3228849
62. Phommasone K, van Leth F, Imwong M, Henriques G, Pongvongsa T, Adhikari B, et al. The use of ultra-sensitive quantitative-PCR to assess the impact of primaquine on asymptomatic relapse of *Plasmodium vivax* infections: a randomized, controlled trial in Lao PDR. *Malar J*. 2020; 19(1): 4. <https://doi.org/10.1186/s12936-019-3091-5> PMID: [31900172](https://pubmed.ncbi.nlm.nih.gov/31900172/); PubMed Central PMCID: PMC6942400
63. Commons RJ, Simpson JA, Thriemer K, Abreha T, Adam I, Anstey NM, et al. The efficacy of dihydroartemisinin-piperazine and artemether-lumefantrine with and without primaquine on *Plasmodium vivax* recurrence: A systematic review and individual patient data meta-analysis. *PLoS Med*. 2019; 16(10): e1002928. <https://doi.org/10.1371/journal.pmed.1002928> PMID: [31584960](https://pubmed.ncbi.nlm.nih.gov/31584960/); PubMed Central PMCID: PMC6777759
64. von Seidlein L, Peerawaranun P, Mukaka M, Nosten FH, Nguyen TN, Hien TT, et al. The probability of a sequential *Plasmodium vivax* infection following asymptomatic *Plasmodium falciparum* and *P. vivax* infections in Myanmar, Vietnam, Cambodia, and Laos. *Malar J*. 2019; 18(1): 449. <https://doi.org/10.1186/s12936-019-3087-1> PMID: [31888643](https://pubmed.ncbi.nlm.nih.gov/31888643/); PubMed Central PMCID: PMC6937799
65. Mayxay M, Pukrittayakamee S, Newton PN, White NJ. Mixed-species malaria infections in humans. *Trends Parasitol*. 2004; 20(5): 233–40. <https://doi.org/10.1016/j.pt.2004.03.006> PMID: [15105024](https://pubmed.ncbi.nlm.nih.gov/15105024/)
66. Shanks GD, White NJ. The activation of vivax malaria hypnozoites by infectious diseases. *Lancet Infect Dis*. 2013; 13(10): 900–6. [https://doi.org/10.1016/S1473-3099\(13\)70095-1](https://doi.org/10.1016/S1473-3099(13)70095-1) PMID: [23809889](https://pubmed.ncbi.nlm.nih.gov/23809889/)
67. Shanks GD. Hemolysis as a signal to initiate *Plasmodium vivax* relapse (Oral Presentation) Manaus, Brazil: 6th International Conference on Plasmodium vivax Research; 2017.
68. World Health Organisation. Guidelines for the treatment of malaria - 3rd edition [Internet]. Geneva, Switzerland: World Health Organization; 2015 [cited 2020 Aug 26]. Available from <https://www.who.int/malaria/publications/atoz/9789241549127/en/>