**The haematological consequences of *Plasmodium vivax* malaria after chloroquine treatment with and without primaquine: a WorldWide Antimalarial Resistance Network systematic review and individual patient data meta-analysis**

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**Abstract**

*Background:*

Malaria causes haemolysis that can be compounded by primaquine (PQ), particularly in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. This study aimed to delineate the relative contributions to haemolysis of malaria and PQ in patients with uncomplicated *P. vivax* malaria.

*Methods:*

We conducted a systematic review to identify all prospective *P. vivax* therapeutic clinical trials published between January 2000 and March 2017. Individual patient data were pooled using standardised methodology and the haematological response quantified using a multivariable linear mixed effects model with non-linear terms. PROSPERO: CRD42016053312.

*Findings:*

In total, 3,421 patients from 29 studies were included; 49·5% (1,692/3,421) with normal G6PD activity and 49·7% (1,701/3,421) with unknown G6PD status. Of 1,975 patients treated with chloroquine (CQ) alone, the mean haemoglobin fell to a nadir on day 2, before recovering by day 7 and plateauing thereafter. Of 1,446 patients treated with CQ+PQ, the mean haemoglobin was -0·13 g/dL [95%CI -0·27 to 0·01] lower at day of nadir (p=0·072), but 0·49 g/dL [95%CI 0·28 to 0·69] higher by day 42 (p<0·001) compared with patients treated with CQ alone. On day 42, patients with recurrent parasitaemia had a mean haemoglobin concentration 0·72 g/dL [95%CI 0·90 to 0·54] lower than patients without recurrence; p<0·001.

*Interpretation:*

In G6PD normal patients, the fall in haemoglobin following treatment of uncomplicated vivax malaria relates primarily to malaria, rather than treatment with PQ. Treatment with PQ leads to a higher overall haemoglobin by day 42, likely due to the prevention of recurrence.

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**Research in context**

*Evidence before this study*

Using the search terms “vivax”, “chloroquine”, “primaquine” and “haemolysis”, Medline, Web of Science, Embase, and the Cochrane Database of Systematic Reviews were searched for articles published prior to September 11, 2018, that assessed the haematological response following treatment with chloroquine, with or without primaquine, for uncomplicated *Plasmodium vivax* malaria. Multiple studies demonstrate the haemolytic risk of primaquine in patients with G6PD deficiency and a recent study identified primaquine to be associated with haemolysis in females with G6PD heterozygosity. However, there were limited data on the haemolytic response following treatment in patents without G6PD deficiency, and the quantification of the malaria and primaquine-attributable components of haemolysis.

*Added value of this study*

Our pooled analysis includes individual patient data from 29 studies and to our knowledge, is the largest individual patient data meta-analysis of the haematological response following treatment of *P. vivax*. Our findings highlight the relative benefits of primaquine in patients with predominantly normal G6PD activity, whom have a greater haematological recovery by day 42 than patients treated with chloroquine alone.

*Implications of all the available evidence*

In a population with predominantly normal G6PD activity there is no clinically significant haemolysis attributable to primaquine beyond the haemolysis present following treatment of vivax malaria itself. Indeed, primaquine treatment is associated with improved haematological outcomes at day 42, likely related to the prevention of relapse. These results highlight the public health benefits of primaquine radical cure for *P. vivax* and support strengthened implementation in endemic areas, in association with an accurate point of care test for G6PD deficiency.

**Introduction**

Outside of Sub-Saharan Africa *Plasmodium vivax* is a significant cause of morbidity and mortality in malaria endemic regions.1-3 Anaemia is a common manifestation of vivax malaria and compounded by recurrent parasitaemia associated with multiple relapses arising from reactivation of dormant liver stages (hypnozoites).4,5 Each episode of malaria results in haemolysis of infected and uninfected red blood cells, as well as reduced red cell production due to dyserythropoiesis.4 Radical cure of both the erythrocytic and hypnozoite stages of the parasite can prevent recurrent *P. vivax* infections and thus reduce the cumulative risk of anaemia.6

Primaquine (PQ), an 8-aminoquinoline compound in use for over 60 years, remains the only widely available drug with activity against hypnozoites. PQ can cause severe haemolysis in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency, an inherited enzymopathy, caused by genetic polymorphisms on the X chromosome. The risk of drug-induced haemolysis relates to the dose of PQ administered and an individual’s genetic polymorphism and sex.7-9 In areas where routine testing for G6PD deficiency is unavailable, concerns about severe haemolysis are a major barrier to widespread clinical use of PQ.10,11

The relative contributions of parasitaemia and PQ treatment to haemolysis in patients with vivax malaria are poorly defined. The aim of this study was to determine the degree of haemolysis following chloroquine, the standard schizontocidal treatment of vivax malaria,12 and to quantify the additional haemolysis attributable to the co-administration of PQ.

**Methods**

*Search strategy and selection criteria*

A systematic search was undertaken of Medline, Web of Science, Embase and the Cochrane Database of Systematic Reviews according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines; Appendix page 2. Prospective therapeutic efficacy trials of treatment of uncomplicated *Plasmodium vivax* infectionwith a minimum of 28 days follow-up, published between January 1, 2000 and March 22, 2017 in any language were identified; Appendix page 6.13 Investigators of eligible studies were invited to participate in an individual patient data meta-analysis and contribute data from similar unpublished studies.

Studies were included in the analysis if they enrolled patients with *P. vivax* monoinfection treated with CQ, either alone or with PQ during the first 28 days, and recorded haemoglobin (Hb) or haematocrit at baseline. Individual patient data were shared to the WorldWide Antimalarial Resistance Network (WWARN) repository, anonymised and standardised as described previously.14 The review protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO: CRD42016053312).

*Procedures*

The dose of CQ and PQ were calculated from the number of tablets given daily to each patient, or the study protocol if tablet numbers were unavailable. Individual patient records were excluded if the treatment course of CQ was incomplete, no Hb or haematocrit was recorded for the first 42 days or no information was available on the dose given, parasite counts, age or gender of the patient. Patients were not excluded based on G6PD activity.

Study sites were categorised into regions of long or short *P. vivax* relapse periodicity,15 with regions of short relapse periodicity considered to have a median time to relapse of less than or equal to 47 days. To avoid confounding from early treatment failure, recurrence was defined as *P. vivax* parasitaemia between day 7 and 42. Daily PQ mg/kg dose was defined as low dose if less than 0·5 mg/kg/day and high dose if greater than or equal to 0·5 mg/kg/day.

If only the haematocrit was available, it was converted to Hb according to the equation:

Hb (g/dL) = (Haematocrit (%) – 5·62)/2·6.16

Where more than one measurement of Hb was recorded on a single day, the minimum value was used.

*Statistical Analysis*

The primary endpoint of the analysis was the mean drop in Hb, from baseline to the day of the nadir. Secondary endpoints were the mean change in Hb from baseline to day 42 and total red cell loss, as determined by the area under the mean haemoglobin-time curve. In addition, two safety outcomes were considered to identify patients at risk of poor clinical outcome: a Hb fall of >25% from a baseline of ≥7g/dL to a Hb <7g/dL and an absolute fall in Hb of >5g/dL. The safety outcomes were assessed at day 2 or 3 (day 2/3), day 7 ± 2 days (day 7), and day 28 ± 3 days (day 28).

Statistical analyses were undertaken using Stata version 15 (StataCorp, College Station, TX, US) and R version 3·4·0 (R Foundation for Statistical Computing, Vienna, Austria), according to an *a priori* statistical analysis plan.17 The analysis of patients treated with CQ+PQ included only those patients who commenced PQ on day 0. The Hb response over time was estimated using a linear mixed effects model with non-linear terms, derived by fractional polynomial regression, with fixed effects for age, gender, baseline parasitaemia, total CQ dose (mg/kg), relapse periodicity, PQ use and study site. The interaction between PQ use and time was included in order to capture the different time course of Hb responses following the two regimens CQ or CQ+PQ. The effect of the daily mg/kg PQ dose was assessed in a similar linear mixed effect model in the subset of patients treated with PQ on day 0. The primary analysis was repeated in the subgroup of patients with documented normal G6PD activity.

The effect of delayed parasite clearance (defined as persistence of parasitaemia until day 2 or later) on Hb at day of nadir and day 42 and the effect of recurrence between day 7 and 42 on Hb at day 42 were assessed using separate linear mixed effects models. In the model of recurrence between day 7 and 42, patients with early treatment failure, late clinical failure prior to day 7 or persistent parasitaemia between days 4 to 6 were excluded from the analysis. Hb-time models were used to derive the day of the minimum Hb (nadir), and the mean difference in Hb with the addition of PQ at day of nadir, day 7 and day 42.

Estimates of the difference in cumulative haemolysis up to day 3 and day 42 were derived from the area under the mean Hb-time profiles in patients treated with and without PQ, and the ratio of parasitised to non-parasitised red cells undergoing haemolysis up to day 2 in patients treated with CQ alone were estimated as detailed in the Appendix, page 7.

A descriptive table of safety outcomes was presented to provide commonly reported parameters of the Hb response in published clinical trials; the numbers of patients available for these summary statistics varied accordingly to the time point presented. There were insufficient numbers of patients experiencing either of the safety outcomes to conduct multivariable analyses of the haemolytic risk attributable to PQ.

A sensitivity analysis was undertaken to assess bias. One study at a time was removed and the coefficient of variation for main outcomes was calculated. Baseline characteristics of included studies were also compared to studies that were targeted but not available for inclusion.

*Role of funding source*

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

*Ethics*

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

**Results**

Between 1 January 2000 and 22 March 2017 there were 168 published *P. vivax* clinical trials of which 134 (79·8%) included patients treated with CQ and 56 (33·3%) contained information on haemoglobin concentration or haematocrit. Individual patient data were available for 5,640 (49·2%) patients from 25 of these studies plus patients from an additional four unpublished studies. Of the 7,537 patients with available data, 2,685 (32·0%) were not treated with CQ, 405 (4·8%) were treated with PQ after day 0 and 531 (6·3%) were excluded for other reasons; Figure 1 and Appendix pages 8-12. Of the remaining 3,421 patients, 1,975 (57·7%) were treated with CQ alone and 1,446 (42·3%) with CQ+PQ.18-44 Patients were followed for 28 days in 14 studies (n=1,841), 29 to 42 days in seven studies (n=388) and more than 42 days in eight studies (n=1,192). In total, G6PD activity was normal in 1,692 (49·5%) patients, deficient or borderline deficient in 28 (0.8%) and unknown in 1,701 (49·7%). Target PQ regimens are described in the Appendix, page 13.

The majority of patients were male (64·6%, 2,211/3,421). The median age of patients was 19 years (inter-quartile range (IQR) 9-32), with 1,314 (38·4%) patients younger than 15 years; Table 1. Most of the patients were enrolled from the Asia-Pacific region (2,247, 65·7%), with 598 (17·5%) enrolled from The Americas and 576 (16·8%) from Africa; Appendix page 14. Compared to patients treated with CQ, those treated with CQ+PQ tended to be older, had lower baseline parasitaemias and were more likely to come from areas of short relapse periodicity; Table 1. None of the patients enrolled in African studies were treated with CQ+PQ. Compared to the studies that were targeted but not included, those included were conducted more recently, enrolled younger populations and were more balanced in the proportion of male and female patients; Appendix page 15.

*Baseline Haemoglobin*

The mean Hb at baseline was 12·2 g/dL (SD 2·1) in patients receiving CQ and 12·7 g/dL (SD 2·1) in patients receiving CQ+PQ. Overall 11·3% (385/3,421) of patients were anaemic at baseline (Hb<10 g/dL), including 13·1% (259/1,975) in those subsequently treated with CQ and 8·7% (126/1446) in those treated with CQ+PQ. Severe anaemia (Hb<7 g/dL) was present in 0·8% (26/3,421) of patients. The risk of anaemia at baseline was greater in females (Adjusted Odds Ratio (AOR) =1·34 [95%CI 1·05 to 1·71]) and patients who were younger than 5 years (AOR=10·37 [95%CI 6·09 to 17·67]), G6PD deficient (AOR=2·88 [95%CI 1·14 to 7·32]) and who were enrolled in regions of short relapse periodicity (AOR=1·94 [95%CI 1·01 to 3·71]); Appendix page 16.

*Haemoglobin-time profile*

The haemoglobin profile between baseline and day 42 was modelled from 9,684 Hb measurements in 1,975 patients treated with CQ alone and 6,029 Hb measurement in 1,446 patients treated with CQ+PQ. Patients treated with CQ alone had a median [IQR] of 7 [5-9] Hb measurements and patients treated with CQ+PQ had a median [IQR] of 9 [3-10] Hb measurements.

*Haemoglobin profile following treatment with chloroquine alone*

The mean Hb fell from baseline to a nadir on day 2, with a fall of 0·58 g/dL from a predicted mean of 12·22 g/dL [95%CI 11·93 to 12·50] to 11·64 g/dL [95%CI 11·36 to 11·93]; Figure 2A. Following the nadir, the Hb rose before plateauing after day 7. By day 42 the predicted mean Hb was 12·88 g/dL [95%CI 12·60 to 13·17], 0·67 g/dL above baseline.

The magnitude and direction of the change in Hb from baseline to day 2 varied with the baseline Hb. The baseline Hb was correlated positively with the absolute change in Hb (r=0·511 (95%CI 0·457, 0·562), p<0·0001); Appendix page 17. Only 19·5% (136/698) of patients with a baseline Hb less than 11·5 g/dL had a fall below their baseline Hb during the first 7 days; Figure 2B-C.

*Haemoglobin profile following addition of primaquine to chloroquine*

The nadir Hb in patients treated with CQ+PQ occurred on day 3 but thereafter rose and continued to do so throughout the subsequent follow up; Figure 2A.

Compared to patients treated with CQ alone those treated with CQ+PQ had a similar mean Hb at the nadir (-0·13 g/dl [95%CI -0·27 to 0·01]; p=0·072) and day 28 (-0·06 g/dL [95%CI -0·18 to 0·05]; p=0·293), but significantly lower Hb at day 7 (-0·34 g/dL [95%CI -0·46 to -0·23]; p=<0·001). At day 42 patients treated with CQ+PQ had a higher mean Hb (0·49 g/dL [95%CI 0·28 to 0·69]; p<0·001) than those treated with CQ alone. There was no significant difference in mean Hb between patients treated with a high or low daily PQ dose, either at day 3 (0·14 g/dL [95%CI -0·05 to 0·33]; p=0·161) or day 7 (0·18 g/dL [95%CI -0·11 to 0·46]; p=0·227); Appendix page 18.

In a subgroup analysis limited to 1,692 patients known to be G6PD normal, there was no significant difference in mean Hb between treatment regimens at the day of nadir, however by day 42 the mean Hb was 0·89 g/dL [95%CI 0·53 to 1·26] higher in patients treated with CQ+PQ; p<0·001, Appendix page 19.

Overall 17·4% (344/1,975) of patients treated with CQ had recurrent parasitaemia between day 7 and 42, compared to 2·0% (29/1,446) of those treated with CQ+PQ. The mean Hb at day 42 was significantly lower in patients with recurrent parasitaemia compared to those with no recurrence (mean difference -0·72 g/dL [95%CI -0·90 to -0·54]; p<0·001). When recurrences were excluded from the analysis the mean difference in Hb at day 42 was 0·38 g/dL [95%CI 0·17 to 0·58] higher in patients treated with CQ+PQ compared to those treated with CQ alone; p<0·001.

*Effect of delayed parasite clearance on Hb Profile*

In total 37·1% (1,000/2,698) of patients had cleared their parasites by day 1, 76·9% (2,076/2,698) by day 2 and 23·1% (622/2,698) of patients had parasite clearance delayed until after day 2. The risk of delayed parasite clearance until after day 2 was 17·6% (290/1,646) following CQ and 31·6% (332/1,052) following treatment with CQ+PQ. After controlling for confounding factors including PQ treatment, patients with delayed parasite clearance had a significantly lower Hb at the day of nadir (-0·26 g/dL [95%CI -0·45 to -0·06]; p=0·010) and day 42 (-0·23 g/dL, [95%CI -0·39 to -0·07]; p=0·004); Appendix page 20.

*Safety outcomes*

The baseline Hb was correlated negatively with the percentage change in Hb at day 2/3 (r=-0·463 (95%CI -0·509, -0·415), p<0·0001) and day 7 (r=-0·521 (95%CI -0·554, -0·486), p<0·0001); Appendix pages 21-22. Whilst 1·1% (7/610) of patients treated with CQ and 5·7% (27/471) treated with CQ+PQ had a fractional fall in Hb greater than 25% from baseline at day 2/3, 94·1% (32/34) of these patients started with a Hb greater than or equal to 11·5 g/dl. A clinically significant fall in Hb was defined as a fall in Hb greater than 25% resulting in severe anaemia (Hb <7 g/dL). Of the 7 patients treated with CQ alone who had a fall in Hb greater than 25% at day 2/3 none had a clinically significant fall or an absolute fall in Hb greater than 5 g/dL; Table 2 and Appendix pages 23-24. Of the 27 patients treated with CQ+PQ who had a fall in Hb greater than 25% at day 2/3, one of 11 patients with normal G6PD activity had a clinically significant fall in Hb and six of 16 patients with unknown G6PD activity had a reduction in Hb greater than 5 g/dL; Table 2 and Appendix pages 23-24. The risk of haemolysis at day 7 and 28 and for patients with unknown G6PD activity are presented in Table 2 and Appendix page 25.

The unadjusted number needed to harm with PQ, causing an extra patient to have a clinically significant drop at day 2/3, was 1/471 (95%CI 1/159, ∞) in patients with any G6PD activity and 1/334 (95%CI 1/113, ∞) in patients with normal G6PD activity. The corresponding risks at day 7 were 1/539 (95%CI 1/182, ∞) and 1/389 (95%CI 1/132, ∞).

*Red cell loss*

The cumulative red cell loss within three days of treatment was 19·0% greater in patients treated with PQ (1·47 g/dL\*days) compared to those treated with CQ alone (1·19 g/dL\*days). By day 42 the total red cell loss was 10.1% higher after CQ+PQ (48·98 g/dL\*days) compared to after CQ alone (44·49 g/dL\*days).

In patients treated with CQ alone the contribution of parasitaemia to red cell loss was estimated from a mean parasite biomass at baseline of 2·82×1010 and an estimated 8·3×1011 RBCs lost by day 2. For each parasitised RBC that was haemolysed acutely, an additional 29 non-parasitised RBCs were also haemolysed.

**Discussion**

This large meta-analysis of 3,421 individual patient data from 29 studies provides the first detailed evaluation of the haematological consequences of *P. vivax* malaria treated with CQ with and without PQ treatment. In patients with predominantly normal G6PD activity, patients treated with PQ had no additional clinically significant haemolysis beyond patients treated with CQ alone. However, patients treated with PQ had faster haematological recovery and by day 42 their mean Hb was 0·5 g/dL higher than those patients treated without PQ, a difference in part attributable to a reduction in recurrent parasitaemia.

Treatment with PQ reduces the risk of early and late vivax recurrences by over 90%, predominantly related to its ability to prevent reactivation of dormant liver stages.42,44,45 Despite these benefits, clinician concern regarding the risk of severe haemolysis in patients with G6PD deficiency, coupled with a lack of reliable point of care tests for G6PD deficiency has prevented the widespread uptake of PQ radical cure in many vivax endemic regions.10 However, the risk of severe haemolysis attributable to PQ needs to be balanced with the underlying haemolytic risk of malaria itself. Our analysis highlights that in a patient population with predominantly normal G6PD activity the fall in Hb over the first two days of CQ treatment was minor with minimal additional haemolysis attributable to PQ. However, treatment with PQ delayed the Hb nadir from day 2 to day 3 and led to an estimated 19·0% greater cumulative mean red cell loss over the first 3 days compared to patients treated with CQ alone. Nevertheless, consistent with previous studies,46 by day 42 patients treated with PQ had a significantly higher Hb, likely reflecting the prevention of relapse and potentially recrudescence.44

Antimalarial studies commonly use a fall in Hb of >25% as a safety outcome.47,48 Whilst 5·7% treated with CQ+PQ had a fractional fall in Hb greater than 25% at day 2 or 3, this occurred predominantly in patients with high baseline Hb. Hence a large fractional fall in Hb from a high baseline does not necessarily equate to clinically relevant morbidity. We explored two alternative safety measures: a composite measure of a fall >25% from baseline to a Hb below 7 g/dL, and a fall in Hb >5 g/dL. The former reflects haemolysis to a level associated with rising risk or mortality5 and the latter massive intravascular haemolysis associated with an increased risk of high cell free Hb, renal tubular toxicity and acute renal failure.49 The risk of these safety outcomes was approximately 15 per 1000 patients treated. PQ was associated with one additional patient with a clinically relevant fall in Hb at day 2 or 3 for every 471 patients treated and one additional fall >5 g/dL for every 78 patients treated; noting that these estimates were unadjusted for confounding factors including background G6PD allele frequency and mutation type. Hence, whilst PQ does not cause a significantly increased risk of haemolysis at a population level, there is an appreciable risk of severe haemolysis in vulnerable individuals, emphasing the importance of reliable and accurate, point of care testing of G6PD activity prior to radical cure of *P. vivax*, in conjunction with clinical or biochemical monitoring for haematological recovery.

The day of nadir Hb occurred on day 2 in patients treated with CQ alone and day 3 in those treated with CQ+PQ, and yet less than half of the clinical studies sampled Hb on these days routinely. Future studies aiming to quantify PQ induced haemolysis should consider reviewing patients on day 3 after completion of schizontocidal treatment, at which time patients at greatest risk of haemolysis could be identified and appropriate management initiated if indicated.

Our analysis included all patients in the available clinical trials irrespective of their G6PD status. A subgroup analysis restricted to patients with normal G6PD activity was consistent with the overall analysis. Not all studies tested patients for G6PD deficiency, reflecting variations in regional protocols. The small number of adverse safety outcomes were not limited to the group with known G6PD deficiency, since within the first 7 days, 53% (8/15) of events occurred in patients with normal G6PD status and 47% (7/15) in those with unknown status.

Our study results are limited by inclusion of data from only half of the patients from the targeted clinical trials. However, although there were minor epidemiological differences between the populations of studies included and targeted (Appendix page 15), the studies in our analysis were undertaken in a range of populations in vivax endemic areas. Furthermore, the mean baseline Hb was similar between the included and targeted studies suggesting that differences in the haematological profiles of the included and targeted populations were unlikely to be an important source of bias. A sensitivity analysis undertaken by removal of one study site at a time did not identify significant evidence of bias related to the included studies; Appendix page 26. Hence our findings are likely to be generalisable to most regions with vivax malaria.

In summary, the administration of PQ did not result in significantly worse haematological outcomes, the haemolysis after treatment for vivax malaria primarily reflecting the disease itself rather than the treatment. Indeed, patients treated with PQ had better haematological outcomes by day 42, consistent with prevention of repeated haemolytic insults from vivax recurrence. There was a small risk of severe haemolysis after treatment with PQ, even in patients with normal G6PD activity, however, whether this was attributable to PQ treatment could not be determined. Our results highlight the public health benefits of radical cure for the treatment of *P. vivax.* Short course PQ and single-dose tafenoquine regimens are under development and will facilitate widespread implementation of effective vivax radical cure, the safety of which can be assured by accurate point of care testing for G6PD activity.

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**Authors’ contributions**

RJC, RNP, JAS, KT and ND conceived the study, analysed and interpreted the data and drafted the manuscript. TA, SGA, AAn, NMA, AAs, GRA, BEB, IB, CSC, UDA, AD, PJdV, AE, MSGM, MJG, JH, PAK, TK, WAK, MVGL, TL, BL, KL, WMM, FN, DBP, GTP, APP, RNP, MR, KSa, AMS, WRJT, KT, GT, BQT, HTT, JLFV, SW, NJW, TW, and CJW conceived the individual studies, enrolled the patients and undertook the individual studies. JW conceived and undertook an individual study. RJC, PD, PJG, CHS, KSt, and JW provided technical support and RJC and PD, undertook pooling of patient data. All authors revised the manuscript.

**Declaration of interests**

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**Ethics approval**

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; these do not require review from the Ethics Committee according to the guidelines of the Oxford Central University Research Ethics Committee.

**References**

1. Tjitra E, Anstey NM, Sugiarto P, et al. Multidrug-resistant Plasmodium vivax associated with severe and fatal malaria: a prospective study in Papua, Indonesia. *PLoS Med* 2008; **5**(6): e128.

2. Genton B, D'Acremont V, Rare L, et al. Plasmodium vivax and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea. *PLoS Med* 2008; **5**(6): e127.

3. Price RN, Tjitra E, Guerra CA, Yeung S, White NJ, Anstey NM. Vivax malaria: neglected and not benign. *Am J Trop Med Hyg* 2007; **77**(6 Suppl): 79-87.

4. Douglas NM, Anstey NM, Buffet PA, et al. The anaemia of Plasmodium vivax malaria. *Malar J* 2012; **11**: 135.

5. Douglas NM, Lampah DA, Kenangalem E, et al. Major burden of severe anemia from non-falciparum malaria species in Southern Papua: a hospital-based surveillance study. *PLoS Med* 2013; **10**(12): e1001575; discussion e.

6. Price RN, Douglas NM, Anstey NM, von Seidlein L. Plasmodium vivax treatments: what are we looking for? *Curr Opin Infect Dis* 2011; **24**(6): 578-85.

7. von Seidlein L, Auburn S, Espino F, et al. Review of key knowledge gaps in glucose-6-phosphate dehydrogenase deficiency detection with regard to the safe clinical deployment of 8-aminoquinoline treatment regimens: a workshop report. *Malar J* 2013; **12**: 112.

8. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. *Lancet* 2008; **371**(9606): 64-74.

9. Chu CS, Bancone G, Nosten F, White NJ, Luzzatto L. Primaquine-induced haemolysis in females heterozygous for G6PD deficiency. *Malar J* 2018; **17**(1): 101.

10. Thriemer K, Ley B, Bobogare A, et al. Challenges for achieving safe and effective radical cure of Plasmodium vivax: a round table discussion of the APMEN Vivax Working Group. *Malar J* 2017; **16**(1): 141.

11. Recht J, Ashley E, White J. Safety of 8-aminoquinoline antimalarial medicines. 2014. www.who.int/malaria/publications/atoz/9789241506977/en/ (accessed 15th October 2018 2018).

12. World Health Organisation. Guidelines for the treatment of malaria - 3rd edition. Geneva: World Health Organization, 2015.

13. Commons RJ, Thriemer K, Humphreys G, et al. The Vivax Surveyor: Online mapping database for Plasmodium vivax clinical trials. *Int J Parasitol Drugs Drug Resist* 2017; **7**(2): 181-90.

14. WorldWide Antimalarial Resistance Network. Data Management and Statistical Analysis Plan v1.2. 2012. www.wwarn.org/sites/default/files/ClinicalDMSAP.pdf (accessed 29 November 2017 2017).

15. Battle KE, Karhunen MS, Bhatt S, et al. Geographical variation in Plasmodium vivax relapse. *Malar J* 2014; **13**: 144.

16. Lee SJ, Stepniewska K, Anstey N, et al. The relationship between the haemoglobin concentration and the haematocrit in Plasmodium falciparum malaria. *Malar J* 2008; **7**: 149.

17. WorldWide Antimalarial Resistance Network. Statistical Analysis Plan: WWARN Vivax Haematology Study Group v0.2. 2016. http://www.wwarn.org/sites/default/files/attachments/documents/wwarn\_sap\_haematology\_290117.pdf (accessed 20 January 2018 2018).

18. Taylor WR, Widjaja H, Richie TL, et al. Chloroquine/doxycycline combination versus chloroquine alone, and doxycycline alone for the treatment of Plasmodium falciparum and Plasmodium vivax malaria in northeastern Irian Jaya, Indonesia. *Am J Trop Med Hyg* 2001; **64**(5-6): 223-8.

19. Phan GT, de Vries PJ, Tran BQ, et al. Artemisinin or chloroquine for blood stage Plasmodium vivax malaria in Vietnam. *Trop Med Int Health* 2002; **7**(10): 858-64.

20. Leslie T, Mayan MI, Hasan MA, et al. Sulfadoxine-pyrimethamine, chlorproguanil-dapsone, or chloroquine for the treatment of Plasmodium vivax malaria in Afghanistan and Pakistan: a randomized controlled trial. *JAMA* 2007; **297**(20): 2201-9.

21. Ratcliff A, Siswantoro H, Kenangalem E, et al. Therapeutic response of multidrug-resistant Plasmodium falciparum and P. vivax to chloroquine and sulfadoxine-pyrimethamine in southern Papua, Indonesia. *Trans R Soc Trop Med Hyg* 2007; **101**(4): 351-9.

22. Leslie T, Mayan I, Mohammed N, et al. A randomised trial of an eight-week, once weekly primaquine regimen to prevent relapse of plasmodium vivax in Northwest Frontier Province, Pakistan. *PLoS One* 2008; **3**(8): e2861.

23. Ketema T, Bacha K, Birhanu T, Petros B. Chloroquine-resistant Plasmodium vivax malaria in Serbo town, Jimma zone, south-west Ethiopia. *Malar J* 2009; **8**: 177.

24. Awab GR, Pukrittayakamee S, Imwong M, et al. Dihydroartemisinin-piperaquine versus chloroquine to treat vivax malaria in Afghanistan: an open randomized, non-inferiority, trial. *Malar J* 2010; **9**: 105.

25. Phyo AP, Lwin KM, Price RN, et al. Dihydroartemisinin-piperaquine versus chloroquine in the treatment of Plasmodium vivax malaria in Thailand: a randomized controlled trial. *Clin Infect Dis* 2011; **53**(10): 977-84.

26. Poravuth Y, Socheat D, Rueangweerayut R, et al. Pyronaridine-artesunate versus chloroquine in patients with acute Plasmodium vivax malaria: a randomized, double-blind, non-inferiority trial. *PLoS One* 2011; **6**(1): e14501.

27. Barber BE, William T, Grigg MJ, et al. A prospective comparative study of knowlesi, falciparum, and vivax malaria in Sabah, Malaysia: high proportion with severe disease from Plasmodium knowlesi and Plasmodium vivax but no mortality with early referral and artesunate therapy. *Clin Infect Dis* 2013; **56**(3): 383-97.

28. Hwang J, Alemayehu BH, Reithinger R, et al. In vivo efficacy of artemether-lumefantrine and chloroquine against Plasmodium vivax: a randomized open label trial in central Ethiopia. *PLoS One* 2013; **8**(5): e63433.

29. Marques MM, Costa MR, Santana Filho FS, et al. Plasmodium vivax chloroquine resistance and anemia in the western Brazilian Amazon. *Antimicrob Agents Chemother* 2014; **58**(1): 342-7.

30. Anez A, Moscoso M, Laguna A, et al. Resistance of infection by Plasmodium vivax to chloroquine in Bolivia. *Malar J* 2015; **14**: 261.

31. Getachew S, Thriemer K, Auburn S, et al. Chloroquine efficacy for Plasmodium vivax malaria treatment in southern Ethiopia. *Malar J* 2015; **14**: 525.

32. Gomes Mdo S, Vieira JL, Machado RL, et al. Efficacy in the treatment of malaria by Plasmodium vivax in Oiapoque, Brazil, on the border with French Guiana: the importance of control over external factors. *Malar J* 2015; **14**: 402.

33. Lidia K, Dwiprahasto I, Kristin E. Therapeutic effects of dyhidroartemisinin piperaquine versus chloroquine for uncomplicated Vivax Malaria in Kupang, East Nusa Tenggara, Indonesia. *Int J Pharm Sci Rev Res* 2015; **31**(2): 247-51.

34. Rishikesh K, Kamath A, Hande MH, et al. Therapeutic assessment of chloroquine-primaquine combined regimen in adult cohort of Plasmodium vivax malaria from a tertiary care hospital in southwestern India. *Malar J* 2015; **14**: 310.

35. Thanh PV, Hong NV, Van NV, et al. Confirmed Plasmodium vivax Resistance to Chloroquine in Central Vietnam. *Antimicrob Agents Chemother* 2015; **59**(12): 7411-9.

36. Grigg MJ, William T, Menon J, et al. Efficacy of Artesunate-mefloquine for Chloroquine-resistant Plasmodium vivax Malaria in Malaysia: An Open-label, Randomized, Controlled Trial. *Clin Infect Dis* 2016; **62**(11): 1403-11.

37. Ley B, Alam MS, Thriemer K, et al. G6PD Deficiency and Antimalarial Efficacy for Uncomplicated Malaria in Bangladesh: A Prospective Observational Study. *PLoS One* 2016; **11**(4): e0154015.

38. Pereira D, Daher A, Zanini G, et al. Safety, efficacy and pharmacokinetic evaluations of a new coated chloroquine tablet in a single-arm open-label non-comparative trial in Brazil: a step towards a user-friendly malaria vivax treatment. *Malar J* 2016; **15**: 477.

39. Saravu K, Kumar R, Ashok H, et al. Therapeutic Assessment of Chloroquine-Primaquine Combined Regimen in Adult Cohort of Plasmodium vivax Malaria from Primary Care Centres in Southwestern India. *PLoS One* 2016; **11**(6): e0157666.

40. Thuan PD, Ca NT, Van Toi P, et al. A Randomized Comparison of Chloroquine Versus Dihydroartemisinin-Piperaquine for the Treatment of Plasmodium vivax Infection in Vietnam. *Am J Trop Med Hyg* 2016; **94**(4): 879-85.

41. Wangchuk S, Drukpa T, Penjor K, et al. Where chloroquine still works: the genetic make-up and susceptibility of Plasmodium vivax to chloroquine plus primaquine in Bhutan. *Malar J* 2016; **15**(1): 277.

42. Abreha T, Hwang J, Thriemer K, et al. Comparison of artemether-lumefantrine and chloroquine with and without primaquine for the treatment of Plasmodium vivax infection in Ethiopia: A randomized controlled trial. *PLoS Med* 2017; **14**(5): e1002299.

43. Siqueira AM, Alencar AC, Melo GC, et al. Fixed-Dose Artesunate-Amodiaquine Combination vs Chloroquine for Treatment of Uncomplicated Blood Stage P. vivax Infection in the Brazilian Amazon: An Open-Label Randomized, Controlled Trial. *Clin Infect Dis* 2017; **64**(2): 166-74.

44. Chu CS, Phyo AP, Lwin KM, et al. Comparison of the Cumulative Efficacy and Safety of Chloroquine, Artesunate, and Chloroquine-Primaquine in Plasmodium vivax Malaria. *Clin Infect Dis* 2018.

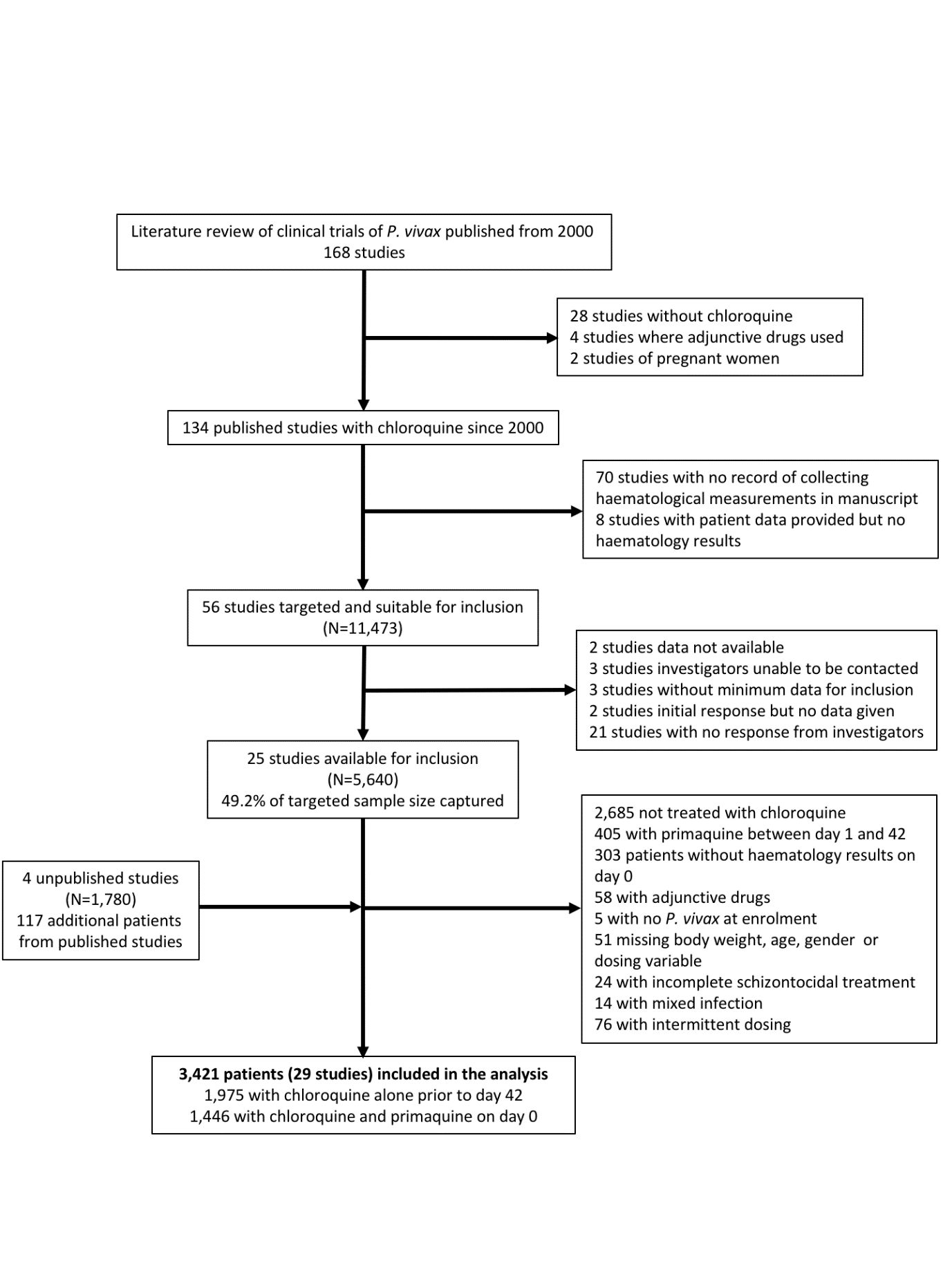
45. Pukrittayakamee S, Vanijanonta S, Chantra A, Clemens R, White NJ. Blood stage antimalarial efficacy of primaquine in Plasmodium vivax malaria. *J Infect Dis* 1994; **169**(4): 932-5.

46. Price RN, Simpson JA, Nosten F, et al. Factors contributing to anemia after uncomplicated falciparum malaria. *Am J Trop Med Hyg* 2001; **65**(5): 614-22.

47. Llanos-Cuentas A, Lacerda MV, Rueangweerayut R, et al. Tafenoquine plus chloroquine for the treatment and relapse prevention of Plasmodium vivax malaria (DETECTIVE): a multicentre, double-blind, randomised, phase 2b dose-selection study. *Lancet* 2014; **383**(9922): 1049-58.

48. Chu CS, Bancone G, Moore KA, et al. Haemolysis in G6PD Heterozygous Females Treated with Primaquine for Plasmodium vivax Malaria: A Nested Cohort in a Trial of Radical Curative Regimens. *PLoS Med* 2017; **14**(2): e1002224.

49. Qian Q, Nath KA, Wu Y, Daoud TM, Sethi S. Hemolysis and acute kidney failure. *Am J Kidney Dis* 2010; **56**(4): 780-4.

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**Figure 1: Study Flowchart**



**B**

**C**

**A**

**Figure 2. Haemoglobin versus time profiles for (A) all patients treated with CQ with (n=1,975) or without PQ (n=1,446), (B) patients with baseline haemoglobin ≥ 11·5 g/dL (n=1,277 for CQ and n=1,063 for CQ+PQ), and (C) patients with baseline haemoglobin <11·5 g/dL (n=698 for CQ and n=383 for CQ+PQ).**

CQ – chloroquine; PQ – primaquine. Profiles for chloroquine alone and chloroquine plus primaquine adjusted to the same baseline haemoglobin. Figure derived from linear mixed effect model with fractional polynomial terms for time. Shaded regions show 95% confidence intervals.

**Table 1: Demographics, baseline characteristics and baseline haemoglobin measurements**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Chloroquine alone** | | | **Chloroquine plus primaquine** | | | **Overall** | | |
|  | **Number (%)\*** | **Mean Hb (SD)** | **Range** | **Number (%)\*** | **Mean Hb (SD)** | **Range** | **Number (%)\*** | **Mean Hb (SD)** | **Range** |
| Overall | 1975 (100%) | 12·2 (2·1) | (6·0 to 18·7) | 1446 (100%) | 12·7 (2·1) | (4·0 to 19·0) | 3421 (100%) | 12·4 (2·1) | (4·0 to 19·0) |
| Parasitaemia, parasites per uL; median (IQR) | 3400 (1261, 8290) |  |  | 2700 (912, 7040) |  |  | 3104 (1137, 8000) |  |  |
| *Gender* |  |  |  |  |  |  |  |  |  |
| Female | 772 (39·1%) | 11·8 (1·9) | (6·0 to 17·4) | 438 (30·3%) | 11·7 (1·8) | (4·0 to 17·4) | 1210 (35·4%) | 11·7 (1·9) | (4·0 to 17·4) |
| Male | 1203 (60·9%) | 12·5 (2·1) | (6·6 to 18·7) | 1008 (69·7%) | 13·1 (2·1) | (4·9 to 19·0) | 2211 (64·6%) | 12·8 (2·1) | (4·9 to 19·0) |
| *Age category, years* |  | |  |  |  |  |  |  |  |
| <5 | 225 (11·4%) | 10·7 (2·0) | (6·0 to 16·6) | 72 (5·0%) | 10·3 (1·8) | (4·9 to 14·1) | 297 (8·7%) | 10·6 (2·0) | (4·9 to 16·6) |
| 5 to <15 | 691 (35·0%) | 11·6 (1·8) | (6·6 to 17·4) | 326 (22·5%) | 11·5 (1·6) | (5·5 to 16·3) | 1017 (29·7%) | 11·6 (1·8) | (5·5 to 17·4) |
| >=15 | 1059 (53·6%) | 13·0 (1·9) | (6·2 to 18·7) | 1048 (72·5%) | 13·2 (2·0) | (4·0 to 19·0) | 2107 (61·6%) | 13·1 (2·0) | (4·0 to 19·0) |
| *Weight category, kg* |  | |  |  |  |  |  |  |  |
| 5 to <15 | 195 (9·9%) | 10·4 (1·9) | (6·0 to 16·3) | 83 (5·7%) | 10·3 (1·6) | (5·2 to 13·4) | 278 (8·1%) | 10·4 (1·8) | (5·2 to 16·3) |
| 15 to <25 | 440 (22·3%) | 11·5 (1·9) | (6·9 to 16·6) | 172 (11·9%) | 11·1 (1·6) | (4·9 to 15·9) | 612 (17·9%) | 11·4 (1·8) | (4·9 to 16·6) |
| 25 to <35 | 182 (9·2%) | 11·7 (1·6) | (6·6 to 16·2) | 94 (6·5%) | 11·7 (1·6) | (7·5 to 15·1) | 276 (8·1%) | 11·7 (1·6) | (6·6 to 16·2) |
| 35 to <45 | 196 (9·9%) | 12·1 (1·9) | (6·5 to 17·4) | 153 (10·6%) | 12·1 (1·9) | (5·8 to 17·1) | 349 (10·2%) | 12·1 (1·9) | (5·8 to 17·4) |
| 45 to <55 | 404 (20·5%) | 12·9 (1·9) | (6·2 to 18·7) | 338 (23·4%) | 12·9 (1·9) | (5·4 to 18·1) | 742 (21·7%) | 12·9 (1·9) | (5·4 to 18·7) |
| 55 to <80 | 484 (24·5%) | 13·1 (1·9) | (7·0 to 18·1) | 508 (35·1%) | 13·6 (1·9) | (4·0 to 19·0) | 992 (29·0%) | 13·3 (1·9) | (4·0 to 19·0) |
| >=80 | 74 (3·7%) | 13·8 (1·3) | (9·9 to 16·5) | 98 (6·8%) | 14·0 (1·7) | (8·2 to 17·9) | 172 (5·0%) | 13·9 (1·5) | (8·2 to 17·9) |
| *G6PD status* |  | |  |  |  |  |  |  |  |
| Normal | 856 (43·3%) | 12·4 (1·9) | (6·5 to 18·1) | 836 (57·8%) | 12·8 (2·0) | (5·4 to 19·0) | 1692 (49·5%) | 12·6 (2·0) | (5·4 to 19·0) |
| Borderline | 3 (0·2%) | 13·9 (1·1) | (13·1 to 15·2) | 0 (0%) | - | - | 3 (0·1%) | 13·9 (1·1) | (13·1 to 15·2) |
| Deficient | 24 (1·2%) | 12·4 (1·8) | (8·6 to 15·7) | 1 (0·1%) | 14·0 (-) | (14·0 to 14·0) | 25 (0·7%) | 12·4 (1·8) | (8·6 to 15·7) |
| Not known | 1092 (55·3%) | 12·1 (2·2) | (6·0 to 18·7) | 609 (42·1%) | 12·5 (2·2) | (4·0 to 18·9) | 1701 (49·7%) | 12·2 (2·2) | (4·0 to 18·9) |
| *Relapse Periodicity* |  | |  |  |  |  |  |  |  |
| Long | 1360 (68·9%) | 12·3 (2·1) | (6·0 to 18·1) | 627 (43·4%) | 13·4 (1·9) | (4·0 to 18·9) | 1987 (58·1%) | 12·6 (2·1) | (4·0 to 18·9) |
| Short | 615 (31·1%) | 12·1 (2·0) | (6·2 to 18·7) | 819 (56·6%) | 12·2 (2·1) | (4·9 to 19·0) | 1434 (41·9%) | 12·2 (2·0) | (4·9 to 19·0) |
| *Geographical region* |  | |  |  |  |  |  |  |  |
| Asia-Pacific | 1114 (56·4%) | 11·9 (1·9) | (6·2 to 18·7) | 1133 (78·4%) | 12·5 (2·1) | (4·9 to 19·0) | 2247 (65·7%) | 12·2 (2·0) | (4·9 to 19·0) |
| The Americas | 285 (14·4%) | 12·5 (2·0) | (7·0 to 17·4) | 313 (21·6%) | 13·5 (1·8) | (4·0 to 18·9) | 598 (17·5%) | 13·0 (2·0) | (4·0 to 18·9) |
| Africa | 576 (29·2%) | 12·7 (2·2) | (6·0 to 18·1) | 0 (0%) | - | - | 576 (16·8%) | 12·7 (2·2) | (6·0 to 18·1) |

Hb – haemoglobin; SD – standard deviation; IQR – interquartile range; \* Number of patients (percentage of total patients in group) unless otherwise specified.

**Table 2. Distribution of absolute and percentage change in haemoglobin and risk of anaemia on days 2/3, 7 and 28 by treatment group·**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Any G6PD activity** | | **Normal G6PD activity** | |
| **Day and metric** | **Chloroquine alone** | **Chloroquine plus primaquine** | **Chloroquine alone** | **Chloroquine plus primaquine** |
| *Day 2/3 (number of patients)* | 610\* | 471† | 338 | 334 |
| Absolute change‡, mean (SD) [range]; g/dL | -0·5 (1·1)  [-4·6 to 3·4] | -1·1 (1·6)  [-6·4 to 5·0] | -0·9 (1·2)  [-4·6 to 3·4] | -1·2 (1·3)  [-4·2 to 5·0] |
| Percentage change‡, mean (SD) [range]; % | -3·5 (8·5)  [-32·3 to 34·3] | -8·0 (11·8)  [-39·4 to 60·8] | -6·7 (9·0)  [-32·3 to 34·3] | -8·8 (10·1)  [-39·4 to 60·8] |
| Percentage fall >25% | 7/610 (1·1%) | 27/471 (5·7%) | 7/338 (2·1%) | 11/334 (3·3%) |
| >25% fall associated with severe anaemia (%)§ | 0/610 (0%) | 1/471 (0·2%) | 0/338 (0%) | 1/334 (0·3%) |
| Absolute fall >5 g/dL¶ | 0/610 (0%) | 6/471 (1·3%) | 0/338 (0%) | 0/334 (0%) |
| *Day 7 ± 2* | 1222 | 539 | 608 | 389 |
| Absolute change‡, mean (SD) [range]; g/dL | -0·1 (1·2)  [-5·6 to 6·5] | -1·0 (1·6)  [-7·3 to 5·4] | -0·2 (1·4)  [-4·6 to 6·5] | -1·0 (1·5)  [-7·3 to 5·4] |
| Percentage change‡, mean (SD) [range]; % | 0·4 (10·5)  [-40·6 to 64·4] | -6·5 (12·3)  [-55·3 to 65·5] | -0·7 (11·3)  [-33·0 to 64·4] | -7·3 (11·5)  [-55·3 to 65·5] |
| Percentage fall >25% | 5/1222 (0·4%) | 33/539 (6·1%) | 4/608 (0·7%) | 20/389 (5·1%) |
| >25% fall associated with severe anaemia (%)§ | 0/1220 (0%) | 1/539 (0·2%) | 0/608 (0%) | 1/389 (0·3%) |
| Absolute fall >5 g/dL¶ | 1/1222 (0·1%) | 8/539 (1·5%) | 0/608 (0%) | 4/389 (1·0%) |
| *Day 28 ± 3* | 1579 | 917 | 731 | 472 |
| Absolute change‡, mean (SD) [range]; g/dL | 0·5 (1·4)  [-6·9 to 6·2] | 0·4 (1·7)  [-6·8 to 6·7] | 0·4 (1·5)  [-4·7 to 6·2] | 0·4 (1·4)  [-4·2 to 6·2] |
| Percentage change‡, mean (SD) [range]; % | 5·0 (13·4)  [-46·3 to 81·7] | 5·1 (16·5)  [-51·1 to 136·7] | 4·2 (13·1)  [-39·5 to 74·7] | 4·2 (12·8)  [-36·2 to 74·8] |
| Percentage fall >25% | 9/1579 (0·6%) | 13/917 (1·4%) | 6/731 (0·8%) | 2/472 (0·4%) |
| >25% fall associated with severe anaemia (%)§ | 1/1576 (0·1%) | 3/906 (0·3%) | 0/730 (0%) | 0/472 (0%) |
| Absolute fall >5 g/dL¶ | 1/1579 (0·1%) | 3/917 (0·3%) | 0/731 (0%) | 0/472 (0%) |

CI = Confidence interval; Hb – haemoglobin; No. – number; SD – standard deviation; \* Includes 338 patients with normal G6PD activity, 258 with unknown activity and 14 with borderline or deficient activity; † Includes 334 patients with normal G6PD activity and 137 with unknown activity; ‡ Results are reported as a change in haemoglobin, with positive results reflecting a rise in Hb and negative results reflecting a fall in Hb; § Patients were considered to develop severe anaemia if their baseline Hb was ≥7 g/dL and their follow up Hb was <7 g/dL, with the denominator the number of people with a Hb recorded for that day who had a baseline ≥7 g/dL. All patients that developed severe anaemia had a Hb fall >25%. Table S7 provides additional patient details; ¶ Table S8 provides additional patient details.