Title

Supervised versus unsupervised primaquine radical cure for the treatment of falciparum and vivax malaria in Papua, Indonesia: a cluster randomised, controlled, open-label superiority trial

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Summary

Background

In co-endemic areas there is a high risk of *P. vivax* recurrence in patients treated for *P. falciparum* malaria. Primaquine radical cure has the potential to reduce *P. vivax* recurrences in patients presenting with *P. falciparum* as well as *P. vivax* malaria, but is undermined by poor adherence to the currently recommended 14 day regimen. We assessed the effectiveness of supervised primaquine radical cure in patients presenting with uncomplicated malaria.

Methods

We conducted a cluster-randomised, controlled, open-label superiority trial in Papua, Indonesia. Twenty one clusters (village health post matched by location, size and malaria transmission) were assigned randomly (1:1) by a statistician independent to the trial to treat patients presenting with uncomplicated *P. falciparum* or *P. vivax* malaria with dihydroartemisinin-piperaquine plus either a supervised or unsupervised 14-day course of primaquine (0.5mg/kg per day). Patients were followed for 6 months and those representing with malaria were retreated with the same drug regimen. The primary outcome was the incidence risk and rate of *P. vivax* recurrent episodes over 6 months assessed in the intention to treat population. The study is registered at ClinicalTrials.gov (NCT 02787070).

Findings

Between September 2016 and July 2018, 419 patients were enrolled. Overall the incidence risk of *P. vivax* recurrence in the 10 unsupervised clusters was 55.8% (95%CI, 32.3-81.8) compared to 29.7% (95%CI, 16.4-49.9) in the 11 supervised clusters (HR 0.23, 95%CI, 0.07-0.76, p=0.016). The incidence rate in unsupervised clusters was 859 (95%CI, 673-1,096) *P. vivax* infections per 1000 person years compared to 539 (95%CI, 390-747) in the supervised

clusters (IRR 0.63, 95%CI, 0.42-0.94, p=0.025). The corresponding rates in the 224 patients presenting with *P. falciparum* malaria were 660 (95%CI, 446-977) and 346 (95%CI, 213-563); IRR 0.52 (95%CI, 0.28-0.98), p=0.043. There were no serious adverse events attributable to primaquine.

Interpretation

In this area of moderate malaria transmission, supervision of primaquine radical cure reduced the risk of *P. vivax* recurrence and this was apparent for patients presenting with either *P. falciparum* or *P. vivax* malaria.

Funding

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Research in context

Evidence before this study

We searched all publications in English language on PubMed, MEDLINE, Web of Science, Embase, and the Cochrane Database of Systematic Reviews from Jan 1, 1960 until May 31, 2016 for relevant clinical trials and systematic reviews of the risk of *P. vivax* recurrence following *P. falciparum* infections using the search terms: "vivax", "falciparum" and "recurrence". The risk of *P. vivax* recurrence within 63 days of treatment for *P. falciparum* malaria exceeded 15% across a spectrum of co-endemic areas, highlighting a potential benefit of primaquine radical cure for *P. falciparum* malaria. The risk of *P. vivax* within 12 months was <10% following supervised high dose primaquine (total dose 7mg/kg) plus dihydroartemisinin-piperaquine, but rose to >88% when primaquine was unsupervised. No trials were identified to determine the efficacy or effectiveness of supervised 14 days primaquine treatment for both vivax and falciparum malaria.

Added value of this study

Our cluster randomized controlled trial demonstrated that alternate daily supervision of primaquine radical cure reduced the risk of *P. vivax* recurrence in patients presenting with either *P. vivax* or *P. falciparum*.

Implications of all available evidence

In areas of moderate malaria transmission, active measures to ensure patient adherence to a complete course of primaquine radical cure and extending its use to all patients presenting with uncomplicated malaria due to either *P. vivax* or *P. falciparum*, has potential to reduce

the risk of recurrent parasitaemia, impacting on ongoing transmission and facilitating malaria elimination.

Introduction

There are between 7.5 and 14.5 million cases of *Plasmodium vivax* malaria worldwide reported each year from 49 endemic countries [1, 2]. The greatest burden of disease is in Asia, where *P. vivax* is becoming the predominant cause of malaria [3]. *P. vivax* is more difficult to eliminate than *P. falciparum*, since it forms dormant liver stages (hypnozoites) that can reactivate weeks to months after an initial infection causing recurrent symptomatic illness (relapses)[4]. The risk and frequency of relapse varies considerably with geographical location, occurring in up to 80% of patients from equatorial regions [4]. Frequent, recurrent *P. vivax* parasitaemia results in a cumulative risk of anaemia, and both direct and indirect morbidity and mortality particularly in young children and pregnant women [5-9]. Relapsing infections are also key contributors to the ongoing transmission of the parasite [10].

The radical cure of malaria refers to a combination of drugs to kill both the blood and liver stages of the parasite. Primaquine (PQ), an 8-aminoquinoline, is currently the only widely available drug that can kill *P. vivax* liver stages and prevent relapse. Supervised administration of artemisinin combination therapy (ACT) and a high dose of primaquine reduces the risk of recurrence, even in areas with high relapse periodicity [11], but its use is limited by the risk of severe haemolysis in patients with glucose-6-phosphate-dehydrogenase (G6PD) deficiency [12]. To improve its tolerability, the total dose of primaquine is usually spread over 14-days, however unsupervised adherence to such a prolonged regimen is poor [13, 14].

Radical cure of malaria is currently restricted to patients presenting with *P. vivax* or *P. ovale*, the only species causing human malaria that form hypnozoites [15]. However a recent systematic review and individual patient data meta-analysis highlight a high risk of *P. vivax* parasitaemia in patients recently treated for *P. falciparum* malaria [16, 17]. We hypothesised that recurrent parasitaemia with *P. vivax* originated from reactivation of occult hypnozoites in patients exposed to both species, potentially triggered by the acute febrile illness of *P. falciparum* malaria [17]. In view of this, extending the indication for primaquine radical cure to patients presenting with all species of malaria has potential to reduce recurrent malaria and accelerate malaria elimination in co-endemic regions [16-18]. We also hypothesized that alternate day supervision of 14 days primaquine would improve treatment adherence and thus reduce the rate of *P. vivax* recurrence compared to the current practice of unsupervised treatment and that this would be apparent in patients presenting with either *P. vivax* or *P. falciparum* uncomplicated malaria.

Methods

Trial Design

We conducted a cluster-randomised, controlled, open-label superiority trial in G6PD-normal patients presenting with uncomplicated falciparum or vivax malaria to outpatient healthcare facilities in Papua province, Indonesia. The climate, geography, malaria endemicity and demographics of the study site have been described previously [19]. In brief, the study area lies in the lowland area of south-central Papua, Indonesia, and has perennial malaria transmission with approximately half of the malaria infections due to *P. vivax* [19, 20].

The unit of randomization was the village health post. A total of 21 village health posts were randomized. Each health post was associated with one of eight public clinics where initial

recruitment and treatment were provided. Clinics were selected to be located within 1.5 hours drive from the research office and from those that treated more than 200 cases of malaria per year (Supplementary material 1).

Ethical approval was obtained from the Human Research Ethics Committee of the Northern Territory Department of Health, Australia (HREC 15.2517) and the Health Research Ethics Committees of the University of Gadjah Mada, Indonesia (KE/FK/522/EC/2016). The trial was registered with the US National Library of Medicine (ID: NCT02787070).

Participants

Consenting febrile patients attending one of the 8 public clinics with suspected malaria were screened for malaria by microscopic examination of Giemsa stained peripheral blood film by a laboratory microscopist at the public clinics. All slides were re-read the same day by an expert trial microscopist and those patients with confirmed uncomplicated falciparum or vivax malaria (including either mono or mixed infections), who were aged > 12 months and weighed > 5kg, were eligible for enrolment. Patients were excluded if they were pregnant or lactating, G6PD deficient (determined by fluorescent blood spot test - FST), anaemic (haemoglobin (Hb) level < 9 g/dL) or had signs of severe malaria. Other exclusion criteria included hypersensitivity to study drugs, or concomitant medication with potential to cause haemolysis or interfere with the pharmacokinetics of the study drugs.

Randomisation

The 21 clusters were matched according to location, size and malaria transmission. Individual clusters were allocated randomly 1:1 to either the supervised or unsupervised primaquine

protocol using Stata v15.1 (StataCorp, College Station, Tx USA). The independent statistician who generated the randomisation list and allocated clusters to treatment groups was not otherwise involved in the conduct of the trial and did not visit any of the study sites.

Procedures

After obtaining informed consent, eligible participants were enrolled and a baseline clinical questionnaire and examination undertaken at the public clinic. All patients were immediately commenced on a three day regimen of oral dihydroartemisinin-piperaquine (DHP) according to national guidelines (Supplementary material 2). All patients were reviewed on days 1 and 2 at the village health posts or at home, to ensure supervised schizontocidal treatment and symptom recovery. On day 2 participants had a repeat finger prick for Hb concentration and if this was ≥9g/dL they were prescribed primaquine according to the study protocol.

Patients residing in the clusters randomized to unsupervised treatment were prescribed a course of primaquine according to local treatment guidelines, at a daily dose of 0.5mg/kg/day, and instructed to take the prescribed tablets once a day for 14 days (Supplementary material 2). In the clusters randomized to supervised treatment, patients were prescribed the same primaquine regimen, but were visited on alternate days by a home visitor who provided them with primaquine tablets for that and the following day. On day 16 all patients were instructed to return either to village health post or were visited at home for clinical review. Patients in the unsupervised arm were asked to return any remaining primaquine tablets for a pill count. In the supervised arm primaquine adherence was based on direct observation on the days of supervised administration and self-reported adherence for the previous unsupervised day. In the unsupervised arm adherence to primaquine was

estimated from the pill count conducted on day 16. Thereafter all participants were followed up on day 28 and then monthly for 6 months.

At each visit, a medical history and symptom questionnaire was completed and any adverse events (AEs) or serious adverse events (SAEs) were recorded. Patients were encouraged to present to the study centre if they became ill. At each routine review or presentation with symptoms compatible with malaria, a capillary blood sample was taken for peripheral blood film examination and measurement of Hb concentration (HemoCue Hb 201^{+}™, Ängelholm, Sweden). Blood film microscopy during follow up was conducted by the study laboratory technicians blinded to treatment allocation.

Patients representing with recurrent episodes of malaria were treated with the same treatment allocation as at enrolment and follow-up continued for a total of 6 months from the day of enrolment. Female patients with recurrence were reviewed for their pregnancy status.

Outcomes

The primary objective of this study was to assess the effectiveness of unsupervised compared to supervised 14-day primaquine treatment in preventing recurrent *P. vivax* parasitaemia in G6PD normal patients presenting with uncomplicated malaria due to *P. vivax* or *P. falciparum* mono-infection or a mixed infection with both species. The co-primary endpoints were the incidence risk of the first recurrent episode of *P. vivax* parasitaemia and the incidence rate of all recurrent episodes of *P. vivax* parasitaemia over 6 months in patients with uncomplicated malaria due to either *P. vivax* and/or *P. falciparum*.

Secondary objectives included assessing the co-primary outcomes amongst those initially presenting with *P. vivax* (either mono infection or mixed) and amongst those initially presenting with *P. falciparum*. Safety endpoints were the vomiting of medication within an hour of administration, experience of symptoms in the supervised arm during primaquine treatment, and adverse events (AEs) within 6 months. Haematological safety endpoints were the incidence risk of severe anaemia (Hb<7g/dl) or transfusion over 6 months, and an acute drop in Hb >5g/dl or fractional fall of >25% in Hb to Hb less than 7 g/dl within 14 days of starting primaquine treatment.

Sample size and statistical analysis

The required sample size was calculated to detect an absolute reduction of 20% in the incidence risk of *P. vivax* recurrence at 6 months from 30% in the unsupervised arm to 10% in supervised arm. Across the 21 clusters, a sample size of 420 participants (20 per cluster) provided 90% power to detect this difference with a two-sided significance level of 5%, assuming 15% loss to follow-up, and a conservative intra-cluster correlation coefficient (ICC) of 0.05 [21].

The combined data from all clusters were analysed to provide a pragmatic comparison of the different treatments using a modified intention-to-treat (ITT) strategy, with analyses conducted for the randomised trial arms, regardless of whether participants were actually supervised or not. Descriptive statistics of patient and disease characteristics at baseline were calculated by intervention arm with frequency and percentage presented for categorical variables, and median with corresponding inter-quartile range (IQR) and range for continuous variables. A clinically relevant fall in Hb was defined as an absolute fall from baseline of >5g/dl or a fractional fall >25% to Hb less than 7g/dl. Overall adherence to primaquine was

evaluated by calculating the proportion of participants in each arm who received a total dosage of at least 5mg/kg of primaquine.

Kaplan-Meier curves were produced to visualise the cumulative incidence risk of the first *P. vivax* recurrence over 6 months in each treatment arm. For the co-primary endpoints comparing supervised versus unsupervised 14-day primaquine: hazard ratios (HR, 95% Confidence Intervals (CI)) were estimated using mixed-effects Cox proportional hazards regression with a time-varying coefficient (to account for clustering and non-proportional hazards respectively) for time to first *P. vivax* recurrence analyses and incidence rate ratios (IRRs) were estimated using negative binomial regression with robust standard error estimation (to account for clustering) for analysis of all *P. vivax* recurrences. Participants missing two or more consecutive months of blood film examination had that period deducted from the total observation time for the incidence rate analyses. Details regarding censoring for the first recurrence of *P. vivax* recurrence, and safety analyses are provided in an a priori statistical plan (Supplementary material 3). All analyses were undertaken using STATA v15.1 (StataCorp LLC, College Station, Tx USA).

Results

The study was conducted over 22 months with the first recruitment on 14th September 2016, the last patient enrolled on 13th February 2018 and the last follow up visit on 31st July 2018. Between September 2016 and July 2018, 436 patients were screened for inclusion and 419 were enrolled into the study: 196 patients in 10 clusters were randomised to unsupervised treatment (PQUnsup), and 223 in 11 clusters for supervised treatment (PQsup); Figure 1. At enrolment 224 (53%) participants were infected with *P. falciparum* mono-infection, 183 (44%) with *P. vivax* mono-infection and 12 (3%) with a mixed species infection. At

presentation the median peripheral asexual parasitaemia was 4,931 parasites μl^{-1} (IQR: 994-12,281) for patients with *P. falciparum* mono-infection, 4,688 μl^{-1} (IQR: 1,313-11,063) for those with *P. vivax* mono-infection and 12,544μl^{-1} (IQR: 6,750-15,656) for those with mixed infections. Baseline characteristics were similar between the supervised and unsupervised treatment arms; Table 1. In total seven patients (five in the PQSup arm and two in the PQUnsup arm) were lost to follow up immediately after their enrolment visit and excluded from further analysis of recurrence outcomes.

The initial response to schizontocidal treatment was rapid. Within 48 hours, 98.5% (399/405) of patients had become afebrile and 92.6% (375/405) had cleared their peripheral parasitaemia, with no significant difference in fever or parasite clearance between treatment arms. Parasite clearance was faster in patients with *P. vivax*, with 95.3% (181/190) of patients becoming aparasitaemic within 24 hours compared to 90.2% (194/215) of those with *P. falciparum* infection. On day 2 review, 95.5% (213/223) patients in the PQSup and 97.4% (191/196) of those in the PQUnsup with Hb $\geq$ 9 g/dl were prescribed primaquine at a median total dose of 7.4mg/kg (range: 3.5 – 15.4) and 7.4mg/kg (range: 4.3 – 14.2) respectively. Overall 161 (72.2%) of patients in PQSup arm completed 6 months of follow up compared to 151 (77.0%) of patients in the PQUnsup arm.

Primary outcomes

At 6 months (day 180) the cumulative incidence of *P. vivax* was 29.7% [95% CI: 16.4%, 49.9%] in the PQSup arm and 55.8% [95% CI: 32.3%, 81.8%] in the PQUnsup arm; Figure 2. Since the Kaplan-Meier survival curves crossed at approximately 60 days, the comparative risk of *P. vivax* recurrence was estimated using a time-varying Hazard Ratio (HR). At 6 months, the overall hazard of *P. vivax* recurrence was significantly lower in the PQSup arm

316 compared to the PQUnsup arm (HR=0.23 [95% CI: 0.07, 0.76]; p=0.016). At day 60 the HR
317 was 0.78 [95% CI: 0.39, 1.55; p=0.480], and 0.43 [95% CI: 0.22, 0.80; p=0.008] at day 120;
318 Table 2.
319
320 At day 28, 4 (0.1%) patients had recurrent parasitaemia, 3 of whom were infected with *P.*
321 *falciparum* and 1 with *P. vivax* and none with mixed infections. By 6 months these numbers
322 had risen to 95, 110 and 6 respectively; Figure 3. The incidence rate was 539 [95%CI: 390,
323 747] *P. vivax* infections per 1000 person-year observed in the PQSup arm compared to 859
324 [95%CI: 673, 1096] in the PQUnsup arm (incidence rate ratio (IRR) of 0.63 [95% CI: 0.42,
325 0.94], p=0.025); Table 2. Overall 62.9% (73/116) of *P. vivax*/mixed recurrences and 70.5%
326 (67/95) of *P. falciparum* recurrences were symptomatic at the time of representation to the
327 clinic.
328
329 *Secondary outcomes*
330 A priori subgroup analyses compared the rate of *P. vivax* recurrences between treatment arms
331 according to the species of infection at presentation. In the 195 patients initially presenting
332 with *P. vivax* infection (mono or mixed), the hazard for the first *P. vivax* recurrence at 6
333 months was significantly lower in PQSup arm compared to the PQUnsup arm (HR = 0.15
334 [95% CI: 0.02, 0.95], p=0.045); Figure 3. Although the incidence rate of all *P. vivax*
335 recurrences was also lower this did not reach statistical significance (IRR = 0.71 [95% CI:
336 0.42, 1.20], p=0.199); Table 2. In the 224 patients who initially presented with *P. falciparum*
337 the corresponding HR at 6 months was 0.33 (95% CI: 0.08, 1.44; p=0.139) and the IRR was
338 0.52 (95% CI: 0.28, 0.98, p=0.043).
339

Post-hoc analyses examined the comparative risks of *P. falciparum* recurrence. The cumulative incidence risk of first *P. falciparum* parasitaemia at 6 months was 27.1% [95%CI: 21.2%, 34.2%] in the PQSup arm and 36.4% [95% CI: 21.8%, 56.5%] in the PQUnsup arm; the corresponding incidence rates for all *P. falciparum* infections of 646 [95% CI: 503, 830] and 544 [95% CI: 405, 730] per 1000 person-year, respectively. Neither the incidence risk or rate of *P. falciparum* differed significantly between treatment arms.

Adherence to treatment

The total dose of primaquine administered was calculated in all 223 patients in the PQSup arm and 155 (79.1%) of patients in the PQUnsup arm. Although the median total prescribed dose of primaquine was similar between arms (7.3mg/kg (IQR: 6.1 to 8.2) and 7.1mg/kg (IQR: 5.7 to 8.0) respectively), only 64.3% (126/196) in the PQUnsup arm took ≥5mg/kg, compared to 89.2%, (199/223) in the PQSup arm.

Haematological Recovery

Overall the mean Hb concentration fell from 11.9g/dl (95% CI: 11.7, 12.1) at baseline to 11.2g/dl (95% CI: 11.1, 11.4) on day 2, prior to administering the first dose of primaquine. The fractional fall in Hb on day 2 was 5.9% (95% CI: 4.3%, 7.5%) in patients infected with *P. falciparum* compared to 4.2% (95% CI: 2.4%, 5.9%) in patients with *P. vivax;* p=0.151. On day 16, after patients had completed primaquine treatment, the mean Hb of patients in the PQSup arm was 11.5g/dL (95% CI: 11.3, 11.7) with 5.6% (11/195) of patients having a fractional fall from baseline >25%. The corresponding figures for patients in the PQUnsup arm were 11.6g/dL (95% CI: 11.4, 11.8) and 4.7% (8/171) respectively. Three patients in each treatment arm, had a fall in Hb from baseline greater than 5g/dl (four with *P. falciparum* infections and two with *P. vivax)*. A 15 year old male had a clinically significant fall in Hb.

He presented with falciparum malaria and was treated with PQSup, but his Hb fell from 9.5 g/dl at baseline to 6.7g/dl on day 16 (Figure 4). He remained asymptomatic and did not require hospitalization, and by day 28 his Hb had risen to 9g/dl without the need for blood transfusion. The haematological profiles during follow up by treatment arm are presented in supplementary figure 4. After day 16, 39.3% (77/196) of patients treated with PQUnsup had at least one episode of anaemia (Hb<10g/dl), compared to 34.2% (76/222) of the patients treated with PQSup.

Tolerability

Since patients in the PQUnsup arm had no routine clinical review between day 2 and day 16, early primaquine tolerability within these days was confined to patients in the PQSup arm. None of the patients in the PQSup arm reported vomiting their primaquine dose within 1 hour of administration. However, 10.7% (23/215) had gastrointestinal symptoms during their course of primaquine treatment; Table 3.

Seven serious adverse events (SAEs) were reported, none of which were related to DHP or primaquine treatment. Four patients were admitted with complications of acute falciparum malaria either at the initial or subsequent presentations and three patients were hospitalized due to non-malaria cases (Supplementary material 5). No patients died or required blood transfusion.

Discussion

Our study demonstrates that supervision of primaquine on alternate days resulted in significantly better efficacy than the current practice of unsupervised primaquine, reducing the risk of any recurrence by 77% and the rate of recurrences by 37%. Importantly the

benefits of supervised primaquine were apparent in patients presenting with either *P. vivax* or *P. falciparum* malaria.

Previously we have shown that 10-15% of patients presenting with either *P. vivax* and *P. falciparum* treated with DHP alone have recurrent *P. vivax* within 42 days [22]. In the current trial the addition of primaquine reduced the risk of *P. vivax* almost 3-fold at a similar time point and this was apparent in patients treated with either supervised or unsupervised primaquine. Although a recent large scale population analysis estimated that the effectiveness of unsupervised primaquine in routine clinical practice was only 12% [10], the effectiveness of unsupervised primaquine in our current study was considerably higher. It is likely that selection and consent of patients into a formal clinical trial and supervision of the initial three days of schizontocidal therapy may have enhanced patient adherence to a full treatment course despite minimal supervision of treatment thereafter. This raises the possibility that supervision of the first dose of primaquine and provision of education may provide a pragmatic approach to improving treatment adherence.

In the current study patient follow up was continued for 6 months with each recurrence treated with the same regimen. At the end of follow up there were significantly more *P. vivax* recurrences in those receiving unsupervised compared to supervised primaquine (859 vs 539 per 1000 patient years, p=0.025). For individuals residing in an endemic setting recurrent malaria can be due to recrudescence (schizontocidal failure), reinfection from a new mosquito bite or relapse from hypnozoite reactivation. Although we were unable to distinguish between these scenarios, recrudescent infections are likely to be only a minor factor, since they usually occur within 63 days, and recent efficacy trials and genomic analyses have found no evidence of parasite resistance to either artemisinin or piperaquine [23].

Since the clusters were matched according to the level of malaria transmission, the risk of reinfection is likely to have been similar between treatment arms. The most likely explanation is higher anti-relapse efficacy in the supervised arms and this is supported by the higher proportion of patients taking a total dose of primaquine ≥5mg/kg. This concurs with pooled analyses of longitudinal cohorts that have shown relapses account for more than 60-90% across a range of endemic settings [24].

Whilst the primary outcome of the study was the overall risk of recurrence between the supervised and unsupervised arms, a key finding is the significant benefit of providing effective radical cure to patients presenting with *P. falciparum* mono-infection. Antimalarial guidelines currently restrict the use of primaquine in patients presenting with acute *P. falciparum*, to a single dose of PQ (0.25mg/kg) for its gametocytocidal activity and reduction of transmission [25]; however this regimen has no activity against *P. vivax* hypnozoites. In our study patients presenting with *P. falciparum* were treated the same as those presenting with *P. vivax*, with a 14-day high dose regimen. By 6 months the risk of *P. vivax* following initial *P. falciparum* was 47.1 % in the unsupervised arm and 29.5% in the supervised arm, equating to a 67% reduction in the risk of *P. vivax* and a 48% reduction in the rate of recurrences, consistent trends to those presenting with *P. vivax*. In the study site the incidence of malaria is approximately 250 cases per 1000 population per year for both *P. falciparum* and *P. vivax* and recurrent episodes of vivax and falciparum malaria are common, suggesting high burden of latent hypnozoite carriage in all patients presenting with malaria [6, 10, 19]. Our study suggests that opportunistically targeting patients with *P. falciparum* who are at high risk of carrying occult hypnozoites, can reduce recurrent clinical illness and potentially ongoing transmission [26]. Indeed the observed benefits of primaquine radical cure for patients with *P. falciparum* are

likely to be conservative, since they represent comparison of complete adherence to partial adherence to 14 day PQ, rather than to current practice of single dose PQ.

In G6PD normal patients, the high dose 14-day PQ regimen was well tolerated and not associated with severe adverse complications. Six patients had a fall in Hb greater than 5 g/dl, four of whom presented with *P. falciparum* infection (Figure 4), which may have been exacerbated by concomitant administration of primaquine. However, these events occurred in patients who presented with Hb greater than 14g/dl, none of whom became clinically unwell and their Hb level remained normal until the end of follow up. A 15 year old male with falciparum malaria had a fall in Hb to less than 7g/dl from initial Hb of 9.5g/dl, but he remained asymptomatic and his Hb recovered quickly. Acute malaria results in inevitable parasite induced haemolysis and this is usually greater following *P. falciparum* infection than *P. vivax*, but haematological recovery typically occurs within 4-6 weeks [27, 28]. Previous pooled analyses show that although primaquine can cause an initial excess reduction in Hb, recovery is usually rapid and often offset by a reduction in subsequent recurrent parasitaemia and further parasite induced haemolysis [27]. In the current study fewer people in the supervised primaquine arm developed anaemia during follow up than in the unsupervised arm.

Gastrointestinal intolerance is an acknowledged adverse side effect of primaquine, and although dose related, can be reduced by concomitant administration of food. Less than 5% of patients vomited their dose of primaquine within one hour of administration, and subsequent rates of vomiting after clinical recovery were even lower. Two G6PD normal patients reported dark urine, but subsequent investigation demonstrated that the dark colour

was due to dehydration rather than haematuria. None of the patients required hospitalization due to primaquine treatment (Supplementary material 5).

Our study has a number of limitations. Follow up was incomplete with only 72-77% of participants contributing to the primary endpoints. The loss to follow up was mainly attributable to the high patient mobility of migrant workers. This might have selected patients with a more positive attitude towards treatment adherence and thus underestimated the true impact of the intervention. The intervention in the unsupervised treatment clusters provided more consultation time with the study staff compared to those practiced in real life [10] and this is likely to have resulted in a Hawthorne effect, modifying patients' behaviour and higher than expected treatment adherence [29, 30], both of which will also have underestimated the impact of the intervention. A further limitation was the significantly higher risk of *P. falciparum* infections in the supervised arm compared to the unsupervised arm, potentially reflecting greater heterogeneity between clusters than expected. A higher intra-cluster correlation coefficient than predicted may have led to the study being underpowered particularly for the secondary analyses.

In conclusion, partial supervision of 14-day primaquine treatment enhanced adherence and reduced recurrent *P. vivax* infection significantly and this was apparent in patients presenting with either *P. vivax* of *P. falciparum* malaria. Extending the use of safe and effective primaquine radical cure to patients presenting with non-vivax malaria, should be considered, but will need to be tailored to areas with a high risk of *P. viva*x after *P. falciparum* [17]. Since alternate day supervision may not be feasible in routine clinical practice, particularly in areas with high case numbers, more parsimonious strategies should be explored that can combine patient education and early clinical review to promote adherence or curtail treatment if signs

488 of an impending haemolysis. These strategies may have particular relevance to implementing
489 high dose 7 day primaquine regimens [31]. Ultimately greater access to safe and effective
490 radical cure has potential to reduce the burden of *P. vivax* substantially paving the way for
491 ambitious malaria elimination targets to be met.
492
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Article information

Contributors

JRP, KT, BL, RNP were responsible for the conception of the study. JRP, EK, FHB, FC, RI, BL, were responsible for the data collection and cleaning. JRP, EK, FHB, FC, RI, LT and BL were responsible for the clinical trials oversight. NM, DJP, JAS, RNP and JRP did the data analyses and interpretation. JRP, NM, DJP, JAS, RNP wrote the first draft of the manuscript. All authors approved the final submitted version and agreed to publication.

Declaration of Interest

No conflict of interests reported.

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Data Availability Statement: The data are available for access via the WorldWide Antimalarial 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. The WWARN is registered with the Registry of Research Data Repositories (re3data.org).

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Tables

Table 1: Baseline characteristics

	PQSup	PQUnsup	Total
	N=223	N=196	N=419
Sex[a, c]			
Male	122 (55%)	101 (52%)	223 (53%)
Female	101 (45%)	94 (48%)	195 (47%)
Age (years)[b, c]	16.8 (7.8-32.3) [1.0-65.0]	18.0 (7.4-35.5) [1.1-72.8]	17.2 (7.4-33.1) [1.0-72.8]
Age group[a, c]			
<5 years	39 (17%)	36 (18%)	75 (18%)
5 to <15 years	59 (26%)	47 (24%)	106 (25%)
≥15 years	120 (54%)	110 (56%)	230 (55%)
Weight (kg)[b]	46.9 (22.2-58.1) [6.8-92.1]	48.3 (18.9-56.7) [7.4-95.4]	47.8 (20.0-57.5) [6.8-95.4]
Weight group[a]			
<9kg	8 (4%)	6 (3%)	14 (3%)
9 to <18kg	39 (17%)	37 (19%)	76 (18%)
18 to <36kg	42 (19%)	32 (16%)	74 (18%)
≥36kg	134 (60%)	121 (62%)	255 (61%)
Ethnicity[a, c]			
Non-Papuan	128 (57%)	99 (51%)	227 (54%)
Highland Papuan	56 (25%)	25 (13%)	81 (19%)
Lowland Papuan	39 (17%)	71 (36%)	110 (26%)
History of malaria in the last 28 days[a]			
No	204 (91%)	184 (94%)	388 (93%)
Yes	5 (2%)	1 (1%)	6 (1%)
Unsure	14 (6%)	11 (6%)	25 (6%)
Species of infection[a]			
Plasmodium falciparum	120 (54%)	104 (53%)	224 (53%)
Plasmodium vivax	97 (43%)	86 (44%)	183 (44%)
Mixed Infection	6 (3%)	6 (3%)	12 (3%)
Asexual *P. falciparum* Parasitaemia (per µL blood)[b, c]	4,350 (750-12,900) [38-710,474]	5,738 (1,275-11,738) [75-143,573]	4,931 (994-12,281) [38-710,474]
Proportion with *P. falciparum*[a]	126 (57%)	110 (56%)	236 (56%)
Asexual *P. vivax* Parasitaemia (per µL blood)[b]	4,538 (1,838-10,425) [75-149,514]	5,063 (1,125-11,888) [75-113,944]	4,688 (1,313-11,063) [75-149,514]
Proportion with *P. vivax*[a]	103 (46%)	92 (47%)	195 (47%)
Asexual *P. falciparum & P. vivax* Parasitaemia (per µL blood)[b]	9,656 (2,400-13,650) [188-58,344]	14,156 (11,663-16,425) [3,900-72,511]	12,544 (6,750-15,656) [188-72,511]
Gametocytaemia (per µL blood)[b, d]	113 (75-150) [38-375]	150 (75-188) [38-1913]	113 (75-188) [38-1913]
Temperature (°C)[b]	36.6 (36.0-37.8) [36.0-41.3]	36.8 (36.2-38.0) [36.0-42.2]	36.7 (36.1-37.9) [36.0-42.2]
Fever[a]			
<37.5°C	156 (70%)	125 (64%)	281 (67%)
≥37.5°C	66 (30%)	71 (36%)	137 (33%)

Haemoglobin (g/dL)[b]	11.7 (10.2-13.7) [9.0-21.4]	11.4 (10.3-12.9) [8.5-16.6]	11.5 (10.3-13.3) [8.5-21.4]

Note: [a] Categorical data are presented as number/valid data (%); [b] Continuous data are presented as median (interquartile range) [range]; [c] Missing data for <1% observations; [d] - Missing observations: 181 for PQSup and 152 for PQUnsup.

Table 2. Cumulative incidence risk of first *P. vivax* recurrence and incidence rate of all *P. vivax* recurrences during follow up

	PQSup	PQUnsup		P value
Overall				
Cumulative Incidence Risk (%)			**Hazards Ratio**	
Day 60	5.2 [2.8, 9.5]	5.9 [3.2, 10.7]	0.78 [0.39-1.55]	0.480
Day 120	14.9 [10.4, 20.9]	25.3 [19.2, 32.9]	0.43 [0.22-0.80]	0.008
Day 180	29.7 [16.4, 49.9]	55.8 [32.3, 81.8]	0.23 [0.07-0.76]	0.016
Incidence Rate (per 1000 person years)			**Incidence Rate Ratio**	
Day 180	539 [390, 747]	859 [673, 1,096]	0.63 [0.42-0.94]	0.025
Patients presenting with *P. vivax* (mono or mixed infections)				
Cumulative Incidence Risk (%)			**Hazards Ratio**	
Day 60	5.9 [2.5, 13.5]	5.1 [2.0, 13.1]	0.82 [0.33-2.08]	0.680
Day 120	19.6 [12.5, 30.0]	37.1 [26.9, 49.7]	0.35 [0.14-0.89]	0.027
Day 180	29.5 [16.9, 48.2]	47.1 [33.9, 62.5]	0.15 [0.02-0.95]	0.045
Incidence Rate (per 1000 person years)			**Incidence Rate Ratio**	
Day 180	778 [512, 1,182]	1,095 [807, 1,485]	0.71 [0.42-1.20]	0.199
Patients presenting with *P. falciparum* (mono-infections)*				
Cumulative Incidence Risk (%)			**Hazards Ratio**	
Day 60	4.7 [2.0, 11.0]	6.6 [3.0, 14.1]	0.76 [0.31-1.89]	0.556
Day 120	11.0 [6.2, 19.0]	16.0 [9.8, 25.5]	0.50 [0.24-1.05]	0.068
Day 180	31.5 [11.0, 70.8]	56.4 [26.1, 89.7]	0.33 [0.08-1.44]	0.139
Incidence Rate (per 1000 person years)			**Incidence Rate Ratio**	
Day 180	346 [213, 563]	660 [446, 977]	0.52 [0.28-0.98]	0.043

Note: * Because of zero events in some clusters, it was not possible to include a shared frailty term for the Cox regression model in the *P. falciparum* subgroup.

Table 3. Safety endpoints

Time Point	Outcome measure	PQSup		PQUnsup	
		N=223		N=196	
1 hour	Vomiting study drug within 1 hour	7/222 (3.2%)		11/190 (5.8%)	
		Day 2 or 16	Inclusive*	Day 2 or 16	Inclusive
Days 2-16 [1]	Vomiting	1/212 (0.5%)	4/215 (1.9%)	1/193 (0.5%)	..
	Headache	19/212 (9.0%)	36/215 (16.7%)	21/193 (10.9%)	..
	Nausea	6/212 (2.8%)	6/215 (2.8%)	7/193 (3.6%)	..
	Diarrhoea	1/212 (0.5%)	4/215 (1.9%)	0/193 (0.0%)	..
	Skin Rash or Itching	0/212 (0.0%)	1/215 (0.5%)	1/193 (0.5%)	..
	Poor appetite	3/212 (1.4%)	10/215 (4.7%)	4/193 (2.1%)	..
	Abdominal Pain	2/212 (0.9%)	6/215 (2.8%)	1/193 (0.5%)	..
	Myalgia / Arthralgia	12/212 (5.7%)	29/215 (13.5%)	8/193 (4.1%)	..
	Fever	19/212 (9.0%)	35/215 (16.3%)	13/193 (6.7%)	..
	Passing dark urine	1/212 (0.5%)	2/215 (0.9%)	0/193 (0.0%)	..
	Dizziness	6/212 (2.8%)	16/215 (7.4%)	13/193 (6.7%)	..
	Any gastrointestinal symptoms [2]	11/212 (5.2%)	23/215 (10.7%)	11/193 (5.7%)	..
Within 28 days	SAE (PQ related - All severities) [3]	0/222 (0%)		0/196 (0%)	
	SAE (PQ unrelated - All severities)	0/222 (0%)		0/196 (0%)	
Within 6 months	SAE PQ related - All severities	0/222 (0%)		0/196 (0%)	
	SAE PQ unrelated - All severities	3/222 (1.4%)		4/196 (2.0%)	

Note: [1] The proportion of patients reporting each symptom at least once between day 2 and day 16. Symptoms elicited from daily questionnaires during treatment. Participants in the unsupervised PQ arm were assessed on days 2 and 16 only, while those in the supervised PQ arm were assessed at each supervised visit (day 2, 4, 6, 8, 10, 12, 14 and 16); [2]: Composite of any of the following: nausea, vomiting, anorexia, diarrhoea or abdominal pain; [3]: Related to PQ includes 'possibly', 'probably' and 'definitely' related

* Inclusive refers to presence on any day between days 2 and 16

Figures Legends

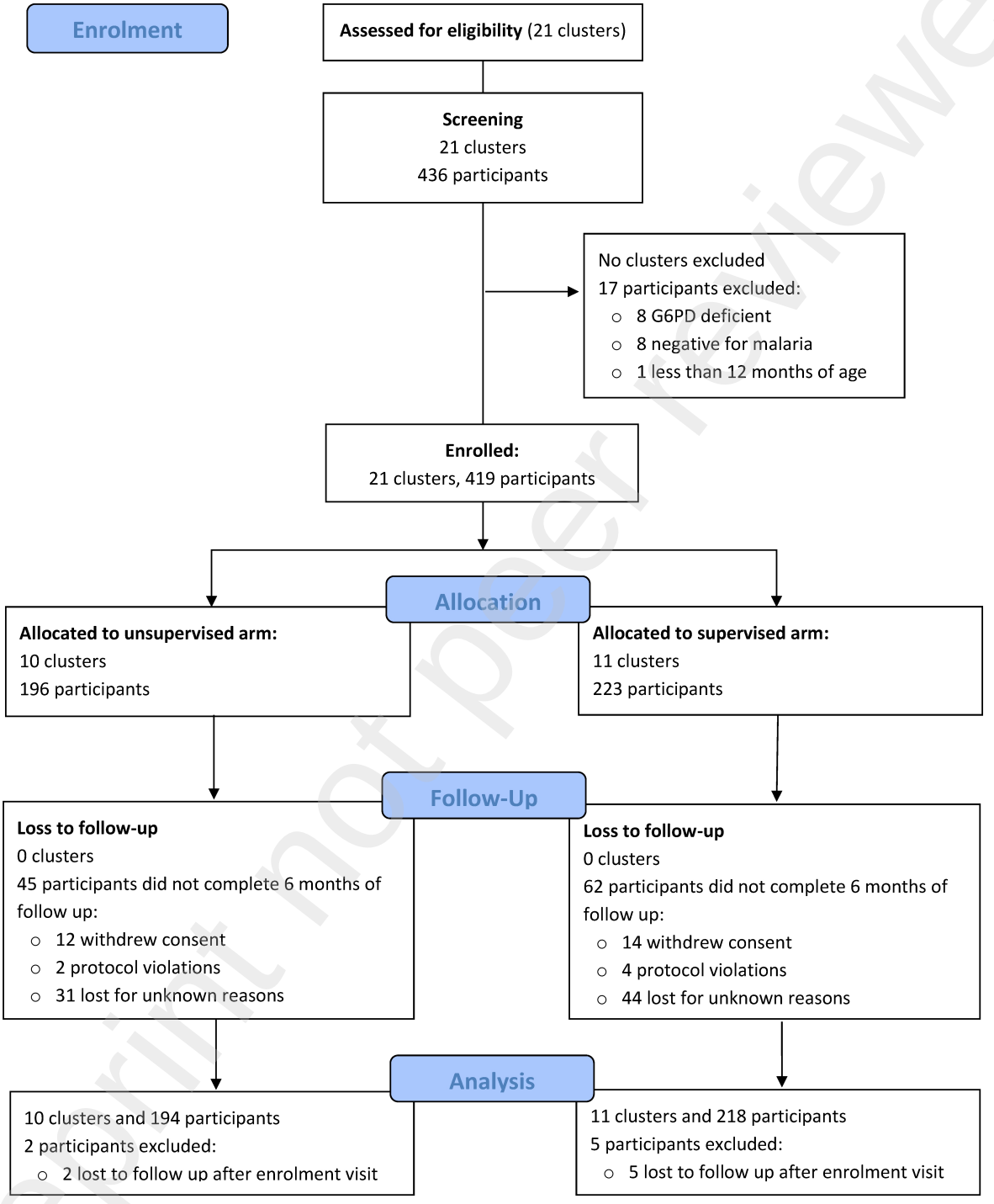
Figure 1. Study profile

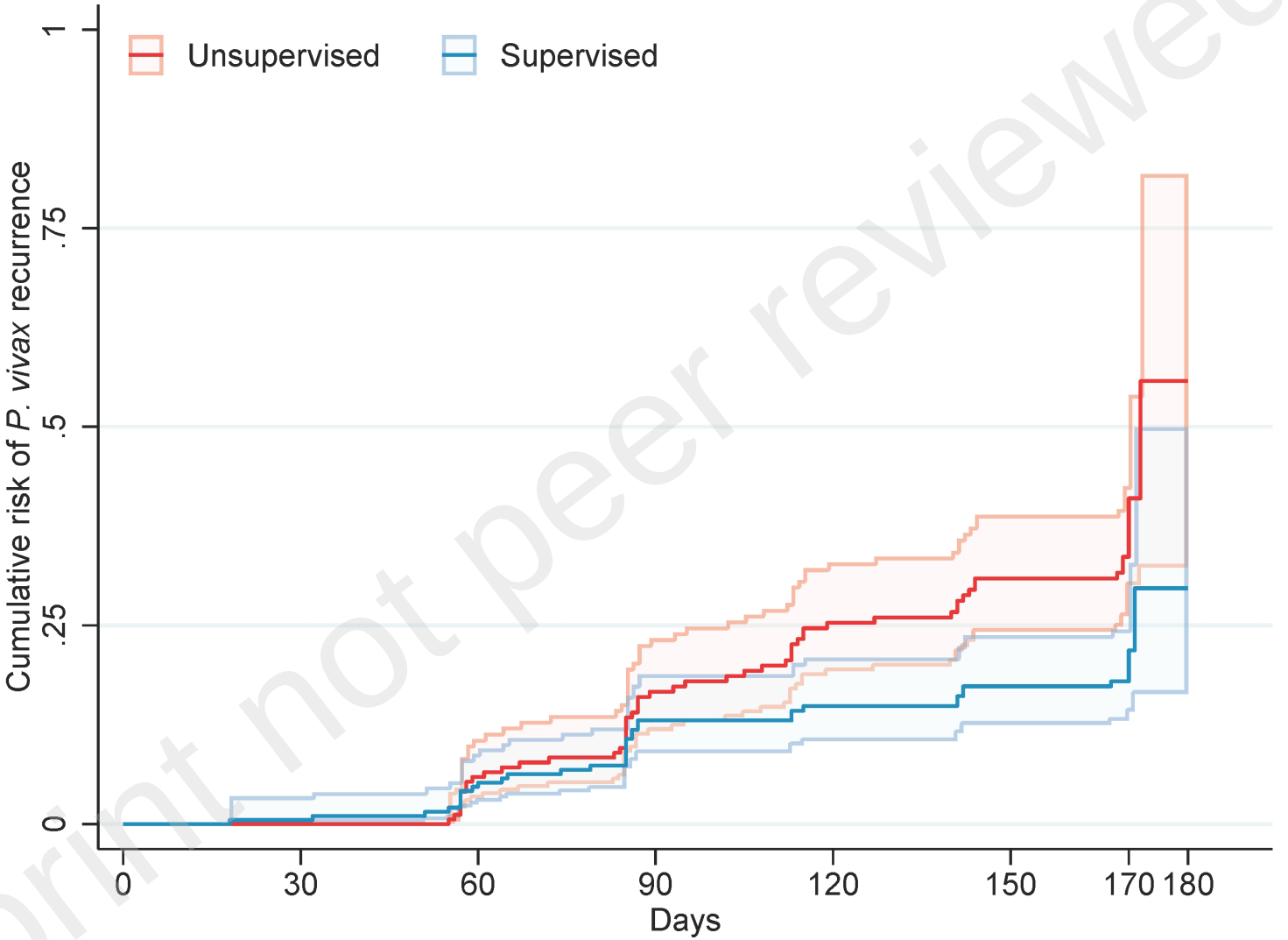
Figure 2. Cumulative incidence of the first recurrence of *Plasmodium vivax* parasitaemia

Figure 3. Histogram of the number of *Plasmodium vivax* recurrences (red columns) and *P. falciparum* recurrences (blue columns) stratified by (**A**) treatment arm and (**B**) species of infection at enrolment.

Figure 4. (**A**) Relative and (**B**) absolute change in haemoglobin (Hb; g/dL) from before (day 0) to 2 days after 14 days of primaquine treatment.

Footnote: 171 participants in the unsupervised arm (blue) and 195 in the supervised arm (red) had measurements at both timepoints. Circles denote participants enrolled with *Plasmodium vivax*, triangles denote those enrolled with *P. falciparum* and squares show those enrolled with mixed *P. vivax*/*P. falciparum* infection. The dashed orange lines represent a fractional fall of 25%.





Number at risk

Unsupervised	194	176	155	127	110	98	18	0
Supervised	218	193	179	147	141	132	21	0