The effect of chloroquine dose and primaquine on Plasmodium vivax recurrence: a WorldWide Antimalarial Resistance Network systematic review and individual patient pooled meta-analysis

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Summary

Background Chloroquine remains the mainstay of treatment for Plasmodium vivax malaria despite increasing reports of treatment failure. We did a systematic review and meta-analysis to investigate the effect of chloroquine dose and the addition of primaquine on the risk of recurrent parasitaemia across different settings.

Methods A systematic review done in MEDLINE, Web of Science, Embase, and Cochrane Database of Systematic Reviews identified P vivax clinical trials published between Jan 1, 2000, and March 22, 2017. Principal investigators were invited to share individual patient data, which were pooled using standardised methods. Cox regression analyses with random effects for study site were used to investigate the roles of chloroquine dose and primaquine use on rate of recurrence between day 7 and day 42 (primary outcome). The review protocol is registered in PROSPERO, number CRD42016053310.

Findings Of 134 identified chloroquine studies, 37 studies (from 17 countries) and 5240 patients were included. 2990 patients were treated with chloroquine alone, of whom 1041 (34·8%) received a dose below the target 25 mg/kg. The risk of recurrence was 32·4% (95% CI 29·8–35·1) by day 42. After controlling for confounders, a 5 mg/kg higher chloroquine dose reduced the rate of recurrence overall (adjusted hazard ratio [AHR] 0·82, 95% CI 0·69–0·97; p=0·021) and in children younger than 5 years (0·59, 0·41–0·86; p=0·0058). Adding primaquine reduced the risk of recurrence to 4·9% (95% CI 3·1–7·7) by day 42, which is lower than with chloroquine alone (AHR 0·10, 0·05–0·17; p<0·0001).

Interpretation Chloroquine is commonly under-dosed in the treatment of vivax malaria. Increasing the recommended dose to 30 mg/kg in children younger than 5 years could reduce substantially the risk of early recurrence when primaquine is not given. Radical cure with primaquine was highly effective in preventing early recurrence and may also improve blood schizontocidal efficacy against chloroquine-resistant P vivax.

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increasing the dose or duration of chloroquine, or by
combining chloroquine with an additional drug with blood
schizontocidal activity or the ability to reverse chloroquine
resistance. Although early dose-finding studies showed
excellent efficacy against \textit{P} \textit{vivax} at doses below 25 mg/kg,
higher doses are well tolerated and might provide increased
efficacy. Alternatively, the addition of a hypnozoitocidal
agent such as primaquine to chloroquine improves blood
schizontocidal efficacy and reduces relapse.

To explore alternative strategies for improving
chloroquine efficacy, we did a pooled analysis of
individual patient data from prospective \textit{P} \textit{vivax} clinical
trials to investigate the effect of chloroquine dose and
primaquine co-administration on the risks of \textit{P} \textit{vivax}
recurrence between day 7 and day 42.

### Methods

**Search strategy and selection criteria**

We searched MEDLINE, Web of Science, Embase, and
Cochrane Database of Systematic Reviews, according to
the Preferred Reporting Items for Systematic Reviews and
Meta-Analyses statement (appendix pp 2–5). Prospective
therapeutic efficacy trials of uncomplicated \textit{P} \textit{vivax}
malaria published in any language between
Jan 1, 1960, and March 22, 2017, were identified using
the following search terms:\ malaria OR plasmodium
AND (amodiaquine OR atovaquone OR artesinin OR
artether OR artesunate OR arteether OR artemotil
OR azithromycin OR artikin OR chloroquine OR
chloro- proguanil OR cycloguanil OR clindamycin OR
cortem OR dapsone OR dihydroartemisinin OR
duo-cotecin OR doxycycline OR halofantrine OR
lumefantrine OR lariam OR malaron OR mefloquine OR
naphthoquine OR naphthquinone OR piperazine OR
primaquine OR proguanil OR pyrimethane OR pyronaridine OR
quinine OR quinine OR riamet OR sulphadoxine OR
tetracycline OR tafenoquine). Further details are
provided in the appendix (p 6).

The review process was done by two independent
investigators (RJC and RNP), who also extracted the data.
Disagreement was resolved through discussion. To
ensure results were relevant to the current clinical
landscape, only studies published after 2000 were
included. Principal investigators were contacted and
invited to share individual patient data and any additional
unpublished data.

### Evidence before this study

Using the search terms “vivax” and “chloroquine”, MEDLINE,
Web of Science, Embase, and the Cochrane Database of
Systematic Reviews were searched for articles published before
Nov 29, 2017, that assessed the efficacy of chloroquine, with or
without primaquine, for uncomplicated \textit{Plasmodium vivax}
malaria. A systematic review and meta-analysis showed that
there was evidence of reduced chloroquine efficacy for \textit{P} \textit{vivax}
present in most \textit{P} \textit{vivax} endemic countries. No reviews or
pooled analyses had assessed the effect of chloroquine dose on
the risk of recurrence.

### Added value of this study

Our pooled analysis of individual patient data from 37 studies
across 17 countries is, to our knowledge, the largest individual
pooled analysis of \textit{P} \textit{vivax} clinical trials so far. Our findings
highlight the substantial benefit of increasing the dose
of chloroquine in children younger than 5 years and the additional
benefit of adding primaquine to chloroquine.

### Implications of all the available evidence

Chloroquine is currently under-dosed in children younger than
5 years. Increasing the target dose of chloroquine from
25 mg/kg to 30 mg/kg could significantly reduce the risk of
\textit{P} \textit{vivax} recurrence within 42 days in children younger than
5 years who are not given primaquine. The risk of \textit{P} \textit{vivax}
recurrence was reduced by an even greater degree by the
addition of primaquine to chloroquine in all age groups,
through prevention of relapse and probably improvement in
blood schizontocidal efficacy. These measures warrant
consideration by regional and global policy makers to reduce
the risk of early \textit{P} \textit{vivax} recurrence.

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chloro-proguanil OR cycloguanil OR clindamycin OR
cortem OR dapsone OR dihydroartemisinin OR
duo-cotecin OR doxycycline OR halofantrine OR
lumefantrine OR lariam OR malaron OR mefloquine OR
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quinine OR quinine OR riamet OR sulphadoxine OR
tetracycline OR tafenoquine). Further details are
provided in the appendix (p 6).
observed study site reinfection rates (appendix pp 16–17). Study sites were also categorised as having long or short 
P vivax relapse periodicity according to geographical location. Regions with short relapse periodicity were defined as having a median time to patent relapse of 47 days or fewer.17

Outcomes
The primary outcome was the risk of 
P vivax recurrence between day 7 and day 42. Secondary outcomes were the risk of recurrence between day 7 and day 28 and early parasitological clearance, defined as the prevalence of parasitaemia on days 1, 2, and 3.15

Statistical analysis
The risk of recurrence was calculated using Kaplan-Meier survival analyses. Patients were right censored at the day of their first recurrence, the day they were last seen, the day before a more than 18-day blood smear gap, or day 42, depending on which came first.15

Cox’s proportional hazards regression was used to estimate the association between chloroquine dose and co-administration of primaquine with the rate of recurrence, adjusting for the potential confounders of age, sex, baseline parasitaemia, and regional relapse periodicity, and applying shared frailty for study sites to account for additional variation related to different sites. A linear association between chloroquine dose and the log rate of recurrence was checked visually, and the proportional hazards assumption tested using Schoenfeld residuals. If non-proportional hazards were present, interactions between terms and time were assessed. Owing to collinearity with relapse periodicity, geographical region and parasite prevalence were not included. Age was categorised into three groups (<5 years, 5 to <15 years, and ≥15 years) when a linear association with outcome was not present. Figures of risk of recurrence were estimated according to chloroquine dose and primaquine co-administration, adjusted for other confounders and assuming no study site effect.

The associations between chloroquine dose and microscopy-detectable parasite positivity in patients treated with chloroquine alone were analysed by logistic regression, with study sites included as a random effect. The association between the first day of parasite clearance and parasitaemia recurrence between day 7 and day 28 was assessed by Cox’s proportional hazards regression.

Heterogeneity of studies was assessed by removal of one study site at a time and calculation of the coefficient of variation around parameter estimates. Additionally, baseline characteristics of included studies were compared with targeted studies that were not present. Statistical analyses were done in Stata (version 15.0) and R (version 3.4.0), according to an a-priori statistical analysis plan. The review protocol is registered in PROSPERO, number CRD42016053310.

Role of the funding source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. RNP had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
232 published 
P vivax clinical trials were identified, 134 of which included patients treated with chloroquine, and were published between Jan 1, 2000, and March 22, 2017 (figure 1). Individual patient data were
Chloroquine alone (n=2990)  | Chloroquine and early primaquine (n=1790)  | Overall (n=5240) *
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**Sex**
Female | 1104 (36·3%) | 571 (31·9%) | 1786 (34·1%)
Male | 1886 (63·3%) | 1219 (68·1%) | 3454 (65·9%)
**Age (years)**
Median (IQR) | 17 (0·0–28·0) | 23·5 (13·0–36·0) | 20·0 (10·0–31·0)
<5 | 350 (12·0%) | 88 (4·9%) | 458 (8·6%)
5 to <15 | 916 (30·6%) | 413 (23·1%) | 1403 (26·8%)
x15 | 1715 (57·4%) | 1289 (72·0%) | 3387 (64·6%)
**Weight (kg)**
Median (IQR) | 45 (0·0–56·0) | 51 (36·0–62·0) | 48 (25·0–58·0)
<5 | 342 (11·4%) | 101 (5·6%) | 443 (8·5%)
5 to <15 | 574 (19·2%) | 208 (11·6%) | 782 (15·0%)
25 to <35 | 235 (7·9%) | 120 (6·7%) | 355 (6·8%)
35 to <45 | 320 (10·7%) | 185 (10·3%) | 505 (9·7%)
45 to <55 | 649 (21·7%) | 412 (22·1%) | 1261 (23·9%)
≥55 | 777 (26·0%) | 656 (36·6%) | 1433 (27·2%)
x≥50 | 95 (3·1%) | 107 (6·0%) | 203 (3·9%)
**Relapse periodicity**
Long | 2914 (64·0%) | 902 (50·4%) | 3016 (57·6%)
Short | 1076 (36·0%) | 888 (49·6%) | 2164 (42·4%)
**Geographical region**
Asa-Pacific | 2112 (70·6%) | 1203 (67·2%) | 3315 (63·5%)
The Americas | 289 (9·7%) | 487 (27·2%) | 776 (14·8%)
Africa | 580 (19·7%) | 100 (5·6%) | 691 (13·2%)
**Prevalence of Plasmodium vivax**
Low | 1243 (41·6%) | 195 (10·9%) | 1438 (27·2%)
Moderate | 607 (20·3%) | 744 (41·6%) | 1251 (23·9%)
High | 1140 (38·1%) | 851 (47·5%) | 2001 (38·9%)
**Enrolment clinical variables**
Parasitaemia, parasites per µL | 4000 (1480–8290) | 3000 (1000–7520) | 3809 (1380–8360)
Haemoglobin, g/dL† | 12 (2–1) | 12 (2–1) | 12 (2–1)
Anaemia, haemoglobin <10 g/dL | 263 (19·1%) (13·7%) | 138 (65·5%) (8·6%) | 428 (340·1%) (11·1%)
Gametocytes present | 147 (26·8%) (89·7%) | 85 (95·2%) (98·2%) | 250 (276·3%) (90·6%)
Fever, temperature >37.5°C | 128 (27·5%) (46·4%) | 68 (15·6%) (44·4%) | 226 (47·2%) (47·2%)

Data are number (%), median (IQR), mean (SD), or n/N (%). Some percentages do not add up to 100 because of rounding. *Includes 450 patients treated with chloroquine and primaquine who started primaquine after the first 3 days. †Data not available for 1600 of 5240 patients. 999 in the chloroquine alone group and 181 in the chloroquine and primaquine group.

Table 1: Demographics and baseline characteristics
Within 24 h of starting treatment, 1169 (56.5%) of 2070 patients had cleared their detectable parasitaemia, increasing to 2095 (80.9%) of 2590 on day 2 and 2369 (94.8%) of 2499 on day 3. Low chloroquine dose (<25 mg/kg) was a risk factor for parasitaemia on day 1 in the univariable analysis (odds ratio 2.09, 95% CI 1.24–3.51; p=0.0056), as were male sex, older age, and higher baseline parasitaemia (appendix p 26). After controlling for confounding factors, the association between low chloroquine dose and parasitaemia by day 42 was smaller and statistically significant for age 5 to <15 years (AHR 0.66, 95% CI 0.45–0.96; p=0.030) and ≥15 years 0.83, 0.61–1.15; p=0.27), but there was no reduction with dose up to day 21 (appendix p 25). Sensitivity analyses in which one study site was removed at a time revealed no apparent bias relating to individual study sites from included studies (appendix pp 33–36).

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See Online for appendix

**Figure 1:** Risk of recurrence in patients younger than 5 years receiving chloroquine alone with (A) varied chloroquine doses, and in (B) long periodicity and (C) short periodicity regions

Dashed lines are the 95% CIs. Adjusted for age, sex, and baseline parasitaemia. Assumes zero effect from study site. p values are derived from a Cox model.

**Figure 2:** Risk of recurrence according to day of parasite clearance in patients receiving chloroquine alone in (A) long and (B) short periodicity regions

Dashed lines are the 95% CIs. Adjusted for age, sex, baseline parasitaemia, and chloroquine dose. Assumes zero effect from study site. p values are derived from a Cox model.

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dose and parasitaemia on day 1 was attenuated (AOR 1·65, 95% CI 0·98–2·78; p=0·060; appendix p 26). There was no relationship between chloroquine dose and parasite clearance on day 2 (AOR 1·52, 95% CI 0·78–2·97; p=0·22) or day 3 (1·39, 0·60–3·22; p=0·44).

In patients treated with chloroquine alone who were assessed at day 28, 32 (23%) of 139 who were parasitaemic on day 3 had recurrent *P vivax* between day 7 and day 28, compared with 229 (99%) of 2657 who had already cleared their parasitaemia (p<0·0001). After controlling for confounding factors, parasite clearance on or after day 3 was associated with an increased rate of recurrence between day 7 and day 28 compared with parasite clearance on day 1 (AHR 3·57, 95% CI 2·09–6·11; p<0·0001; appendix p 27). The higher rate of recurrence with delayed parasite clearance was more apparent in studies from short periodicity regions (figure 3).

In 17 studies, 1790 patients were treated with chloroquine and early primaquine. 917 (51·2%) patients from 11 of these studies had a target dose of primaquine between 3·5 mg/kg and less than 5·0 mg/kg, and 873 (48·8%) from six studies had a target dose of at least 5·0 mg/kg. Overall, patients were administered a median dose of primaquine of 4·7 mg/kg (IQR 3·4–6·7; range 0·3–13·1; appendix pp 10, 23). 1046 (58·4%) of 1790 primaquine regimens were 14 days long (range 7–14 days; appendix p 28).

31 patients had recurrent parasitaemia by day 42, with a cumulative risk of 1·4% (95% CI 0·9–2·1) at day 28 and 4·9% (3·1–7·7) at day 42. When patients treated with chloroquine plus early primaquine were added to the previous Cox regression model, the addition of early primaquine was associated with a reduction in the rate of recurrent parasitaemia (AHR 0·10, 95% CI 0·05–0·17; p<0·0001; figure 4; appendix p 29). This reduction did not vary significantly with time; early primaquine was associated with a reduced rate of recurrence up to day 21 (AHR 0·07, 95% CI 0·03–0·18; p<0·0001) and between...
day 22 and day 42 (0.10, 0.06–0.18; p=0.0001). In a multivariable model of patients only treated with chloroquine plus early primaquine, neither primaquine dose nor chloroquine dose were significantly associated with a lower rate of recurrent parasitaemia (appendix p 30).

Discussion
In this pooled analysis of individual patient data, a high proportion of patients, especially older males, received a suboptimal dose of chloroquine (<25 mg/kg); increasing the total mg/kg chloroquine dose reduced early recurrences if primaquine was not given, especially in children younger than 5 years; and, the risk of early recurrent parasitaemia was markedly reduced by co-administration of primaquine.

Increasing reports of declining chloroquine efficacy have highlighted the need for alternative treatment strategies for P vivax.5 In countries where there are high levels of chloroquine resistance, national guidelines have changed to ACT as first-line therapy for P vivax.52 Other countries have included primaquine as adjunctive therapy to prevent P vivax relapses, with the added benefit of providing additional blood schizontocidal activity.53,54 However, the risk of substantial haemolysis, coupled with poor adherence, have prevented widespread effective implementation.53,54 The results of this individual pooled data meta-analysis suggest that in the absence of primaquine, an increased dose of chloroquine would also decrease P vivax recurrence substantially in children younger than 5 years.

Previous pharmacokinetic studies have shown that chloroquine is under-dosed in children and have suggested that an increase in the chloroquine dose or dosing based on body surface area would be more appropriate and effective.55–58 In children younger than 2 years, approximately twice the dose of chloroquine was required to reach the same chloroquine blood concentration as children aged 10–14 years.55 In addition, Añez and colleagues56 found that children had the greatest variation between dose per kg of bodyweight and theoretical dose calculated by body surface area. Chloroquine blood concentrations are also lowest in children, in whom the risk of recurrence is greatest.56

Our data are in keeping with these findings and suggest that increasing the total chloroquine dose from 25 mg/kg to 30 mg/kg in children younger than 5 years would decrease the risk of early recurrence by more than 40% if chloroquine was used alone. Although increasing the target dose might reduce tolerability, substantial data support the safety of 30 mg/kg in children. In Guinea-Bissau, chloroquine doses of 50 mg/kg against drug-resistant Plasmodium falciparum were well tolerated in children younger than 15 years.59–62 Even higher doses have been used for amoebic liver abscess (21 mg/kg daily for 3 weeks)63 and Giardia lamblia (10 mg/kg twice daily for 5 days).64 Our pooled analysis did not include a comprehensive safety analysis, but, reassuringly, the risk of vomiting after chloroquine treatment was low and was not associated with chloroquine dose.

Current molecular analyses cannot differentiate reliably between the three causes of recurrent P vivax parasitaemia: recrudescence, relapse, and new infections.65 Hence, increasing the dose of chloroquine might simply provide a prolonged period of chemoprophylaxis, delaying recurrent infection rather than preventing recrudescence. Although this prolonged chemo-prophylaxis is likely to account for some of the reduction in recurrences after a higher chloroquine dose, two factors suggest that there is also a reduction in the risk of recrudescence. First, regions with long relapse periodicity have a low risk of relapse within 6 weeks of treatment, increasing the likelihood that recurrences during this period are attributable to recrudescence. A subgroup analysis of patients from long relapse periodicity regions showed that a higher dose of chloroquine was protective against recrudescence even in this setting (AHR per 5 mg/kg increase 0.63, 95% CI 0.42–0.96; p=0.031; appendix p 31). Second, the reduction in rate of recurrence associated with chloroquine dose in children younger than 5 years did not vary significantly over the follow-up period. By contrast, for older patients, the hazard ratio decreased after day 21 of follow-up compared with earlier. Between days 7 and 21, recurrences are more likely to be due to recrudescence, compared with
relapses or new infections after this time.\textsuperscript{24}–\textsuperscript{26} Hence, in older patients, a higher chloroquine dose might afford greater prevention of relapse or new infection between days 22 and 42, but have minimal effect on true recrudescent infections. Conversely, in younger patients, a higher chloroquine dose probably also reduces recrudescent infections as a result of relative under-dosing of chloroquine despite delivery of the recommended chloroquine dose in this age group. Although our study design did not allow us to establish conclusively whether an increased dose of chloroquine prevents or delays parasite recurrence, either response is likely to be of substantial clinical benefit to the patient. Both responses allow greater time for haematological recovery after the initial infection, a reduced risk of cumulative anaemia, and thus the potential to reduce associated morbidity and mortality.\textsuperscript{3} However, prospective studies with prolonged follow-up are warranted.

The addition of primaquine to chloroquine reduced early recurrences before day 42 by 90% compared with chloroquine alone; probably in large part as a result of prevention of early relapse related to primaquine. However, addition of primaquine probably also reduces recrudescence through its blood schizontocidal activity, potentially in patients with low-grade chloroquine resistance. In the current pooled analysis, the reduction with chloroquine and primaquine did not vary before and after day 21, consistent with a reduction in both recrudescences and relapses.

Delayed parasite clearance predicts treatment failure in \textit{P falciparum} malaria.\textsuperscript{39–41} Similar associations have been described in \textit{P vivax}.\textsuperscript{1} In the current study, we confirm that delayed parasite clearance is associated with a higher risk of recurrence at day 28, consistent with an association with recrudescence. Although the specificity of persistent parasitaemia on day 3 for predicting risk of recurrence was 95\%-8\%, the positive predictive value was only 23\%-0\% (appendix p 32), showing the difficulty in using delayed parasite clearance as a measure of an individual’s risk of recurrence. However, if parasite clearance was delayed until day 3, there was a three-times increased risk of recurrence at day 28. This association between delayed parasite clearance and recurrence is a potential parameter for identifying sites of possible chloroquine resistance, since this approach would avoid the confounding effect of relapses and reinfections that currently cannot be avoided in formal antimalarial efficacy studies.

Our study has several limitations. First, the analysis only included about 20\% of patients from the clinical trials targeted. However, a sensitivity analysis in which one study site was removed at a time revealed no apparent bias relating to individual study sites that were included, and baseline characteristics of patients included had similar characteristics to those from all targeted studies (appendix p 21). Second, the number of tablets given was only available for about 60\% of patients, with the remainder extrapolated from the protocol and assuming complete adherence. However, when the method used to calculate dose was included in the multivariable analyses, the results remained unchanged (data not shown).

In summary, although the risk of early recurrence of \textit{P vivax} after chloroquine monotherapy is high, it can be reduced by a modest increase in the dose of chloroquine, particularly in children younger than 5 years, and by the additional administration of primaquine. As reports of chloroquine treatment failure for \textit{P vivax} increase, we recommend that the dose of chloroquine be increased to 30 mg/kg in children younger than 5 years, and health-care providers should be encouraged to provide adjunctive primaquine radical therapy to reduce the risk of both recrudescent and relapsing infections. Alternatively, a universal policy of ACT for uncomplicated malaria, with additional primaquine for \textit{vivax} malaria, should be considered in regions where there is a high risk of recurrent \textit{P vivax} after chloroquine treatment.

Contributors

RJC, JAS, KT, and RNP conceived the study, analysed and interpreted the data, and drafted the manuscript. RJC, GSHP, PD, CHS, PGJ, and KST provided technical support and undertook pooling of patient data. KT, TA, SGA, AA, NMA, GRA, JKB, BEB, IB-F, CSC, UD-A, AD, PJIV, AE, MSMG, LG-C, MGJ, AH, JH, PAK, TX, WAK, MVCL, TL, BI, KL, WMM, FN, DBP, GTP, APP, MR, KSA, AMS, IS, WRJT, GT, BQT, HTT, NV, JLFV, SW, TW, CJW, LZ-I, NJW, and RNP conceived and undertook the individual studies and enrolled the patients. All authors revised the manuscript.

Declaration of interests

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