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1 **High versus low dose of 14 days treatment of primaquine in *Plasmodium vivax* infected patients in**
2 **Cambodia: a randomised open-label efficacy study**

3
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19

20 **Summary**

21 **Background**

22 The WHO malaria treatment guidelines recommend a total dose in the range of 3.5 to 7.0 mg/kg of
23 primaquine to eliminate *Plasmodium vivax* (*P. vivax*) hypnozoites and prevent relapses. There are
24 however indications that for tropical *P. vivax* isolates, notably from Southeast Asia, the lower dose of 3.5
25 mg/kg is insufficient. Determining the most effective regimen to eliminate *P. vivax* hypnozoites is
26 needed to achieve elimination of this malaria parasite.

27 **Methods**

28 We conducted an open-label randomised controlled trial in Kampong Speu province, Western
29 Cambodia. *P. vivax* infected patients with uncomplicated malaria, diagnosed at the community level or
30 in health centres of the province, were offered to participate. Patients aged less than 15 years old, and
31 pregnant or breastfeeding women were excluded. Enrolled patients were treated with a blood
32 schizonticidal artesunate regimen of 2 mg/kg/day for 7 days. Upon enrolment, patients' glucose-6-
33 phosphate dehydrogenase (G6PD) activity was determined. G6PD normal patients were randomly
34 assigned (2:2:1) to receive either (i) 3.5 mg/kg (low dose as 0.25 mg/kg/day) or (ii) 7.0 mg/kg (high dose
35 as 0.5 mg/kg/day) of primaquine over 14 days or (iii) no primaquine as comparator arm. G6PD deficient
36 patients were assigned to the no-primaquine comparator arm. Randomisation was done by blocks of 5
37 using sealed envelopes. Upon enrolment, patients were relocated to the study site in Aoral town where
38 no malaria transmission occurs to ensure that they were not reinfected during their 90-day follow-up.
39 After 90 days of relocation, G6PD normal patients in the no-primaquine arm were provided 3.5 mg/kg
40 for 14 days of primaquine to be taken unsupervised. At day 90, all the patients returned home and they
41 were further followed monthly for three months until day 180. The primary outcome was the treatment
42 failure rate defined as the proportion of patients with at least one *P. vivax* recurrence within 90 days of
43 relocated follow-up. All patients that completed treatment and complied with relocation without
44 interruption before any recurrence was detected were included in the primary efficacy analysis. All
45 patients enrolled and assigned to an intervention arm were included in the safety analysis. The study is
46 registered on ClinicalTrials.gov (NCT04706130).

47 **Findings**

48 Between Nov 10, 2021, and Feb 10, 2024, a total of 160 patients were enrolled and 156 were allocated
49 to one of the three study arms. Of these, 37 G6PD deficient patients were assigned to the no primaquine
50 arm and 119 G6PD normal patients were randomised: 24 in the no primaquine arm, 49 in the
51 primaquine 3.5 mg/kg arm, and 46 in the primaquine 7.0 mg/kg arm. The proportion of participants with
52 at least one *P. vivax* recurrence within 90 days in the no primaquine arm was 81.4% (95% CI 69.6-89.2).
53 The proportion of participants with recurrence was higher in the low dose primaquine arm (24.4%, 95%
54 CI 14.2-38.7) compared to the high primaquine arm (4.7%, 95% CI 0.8-15.5, $p=0.0141$) resulting in a
55 hazard ratio of high dose primaquine compared to low dose of 0.17 (95% CI 0.04-0.79, $p=0.0229$). Both
56 primaquine arms were well tolerated.

57 **Interpretation**

58 Not providing primaquine to patients led to a considerable rate of *P. vivax* recurrence. The risk of *P.*
59 *vivax* recurrence was 5.9 times lower for the 7.0 mg/kg of primaquine treatment compared to 3.5

60 mg/kg. Tolerability and safety of both primaquine regimens in G6PD normal individuals was comparable.
61 Policy makers in Cambodia and most likely in other Southeast Asian countries should endorse the 7·0
62 mg/kg of primaquine regimen to reduce the risk of *P. vivax* relapses.

63 **Funding**

64 National Institutes of Health (R01AI146590)

65

66 **Research in context**

67 **Evidence before this study**

68 The WHO treatment guidelines for preventing *Plasmodium vivax* relapses using primaquine recommend
69 a range of 3.5-7.0 mg/kg. These guidelines mention that for *P. vivax* infections acquired in Southeast
70 Asia and Oceania, 7.0 mg/kg should be preferred. We searched Pubmed for randomized controlled trials
71 studies containing the terms “vivax” and “primaquine” published between 1990 and November 2024,
72 with no language restrictions, to identify studies comparing primaquine regimen to treat *P. vivax*
73 infections. Our search retrieved only two studies comparing head-to-head the efficacy of 3.5mg/kg with
74 7.0 mg/kg primaquine administered over a same duration of 14 days but were both conducted in South
75 Asia and both showed similar efficacy of high dose compared to low dose primaquine. No studies
76 compared 3.5 and 7.0 mg/kg of primaquine administered over 14 days in South East Asian countries.

77 **Added value of this study**

78 This randomised controlled trial compared the efficacy of a total dose of 3.5 mg/kg and 7.0 mg/kg of
79 primaquine over 14 days to prevent relapses of *P. vivax* in Cambodia. Our study design minimized
80 confounding factors affecting therapeutic efficacy evaluation. This study confirms that 7.0 mg/kg has
81 greater efficacy to prevent recurrences than the low 3.5mg/kg regimen, with comparable safety and
82 tolerability in G6PD normal patients from Cambodia.

83 **Implications of all the available evidence**

84 Our results confirm that 7.0 mg/kg of primaquine should be recommended to prevent relapses in *P.*
85 *vivax* infections acquired in Cambodia and most likely in South East Asia. National treatment guidelines
86 in those countries should be changed to endorse this regimen rather than the 3.5 mg/kg.

87

88 Introduction

89 *Plasmodium vivax* is the parasite responsible for the majority of malaria cases outside Africa, with more
90 than three billion people living within *P. vivax* transmission limits.¹ Upon mosquito inoculation, a
91 fraction of the injected parasites remain dormant in human hepatocytes as hypnozoites and can
92 reactivate after the initial infection causing relapses.^{2,3} Repeated relapses of *P. vivax* cause significant
93 morbidity and mortality in individuals living in endemic areas.⁴⁻⁶ In addition, the hypnozoite reservoir of
94 *P. vivax* make this parasite resilient to elimination efforts and the proportion of *P. vivax* malaria tends to
95 increase during malaria elimination campaigns.⁷⁻¹² In many endemic countries, *P. vivax* is now the most
96 prevalent malaria parasite and dedicated strategies are needed for its elimination.¹³

97 Only 8-aminoquinolines such as primaquine and more recently tafenoquine, are able to eliminate
98 hypnozoites. Despite primaquine being used for decades, the most appropriate regimen for achieving
99 complete elimination of hypnozoites is still unclear (referred to as radical cure when combined to a
100 schizonticide drug killing blood-stage parasites). The WHO malaria treatment guidelines recommend to
101 administer 0.25 to 0.5 mg/kg/day of primaquine over 14 days (total dose of 3.5 to 7.0 mg/kg) to achieve
102 radical cure, and state that tropical *P. vivax* prevalent in Southeast Asia and Oceania should be treated
103 with a total dose of 7.0 mg/kg rather than 3.5 mg/kg.¹⁴ Nonetheless, the guidelines also acknowledges
104 that “No direct comparison has been made of higher doses (0.5 mg/kg body weight for 14 days) with the
105 standard regimen (0.25 mg/kg body weight for 14 days)”. A number of trials conducted in South East
106 Asia and Oceania evaluated different primaquine doses administered over variable durations¹⁵⁻¹⁸, but
107 none compared 3.5 and 7.0 mg/kg total dose of primaquine administered over 14 days. Two studies
108 compared 3.5 and 7.0 mg/kg of primaquine over 14 days, but both were conducted in India and not in
109 South East Asia or Oceania^{19,20}. Both concluded similar efficacy between the two primaquine regimens.
110 To improve compliance of primaquine treatment, the WHO recently added a recommendation to
111 administer a total of 3.5 mg/kg over 7 days instead of 14 days.

112 Many endemic countries have been reluctant to roll out primaquine, especially at the high 7.0 mg/kg
113 total dose, due to safety concerns in glucose-6-phosphate dehydrogenase (G6PD) deficient individuals in
114 whom primaquine can cause severe haemolytic anaemia.^{21,22} For many endemic countries where G6PD
115 deficiency is common, such as in Cambodia and in general in Southeast Asia, the treatment guidelines
116 require that the G6PD status of an individual is determined prior to administration of primaquine and
117 most countries recommend a total primaquine dose of 3.5 mg/kg.^{22,23} It is important to rigorously
118 evaluate the efficacy of the different primaquine regimens for radical cure treatment as substandard
119 treatment will result in relapses within the same individual but will also contribute to ongoing
120 transmission in the population, thereby hindering malaria elimination efforts.

121 *P. vivax* drug efficacy studies are complicated since, following initial treatment, parasite recurrences can
122 arise from blood-stage parasite recrudescence, reinfection from a new mosquito inoculation or relapses
123 from hypnozoite reactivation.^{24,25} In this study, we overcome this difficulty by relocating patients from a
124 malaria endemic region to a non-endemic area. We report the results of a randomised controlled trial
125 evaluating the efficacy of 3.5 versus 7.0 mg/kg total dose over 14 days of primaquine to prevent *P. vivax*
126 recurrences.

127

128

129 **Methods**

130 **Study design**

131 We conducted an open-label randomised controlled trial to compare the efficacy of low (3.5 mg/kg total
132 dose as 0.25 mg/kg/day for 14 days) and high dose (7.0 mg/kg total as 0.5 mg/kg/day for 14 days)
133 primaquine in G6PD normal *P. vivax* infected individuals. The study was conducted in our field site
134 located in Aoral town, Kampong Speu Province, Western Cambodia. The protocol was approved by the
135 National Ethics Committee for Health Research of Cambodia (158-NECHR, 06/29/2020) and by the
136 University of Maryland IRB (HP-00091095). The study was overseen by the NIH DMID (Protocol 20-0010)

137

138 **Participants**

139 Patients aged more than 15 years old, able to provide consent and willing to participate, seeking
140 treatment and diagnosed with an acute symptomatic *P. vivax* infection by rapid diagnostic test at the
141 community or health centre level were eligible for inclusion. Exclusion criteria were: aged less than 15
142 years old, sign of severe malaria, haemoglobin concentration below 8.0 g/dL, pregnant and
143 breastfeeding women, known sensitivity to the study drugs and intake of antimalarial drug in the past
144 month prior to inclusion. Since prolonged artesunate treatment, as administered in this study prior to
145 primaquine, has been shown to possibly lead to neutropenia²⁶, initially patients were excluded if they
146 presented with a neutrophil count of less than 1.5×10^9 cells/L. However, after approval from the Safety
147 Monitoring Committee of the study an amendment to the study protocol was made to remove this
148 exclusion criterion and instead monitor clinical signs of neutropenia. Only PCR-confirmed *P. vivax* mono-
149 infections were initially eligible. However, we amended the study protocol to include mixed *P. vivax* with
150 other *Plasmodium* species infections detected by PCR to ensure faster enrolment rates. Only patients
151 with a G6PD activity of more than 4 U/gHb for males or 6 U/gHb for females were eligible for
152 primaquine administration according to the Cambodian National Treatment Guidelines. G6PD activity
153 was measured by spectrophotometry (Trinity Biotech quantitative G6PD assay, adapted for use on the
154 Integra 400 analyzer, Roche Diagnostics). Patients with G6PD levels below these thresholds were
155 assigned to the no-primaquine arm. All patients or their guardians provided written informed consent
156 and written assent was obtained for all patients aged 15-18 years old.

157 **Randomisation and masking**

158 Enrolled patients were treated with 2 mg/kg/day of artesunate for seven days (total dose 14 mg/kg).
159 Males with a G6PD activity below 4 U/gHb and females with activity below 6 U/gHb were allocated to
160 the no primaquine comparator arm. Male and female patients with G6PD values above these thresholds
161 were randomly assigned to either low dose primaquine (0.25 mg/kg for 14 days, total dose 3.5 mg/kg),
162 high dose primaquine (0.5 mg/kg for 14 days, total dose 7.0 mg/kg) or no primaquine, in a 2:2:1 ratio.
163 Randomisation was done using sealed envelopes in blocks of five and was performed by an independent
164 team member who communicated the allocation to the clinician on-site after screening and enrolment
165 was completed. Randomization was performed in all cases within the first 6 days of artesunate
166 treatment so all participants randomized to the primaquine arms started their treatment on day 7. The
167 clinician on-site was responsible for the enrolment of patients in the study. All treatments were open
168 labelled.

169 Procedures

170 All patients started the blood schizonticidal artesunate regimen (RT Nate, Bonn Schtering Bio Sciences,
171 India) on the day of inclusion before the screening results were available. This artesunate regimen was
172 adapted from a previous study to ensure complete elimination of blood-stage parasites while being
173 rapidly eliminated and not providing post-treatment prophylaxis that could mask failure of
174 primaquine.²⁷ In case the screening led to an exclusion of patients, artesunate regimen was interrupted
175 and a complete artesunate-mefloquine 3-day regimen was provided following the Cambodian National
176 Treatment Guidelines. Excluded patients were then referred to the Aoral Health Centre for G6PD activity
177 determination and primaquine administration. For enrolled patients, all treatments were directly
178 supervised at all times. Artesunate treatment was provided at 2 mg/kg/day for seven days from day 0 to
179 day 6 (appendix Table S1). Following results of screening and arm allocation, patients in the two
180 primaquine arms started primaquine (Remedica, Ltd., Cyprus) on day 7 after the last dose of artesunate.
181 Patients receiving primaquine were treated daily for 14 days from day 7 to day 20 with either low dose
182 (0.25 mg/kg/day) or high dose of primaquine (0.5 mg/kg/day, appendix Table S2). Only antipyretic
183 treatment (paracetamol) was provided concomitantly to some patients at the discretion of the clinical
184 physician.

185 Upon enrolment, a physical examination was performed. A venous blood sample was collected before
186 treatment to perform a full blood count and determine G6PD activity. From the day of inclusion,
187 patients remained relocated for 90 days in our study site located in Aoral, a malaria transmission-free
188 town. Patients could go in and out of the study site during day time but had to spend the night there to
189 ensure they do not travel to remote forested areas during the follow-up. Clearance of blood-stage
190 parasites was monitored by capillary blood collection 1h, 2h, 4h, 8h, 16h, 24h after the first dose and
191 then daily from day 2 to day 6. Follow-up for patients not receiving primaquine was then done every 48h
192 until day 90 and the end of relocation. Patients receiving primaquine had daily follow-up during the 14
193 days of primaquine treatment after which follow-up was performed every 48h until day 90. At each
194 follow-up, a physical examination was performed, any adverse event (AE) was recorded and a capillary
195 blood sample was collected by fingerprick into an EDTA microtainer tube. From each sample, thick and
196 thin smears were prepared on microscopy slides for Giemsa staining (3%, 45 min) and 50µL of blood was
197 used for DNA extraction and qPCR detection of malaria parasites performed in the following 24h.

198 During the follow-up, in case of *P. vivax* recurrence detected by qPCR and confirmed by microscopy,
199 patients were re-treated with the same artesunate regimen received upon inclusion (14 mg/kg over 7
200 days) but not primaquine. Follow-up was then resumed until the end of the 90 days of relocation. On
201 the last day of relocation, G6PD normal patients who did not receive primaquine were provided the
202 standard 14-day primaquine treatment of 0.25 mg/kg/day, to be taken unsupervised. Participants were
203 further followed for 3 additional monthly visits on day 120, day 150 and day 180 for capillary blood
204 collection.

205 Outcomes

206 The primary outcome of the study was the proportion of patients experiencing at least one *P. vivax*
207 recurrence during the relocated 90-day follow-up. Secondary outcomes included the proportion of
208 patients with *P. vivax* recurrence detected after the end of relocation on day 180, the total number of
209 recurrences occurring during (i) the 90-day relocation (ii) the 180-day complete follow-up, the time to
210 the first *P. vivax* recurrence and clearance of blood-stage parasites following artesunate treatment.

211 Safety assessment and grading of adverse events (AE) were performed following the NIAID/NIH DMID
212 toxicity table (Appendix Table S3). A clinical examination was performed every 24 or 48h during the 90-
213 day follow-up. Haematological AEs and other drug related AEs that are the known expected risks
214 associated to artesunate and primaquine treatments were particularly monitored. Neutrophil counts
215 were determined before and 7 and 14 days after any artesunate treatment. Haemoglobin concentration
216 (using a HemoCue) and methaemoglobin percentage (measured using a Masimo oximeter) were
217 recorded daily over the course of any treatment and then every 48h during the 90-day follow-up.

218 **Sample size**

219 We anticipated that at least 50% of patients would have *P. vivax* recurrence during the 90 days follow-
220 up in absence of any radical cure^{27,28}. Assuming an efficacy of 92% for the 0.5 mg/kg/day and 73% for
221 the 0.25 mg/kg/day, a Type I error of 5% and a power (1- β) of 90%, a sample size of 80 individuals per
222 arm was required (total 160) to detect statistically significant differences in therapeutic efficacies (target
223 effect)^{27,29,30}. With a 20% loss during follow-up estimated, 100 patients per primaquine arm (total 200)
224 was defined. For the no primaquine arm, assuming a Type I error of 5%, a power (1- β) of 90% and a loss
225 to follow-up rate of 20%, a sample size of 60 patients in this arm (expected ~ 30 patients with relapses
226 and ~30 patients without) was defined to provide sufficient power. This arm was not intended as
227 therapy for practice, but aimed to serve as comparator as well as to study relapses in vivo.

228 **Statistical analyses**

229 Difference in efficacy of the two primaquine regimens was assessed by two-sided Fisher's exact test
230 using the per protocol population that excluded participants who did not complete the treatment
231 (either because of mixed infections or withdrawal of consent) or interrupted relocated follow-up before
232 any recurrence was detected. The intention-to-treat population was used in a sensitivity analysis where
233 all dropouts were imputed either as treatment success or treatment failure. These analyses were
234 complemented by comparing Kaplan-Meier recurrence-free survival using log-rank analysis. All
235 participants assigned to one of the three study arms were included in the Kaplan Meier analysis.
236 Patients were censored in the Kaplan-Meier analysis at the day of the following events: withdrawal of
237 consent (with or without discontinuation of study drug), mixed *Plasmodium* infections (until this
238 exclusion criterion was removed) and loss to follow-up. Before it was removed from exclusion criteria, in
239 case of mixed infection during the follow-up, patients were referred to the local health centre to be
240 treated with first-line treatment for malaria (artesunate-mefloquine). Interruption of relocation
241 (absence at night from the study site) was tolerated if it lasted less than 4 days (two consecutive 48h
242 follow-up time points). Patients were advised that breach of relocation was not tolerated for more than
243 4 days and this was considered as withdrawal of consent. No patient had more than 1 relocation
244 interruption during the 90 days follow-up. Missing visits after the 90-day of relocation on day 120, 150
245 or 180 were all tolerated. Hazard ratios for time to first recurrence were determined by Cox regression.
246 A Prentice-Williams-Peterson (PWP)^{31,32} extended Cox regression analysis was used to compare risks of
247 all recurrences between the three arms taking into account repeated *P. vivax* recurrences. PWP model
248 stratifies the analysis by the order of events, allowing the baseline hazard to vary for each event.
249 Differences in the number of recurrences, the time to first recurrence and the time to any recurrence (i)
250 between randomized and non-randomized patients in the no primaquine arm and (ii) between low dose
251 primaquine and high dose primaquine arms, were all assessed by Mann Whitney U test. All tests were
252 two-sided. All patients enrolled and assigned to a study arm were included in the safety analysis. All

253 analyses were done using R V12.1 or GraphPad Prism V10.3. There was a Safety Monitoring Committee
254 for this study with pre-planned annual meetings. The study is registered on ClinicalTrials.gov
255 (NCT04706130).

256 **Role of the funding source**

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

260 **Results**

261 Between Nov 10, 2021, and Feb 10, 2024, 312 patients were screened for inclusion, of whom 129
262 declined to participate, 23 did not meet inclusion criteria and 160 were enrolled in the study, of whom
263 four were later excluded as they were not filling laboratory screening criteria (figure 1). All patients were
264 Cambodian. Enrolment was halted following consultation with the Safety Monitoring Committee despite
265 not have reached the initially planned sample size as the study reached its end date and concomitantly
266 with decrease in malaria transmission and enrolment rates in the study area. Of the 156 enrolled
267 patients, 37 (23.7%) had a G6PD activity below the thresholds for primaquine administration and were
268 assigned to the no-primaquine arm. The 119 remaining G6PD normal patients were randomly assigned
269 to one of the three arms: 49 in the low-dose primaquine arm, 46 in the high dose and 24 in the no
270 primaquine arm. The final sample size was 49, 46 and 61 patients enrolled in the low dose primaquine,
271 high dose primaquine and no primaquine arms respectively. Baseline patients' characteristics were
272 similar between the three study arms and between G6PD normal patients randomised to the no
273 primaquine arm and G6PD deficient patients assigned to the no primaquine arm (table 1). The majority
274 of enrolled participants were males reflecting the current malaria epidemiology in Cambodia where
275 adult males are the most at-risk individuals due to occupational exposure.³³ The median parasitemia was
276 5573 parasites per μL (IQR 3223-10795) in the low dose primaquine arm, 6470 parasites per μL (IQR
277 2815-12557) in the high dose primaquine arm, 4890 parasites per μL (IQR 2484-16465) in participants
278 randomised to the no primaquine arm and 5976 parasites per μL (IQR 3897-11561) in G6PD deficient
279 participants assigned to the no primaquine arm.

280 In the per protocol population, the proportion of participants with at least one *P. vivax* recurrences
281 detected by day 90 was 81.4% (48/59, 95% CI 69.6-89.2) in the no primaquine arm. There was no
282 statistically significant difference in recurrence rates between randomized G6PD normal patients
283 (77.3%, 17/22, 95% CI 56.6-89.9) and non-randomized G6PD deficient patients (83.8%, 31/37, 95% CI
284 68.9-92.3, $p=0.7309$) (table 2). Among those receiving primaquine, 24.4% (11/45, 95% CI 14.2-38.7) of
285 the participants in the low dose primaquine arm had a recurrence, statistically significantly higher than
286 those in the high dose primaquine arm 4.7% (2/43, 95% CI 0.8-15.5, $p=0.0141$). Efficacy of primaquine
287 obtained here are similar to those that were anticipated. The ITT analysis led to similar results with
288 statistically significantly higher recurrences in the low dose primaquine arm compared to the high dose
289 arm, whether dropouts were imputed as treatment success ($p=0.0150$) or treatment failure ($p=0.0236$)
290 (Appendix Table S4). Missing data are listed in Appendix Table S5. Log-rank analysis confirms that *P.*
291 *vivax* recurrence-free survival by day 90 was statistically significantly different between low and high
292 dose primaquine arms ($p=0.0100$) (figure 2A). The same results were observed when restricting this
293 analysis to randomized patients only (Appendix figure S1). The hazard ratios (HR) for recurrence
294 compared to no primaquine were 0.14 (95% CI 0.07-0.27) for the low-dose primaquine arm and 0.03

295 (95% CI 0.01-0.10) for the high-dose arm ($p < 0.0001$). The HR of high dose primaquine compared to low
296 dose was 0.17 (95% CI 0.04-0.79, $p = 0.0229$).

297 The Cox model accounting for repeated *P. vivax* recurrence events showed that compared to the no
298 primaquine arm, the risk of recurrence was 4.9 times lower (95%CI 2.49-9.55, $p < 0.0001$) in the low dose
299 primaquine arm, and 36 times lower (95%CI 9.28-142, $p < 0.0001$) in the high dose primaquine arm. The
300 risk of recurrence was 7.5 times lower in the high primaquine arm compared to the low primaquine arm
301 (95%CI 1.63-34.2, $p = 0.0067$).

302 Between day 90 and day 180, two additional participants in the low dose primaquine arm and three in
303 the high dose primaquine arm experienced their first *P. vivax* recurrences. Recurrence-free survival
304 analysis resulted in incidence risk of 13.2% (95%CI 5.7-29.1) in the high dose primaquine arm,
305 statistically significantly lower than in the low dose primaquine arm (incidence risk, 29.1%, 95%CI 17.9-
306 45.0, HR 0.37 95%CI 0.15-0.94 $p = 0.0368$) (figure 2B). For the no primaquine arm, we analysed separately
307 randomized G6PD normal individuals that were provided primaquine to be taken unsupervised at day 90
308 and non-randomized G6PD deficient patients who were not provided primaquine at day 90. Recurrence-
309 free survival analysis showed no difference between G6PD normal and deficient individuals with
310 incidence risks of 81.7% (95%CI 63.6-94.3) and 84.2% (70.7-93.8) for G6PD normal and deficient
311 participants, respectively ($p = 0.8916$).

312 The mean number of recurrences per patient during the 90-day follow-up in the no-primaquine arm was
313 1.7 (SD 1.2, range 0-4) and was not statistically significantly different between randomized G6PD normal
314 patients (1.8, SD 1.2, range 0-4) and non-randomized G6PD deficient patients (1.8, SD 1.2, range 0-4,
315 $p = 0.9728$). For patients treated with primaquine, statistically significantly more recurrences per patient
316 occurred in the low primaquine arm (0.3, SD 0.7, range 0-3) compared to the high primaquine arm (0.04,
317 SD 0.21, range 0-1, $p = 0.0193$). Similar trends were observed when considering all recurrences occurring
318 until day 180 with a mean of 2.1 (SD 1.4, range 0-4) recurrences in the no primaquine arm and no
319 statistically significant difference between randomized (2.0, SD 1.3, range 0-4) and non-randomized
320 patients (2.2, SD 1.4, range 0-4, $p = 0.5868$). For primaquine-treated patients, there were statistically
321 significantly more recurrences per patient in the low primaquine arm (0.47, SD 0.94, range 0-4)
322 compared to the high primaquine arm (0.11, SD 0.31, range 0-1, $p = 0.0367$).

323 Among participants with recurrences, the median time from enrolment to the first recurrence was 20.0
324 days (IQR 14.0-36.5, range 10-150), 64.0 days (IQR 32.0-80.0, range 20-150) and 120.0 days (IQR 73.0-
325 165.0, range 68-180) in the no-primaquine, low dose primaquine and high dose primaquine arms,
326 respectively. In the no primaquine arm, the time to first recurrence was not statistically significantly
327 different between randomized (18 days, IQR 14-30, range 12-120) and non-randomized patients (24
328 days, IQR 14-38, range 10-150, $p = 0.4521$). The time to first recurrence was statistically significantly
329 shorter in the low primaquine arm compared to the high primaquine arm ($p = 0.0379$). When considering
330 all 154 recurrences detected during the 180 days follow-up, including repeated recurrences in a same
331 participant, the median day when any recurrence occurred was 46.0 days (IQR 24.0-74.0, range 10-180),
332 73.0 days (IQR 47.5-120.0, range 20-180) and 120 days (IQR 73.0-165.0, range 68-180) in the no
333 primaquine, low dose primaquine and high dose primaquine arms, respectively. In the no primaquine
334 arm, the time to any recurrence was not statistically significantly different between randomized (42
335 days, IQR 18-72, range 12-180) and non-randomized patients (48 days, IQR 29-77, range 10-150,

336 p=0.2956). The time to any recurrence was not statistically significantly different between low dose
337 primaquine arm and high dose primaquine arm (p=0.0742).

338 All blood-stage infections were treated using artesunate for 7 days at 2 mg/kg/day and arm allocation
339 and primaquine administration were performed after the end of artesunate therapy. Of the 55 patients
340 febrile at enrolment, 93% (51/55) became afebrile within 24h of the first artesunate dose. Parasite
341 clearance was observed for 70% (109/156) of patients within 24h of artesunate initiation, 95% (148/156)
342 were cleared by day 2 and all were parasite-free by day 3 (Appendix Table S6). Parasite clearance of
343 recurrences was similar between the three arms with 87% (87/100), 94% (15/16) and 100% (2/2) of
344 recurrences cleared of parasites within 24h of treatment in the no primaquine, low primaquine and high
345 primaquine arms, respectively. By day 2, 98% (98/100), 100% (16/16) and 100% (2/2) of recurrences
346 were cleared in the no primaquine, low dose primaquine and high dose primaquine arms, respectively.
347 All recurrences were cleared by day 3 in all arms.

348 No serious AEs (SAE) were reported and all laboratory grade 3 and 4 AEs were asymptomatic and did not
349 require medical attention therefore not meeting the criteria for a SAE. A total of 208 AEs were reported
350 between day 0 and day 90 (table 3). A clinical examination was not performed during the three
351 additional visits at day 120, day 150 and day 180. The proportion of participants encountering an AE in
352 the no primaquine arm (80%, 49/61) was higher than in the low dose primaquine arm (55%, 27/49,
353 p=0.0044) but was not different than in the high dose primaquine arm (67%, 31/46, p=0.1272). There
354 was no statistically significant difference between the two primaquine arms (p=0.2929). The median
355 number of AEs per patient was higher in the no-primaquine arm (2.0, IQR 1.0-2.8, range 0-7) compared
356 to the low dose (1.0, IQR 0.0-1.0, range 0-7, p=0.0001) and the high dose primaquine one (1.0, IQR 0.0-
357 2.0, range 0-4, p=0.0158). There was no difference between the low and high dose primaquine
358 arms (p=0.7231).

359 Among grade 3 AEs, only one was defined as related to the intervention and was a self-resolved low
360 neutrophil count within 14 days of artesunate treatment upon *P. vivax* recurrence in the no primaquine
361 arm. All other grade 3 AEs were defined unrelated to artesunate or primaquine therapies but most likely
362 resulted from *Plasmodium* infections either at enrolment (fever and anaemia on day 0 and day 1) or
363 upon recurrences (fever and low neutrophil counts prior to artesunate administration). The only patient
364 with a grade 4 AE was in the no primaquine arm at day 7 with a self-resolved low neutrophil count
365 deemed possibly related to the 7-day artesunate regimen.

366 Primaquine administration was always performed with food intake and was globally well tolerated
367 (Appendix Table S7). Regarding haematological safety, increase in methaemoglobin was more frequent
368 in the high dose primaquine arm (35% of participants, 16/46) compared to the low dose one (10%, 5/49,
369 p=0.0039) but was clinically silent for all participants and resolved after the end of primaquine
370 administration. No patient developed severe anaemia and there was no drop of more than 25% in
371 haemoglobin during primaquine administration.

372 Mild self-limiting decrease (grade 1) in haemoglobin levels during the 14-day primaquine course were
373 reported in 2 and 7 participants in the low and high primaquine arms respectively. Participants with mild
374 and moderate anaemia were all asymptomatic. The proportion of patients with anaemia during the 90-
375 day follow-up was statistically significantly different for the three arms (p=0.0056) and was higher in the
376 no primaquine arm (48%, 29/61) compared to the low dose arm (20%, 10/49, p=0.0031) and to the high

377 dose arm (26%, 12/46, $p=0.0238$). There was no statistically significant difference in the proportion of
378 participants with anaemia between the two primaquine regimens ($p=0.5120$).

379 Discussion

380 This randomised controlled trial provides evidence of higher efficacy to prevent recurrences of the 7.0
381 mg/kg primaquine regimen compared to the 3.5 mg/kg (total dose, both over 14 days) for infections by
382 *P. vivax* acquired in Cambodia. Our unique study design where (i) patients were relocated during the 90-
383 day follow-up to prevent reinfections, (ii) blood-stage infections were cleared using a short half-life
384 antimalarial (artesunate) and (iii) a frequent and sensitive monitoring of parasite recurrence every
385 second day using both PCR and microscopy, allowed us to evaluate the efficacy of primaquine to
386 prevent *P. vivax* recurrences while minimizing confounding effects for instance through reinfections and
387 the post-prophylactic effects of schizonticidal drugs. By day 90, 7.0 mg/kg of primaquine reduced the
388 risk of *P. vivax* recurrence by 5.5 fold compared to the 3.5 mg/kg regimen. The higher efficacy of high
389 dose primaquine was still apparent at day 180. These results are in line with a recent meta-analysis
390 evaluating efficacy of primaquine dosage.³⁴

391 Using artesunate monotherapy coupled to tight and sensitive follow-up of patients allowed us to
392 document an extremely high rate of relapses occurring in *P. vivax* infected patients when primaquine is
393 not administered, as well as the high number of consecutive relapses, as observed in other studies²⁷.
394 These successive relapses inflict a high morbidity to infected individuals as illustrated by the overall
395 higher number of AEs identified in the no primaquine arm compared to the two others with primaquine
396 administration. These AEs are the direct consequence of *P. vivax* relapsing infections and are prevented
397 by primaquine administration. In particular, we show here that successive recurrences in absence of
398 primaquine treatment cause repeated anaemia in infected individuals. Note that this relapse-related
399 morbidity is very likely underestimated in our study because of the frequent follow-up performed: all
400 relapsing infections were re-treated before any clinical consequence of anaemia could be observed,
401 while in real-life settings, repeated anaemia following successive *P. vivax* relapses would have been
402 greater as the disease would have progressed.^{5,6,35-39} Anti-relapse therapy, even with partial efficacy
403 such as the 3.5 mg/kg primaquine regimen, thus provides direct clinical benefit to *P. vivax* infected
404 patients.

405 Our results confirm that the total dose of 7.0 mg/kg should be the recommended primaquine regimen
406 for individuals with normal G6PD activity in Cambodia and likely in other countries in Southeast Asia.
407 Our results are in line with those of a study published over the course of our trial that compared low
408 dose primaquine (total dose of 3.5 mg/kg as in our study, but over 7 days) efficacy with high dose
409 primaquine (same regimen as we evaluated here) for *P. vivax* infections acquired in Brazil⁴⁰ suggesting
410 that the superior efficacy of 7.0 mg/kg compared to 3.5 mg/kg is likely universal to all tropical *P. vivax*
411 infections. Recently, the WHO issued a recommendation for a shorter course of primaquine over 7
412 days¹⁴ to improve compliance, but the total dose of primaquine under this new recommendation is
413 3.5 mg/kg, the same as the low dose primaquine we evaluated here over 14 days. As efficacy of
414 primaquine is influenced by the total dose administered, rather than by the duration of the treatment or
415 the maximal concentration achieved in the blood (a phenomenon referred to as the total dose
416 effect)^{24,41,42}, this new recommendation of 3.5 mg/kg over 7 days is likely insufficient to prevent relapses
417 for infections acquired in Southeast Asia, and probably in most tropical countries⁴⁰. However, 14-day
418 regimens are challenging due to compliance concerns and shorter courses provide clear implementation

419 benefit. A short course (7 days) high dose (7.0 mg/kg total) regimen of primaquine was recently
420 evaluated in a large multicentric clinical trial and was shown to be well tolerated in G6PD normal
421 patients and as effective as 7.0 mg/kg total dose over 14 days¹⁵.

422 We have used here 7-day artesunate monotherapy to treat blood-stage infections prior to primaquine
423 administration. While this short half-life antimalarial allowed us to finely characterize any recurrence
424 occurring by not conferring post-treatment prophylaxis, it does not reflect current practices in the vast
425 majority of endemic countries where primaquine is provided concomitantly with treatment with either
426 chloroquine or an artemisinin based combination therapy¹⁴. These schizonticidal antimalarial drugs will
427 provide longer post-treatment prophylaxis than artesunate due to their longer half-life suppressing early
428 relapses.^{27,28,43} However, with perhaps the exception of chloroquine that has been shown to synergize
429 with primaquine in early small studies⁴⁴, but not in larger clinical trials⁴⁵, intrinsic efficacy of primaquine
430 is not affected by the blood-stage drug used and has been shown to be as effective when started after
431 treating blood-stage parasites.⁴⁶

432 Our study has several limitations. First the sample size is small and the target sample size was not
433 attained for logistical reasons (end of funding period concomitant with decrease in malaria transmission
434 in the study area). Second, this was a single centre open label study conducted in one geographic area of
435 Cambodia. Third, and inherently to *P. vivax* biology, we cannot ascertain that all recurrences observed
436 are relapses and not recrudescence. There is currently no method that can unambiguously tease apart
437 both source of recurrences. We however believe that the majority of recurrences observed are relapses
438 because (i) 95% and 100% of all initial infections were cleared within 48h or 72h of artesunate therapy
439 initiation, respectively, (and confirmed by intensive PCR testing) and (ii) a primaquine dose-dependent
440 decrease in the number of recurrences detected is observed. Further genomic analyses will be
441 performed to provide better insights on these recurrences, providing probabilistic classifications of
442 whether a recurrence is a recrudescence or a relapse.⁴⁷ Finally, our study was performed in tightly
443 controlled conditions, with treatment supervised at all time and sequentially administered with first
444 artesunate and second primaquine and as such does not reflect real life practice and ultimately
445 effectiveness of primaquine.

446 Overall, our results indicate that a total dose of 7.0 mg/kg of primaquine over 14 days has superior
447 efficacy to prevent *P. vivax* recurrences compared to 3.5 mg/kg of primaquine over 14 days in infections
448 acquired in Cambodia. Both treatments were well tolerated in G6PD normal patients. This is the first
449 study comparing these two regimens in a randomised controlled trial in South East Asia and although
450 more studies should be conducted in other country of the region, our results suggest that countries in
451 South East Asia should endorse the 7.0 mg/kg regimen.

452

453 Contributors

454 BW, DS and JP conceived the study. VE, SS, Lbfd, AO, JS, DS, SH and NK were responsible for data
455 collection. VE, DL, DS and JP oversaw the study. VE, KT, CF, CS, RWvdP, MW and JP did the data analysis.
456 VE and JP wrote the first draft. All authors had full access to all the data in the study and had final
457 responsibility for the decision to submit for publication.

458

459 Declaration of interests

460 We declare no competing interests.

461

462 Data sharing

463 De-identified patient data will be made available upon reasonable request.

464

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469 and Dr Jessica Lin for overseeing the safety of this trial.

470

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472

473 **Table and Figure legends**

474 Figure 1. CONSORT diagram. G6PD= glucose-6-dehydrogenase, PQ= primaquine

475 Figure 2. Risk of *Plasmodium vivax* recurrence. Kaplan-Meier graphs showing the risk of *P. vivax*
476 recurrences at day 90 (A) and day 180 (B) for patients treated with artesunate and receiving in addition
477 either no primaquine (No PQ), 14 days of 0.25 mg/kg/day of primaquine (PQ 0.25) or 14 days of 0.5
478 mg/kg/day of primaquine (PQ 0.5). Shading represents 95% CI.

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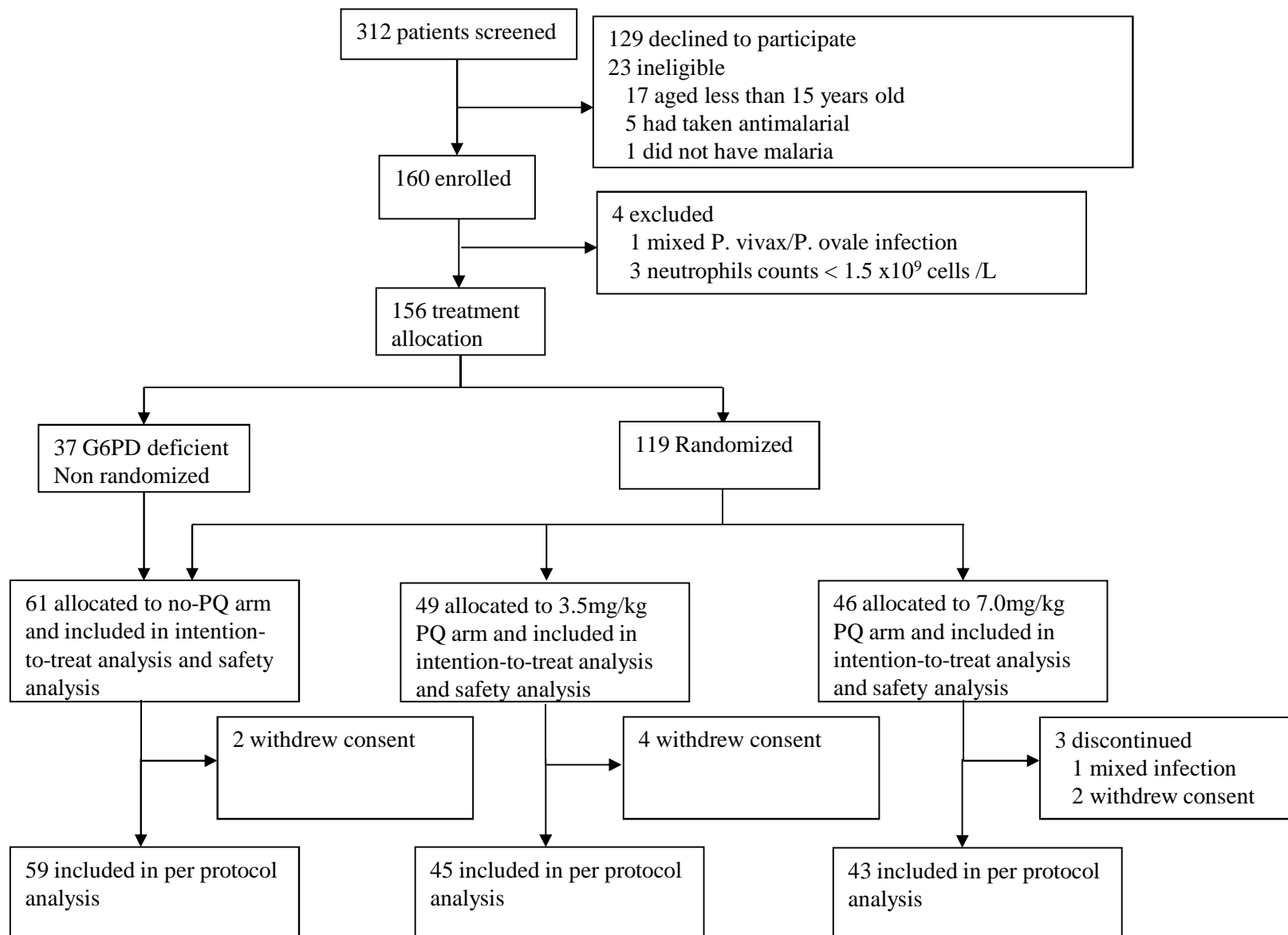
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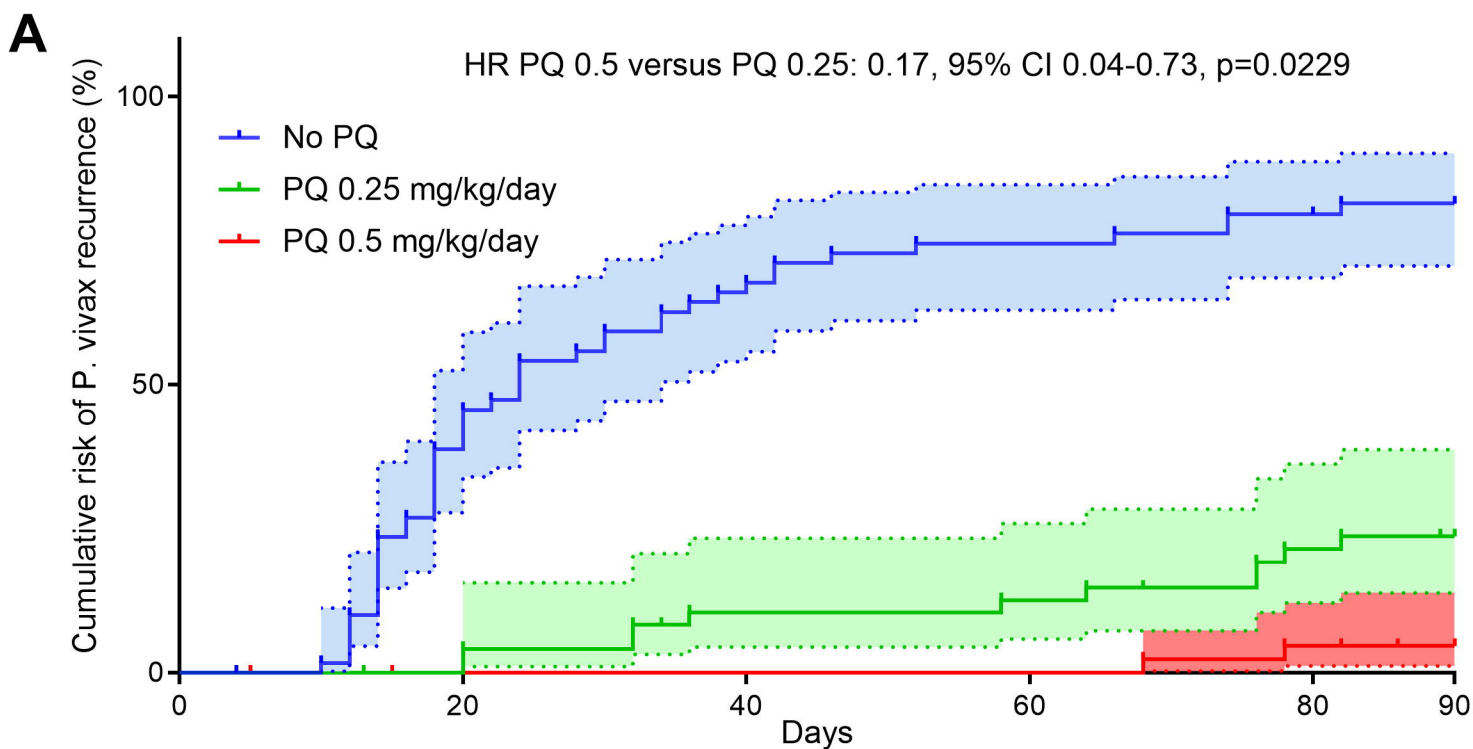
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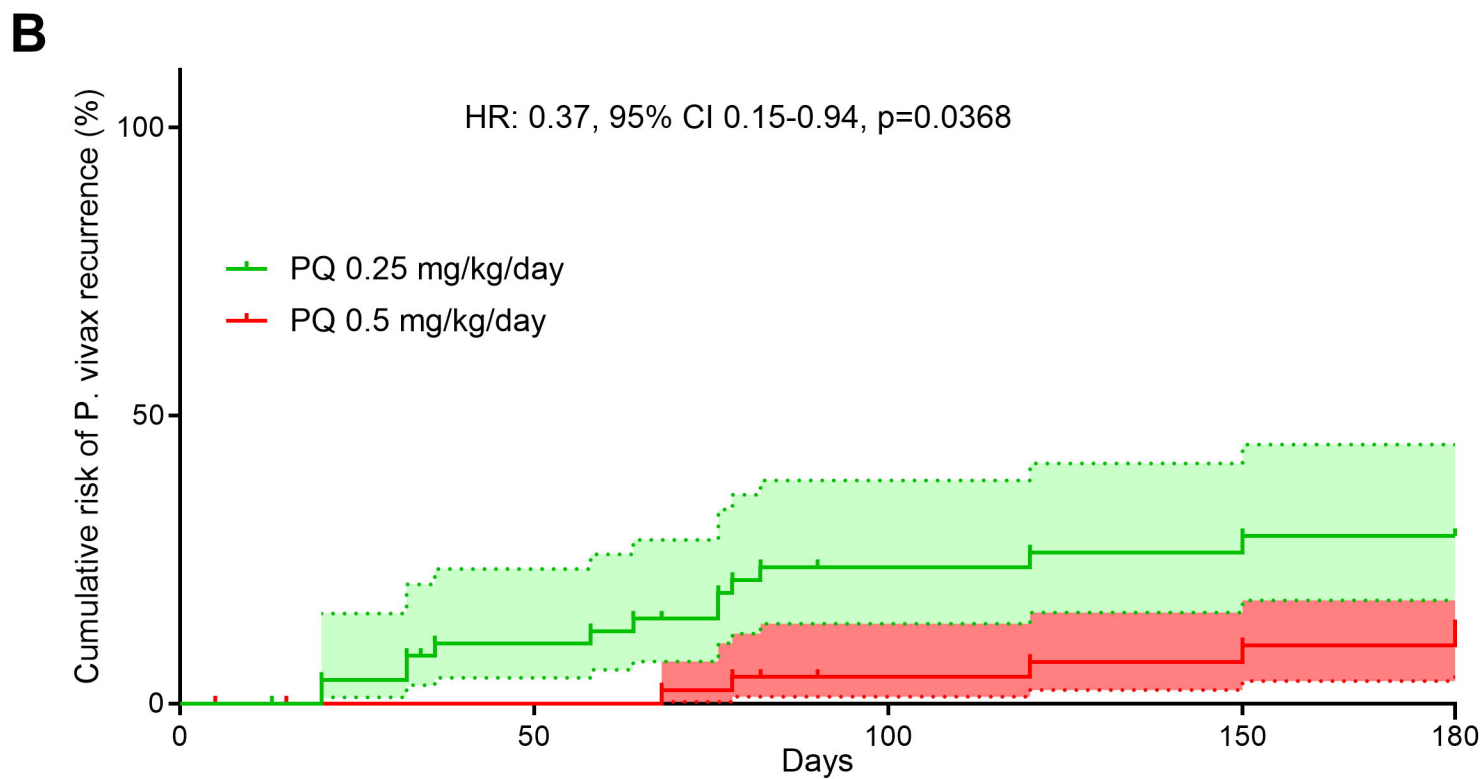
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Number at risk

No PQ	61	36	20	16	12	10
PQ 0.25	49	48	43	42	36	33
PQ 0.5	46	45	45	45	42	39



Number at risk

PQ 0.25	49	43	33	26	22
PQ 0.5	46	45	39	32	29