Article title:

"Electrocardiographic safety of repeated monthly dihydroartemisinin-piperaquine as a candidate for mass drug administration"

Running title: monthly DHA/PQP cardiac safety evaluation.

Names and affiliations of all authors:

Pere Millat-Martínez* [#a,b], Rhoda Ila* [a], Moses Laman [c, d], Leanne Robinson [d,e,f], Harin Karunajeewa [f, g], Haina Abel [h], Kevin Pulai [h], Sergi Sanz [b, i, j], Laurens Manning [k, l], Brioni Moore [k, m], Quique Bassat[¶ b, n, o, p], Oriol Mitjà[¶ b,h,q].

[a] Lihir Malaria Elimination Programme (LMEP), Lihir Island, New Ireland Province, Papua New Guinea.

[b] ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona, Spain.

[c] Department of Paediatrics, Modilon Hospital, Madang, Papua New Guinea.

[d] Papua New Guinea Institute of Medical Research (IMR), Madang, Papua New Guinea.

[e] Burnet Institute, Parkville, Melbourne, Victoria, Australia.

[f] Division of Population Health and Immunity, Walter and Eliza Hall Institute, Parkville, Victoria, Australia.

[g] Western Centre for Health Research and Education, Western Health, Melbourne, Australia.

[h] Lihir Medical Centre, International SOS-Newcrest Mining, Lihir Island, Papua New Guinea

[i] Biostatistics Unit, Department of Public Health, Faculty of Medicine, University of Barcelona, Barcelona, Spain.

[j] CIBER de Epidemiología y Salud Pública CIBERESP, Instituto de Salud Carlos III, Madrid, Spain

[k] Medical School, University of Western Australia, Harry Perkins Research Institute, Fiona Stanley Hospital, Murdoch, Western Australia, Australia.

[l] Department of Infectious Diseases, Fiona Stanley Hospital, Murdoch, Western Australia, Australia.

[m] School of Pharmacy and Biomedical Sciences, Curtin University, Perth, WA, Australia.

[n] Catalan Institution for Research and Advanced Studies (ICREA), Pg Lluís Companys 23, 08010, Barcelona, Spain.

[o] Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique.

[p] Paediatric Infectious Diseases Unit, Paediatrics Department, Hospital Sant Joan de Déu (University of Barcelona), Barcelona, Spain

[q] Division of Public Health, School of Medicine and Health Sciences, University of Papua New Guinea, Port Moresby, Papua New Guinea.

* These authors contributed equally to this work.
¶ These authors are joint senior authors on this work.

#Contact information corresponding author:

Pere Millat-Martínez. Email address: pere.millat@isglobal.org

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Short summary: This interventional study of 3 consecutive monthly treatments of a standard 3-day course of DHA/PQP raised no cardiac safety concerns, including no evidence of QTcF lengthening with cumulative dosing, and overall QTcF prolongation similar to that previously reported after single course treatment.

ABSTRACT

Background: Mass drug administration (MDA) of sequential rounds of antimalarial drugs is being considered as a tool for malaria elimination. As an effective and long-acting antimalarial, Dihydroartemisinin-piperaquine (DHA/PQP) appears suitable as a candidate for MDA. However, absence of cardiac safety data following repeated administration hinders its use in the extended schedules proposed for MDA.

Methods: We conducted an interventional study in Lihir Island, Papua New Guinea, with healthy individuals aged 3 to 60 years who received a standard 3-day course of DHA/PQP on 3 consecutive months. Twelve-lead electrocardiography (ECG) readings were conducted pre-dose and 4h after the final dose of each month. The primary safety endpoint was QTc (using Fridericia's correction; QTcF) prolongation from baseline to 4h post-dosing. We compared the difference in prolongation between the third course post-dose and the first course post-dose.

Results: Of 84 enrolled participants, 69 (82%) completed all treatment courses and ECG measurements. The average increase in QTcF was 19.6 ms (SD 17.8) and 17.1 ms (SD 17.1) for the first-course and third-course post-dosing ECGs [risk difference -2.4 (95%CI - 6.9 to 2.1), p=0.285], respectively. We recorded QTcF prolongation >60 ms from baseline in 3 (4.3%) and 2 (2.9%) participants after the first course and third course (p=1.00), respectively. No participants had QTcF intervals >500 ms at any time point.

Conclusions: Three consecutive monthly courses of DHA/PQP were as safe as a single course. The absence of cumulative cardiotoxicity with repeated dosing support the use of monthly DHA/PQP as part of malaria elimination strategies.

INTRODUCTION

Malaria elimination is set to be a global health priority in coming years and ambitious plans for scaling up from malaria control to elimination already exist in much of the malaria-endemic world. New strategies being actively considered for interrupting malaria transmission include mass drug administration (MDA)(1). This strategy requires the treatment of entire populations with effective antimalarial drugs to reduce the human reservoir of symptomatic and asymptomatic blood stage infections, in addition to conferring post-treatment prophylaxis that prevents re-infection and relapse over a time period that significantly exceeds the lifespan of the anopheline vector (2). Because most individuals treated in an MDA program are asymptomatic parasite carriers or uninfected, the ideal antimalarial for MDA must i) have a prolonged duration of effect that optimises the period of prophylaxis against reinfection and relapse and ii) be demonstrated to be safe when delivered in sequential repeated treatment courses in the manner proposed for MDA deployment (2).

Dihydroartemisinin-piperaquine (DHA/PQP) is a good candidate for MDA. The rapid-acting artemisinin-derivative reduces the parasite biomass of existing infections and the long-acting PQP component exerts an especially long post-treatment prophylactic effect (3). Repeated monthly exposure to standard 3-day treatment courses of DHA/PQP over 3 consecutive months should theoretically prevent new infections for a period of at least 90-120 days. In a meta-analysis of 11 studies, monthly DHA/PQP for high-risk populations was associated with an 84% reduction in the incidence of malaria parasitaemia (4-6).

Nevertheless, the feasibility of DHA/PQP for MDA has been questioned because PQP can cause dose-dependent prolongation of the electrocardiographic QT interval. As such, PQP is contraindicated in patients with congenital long QT syndrome (about one in 2500 children) or those taking other drugs that prolong the QT. Mild QT prolongation is clinically silent, but

extreme prolongation can cause fatal arrhythmias such as Torsades de Pointes (TdP). Prior studies have demonstrated that QTc prolongation associated with DHA/PQP is predominantly mild and not associated with clinical adverse cardiac outcomes (4, 7). However, PQP is eliminated slowly (elimination half-life ~ 23 days) and the risk of QT prolongation may be exacerbated when repeated doses are given, especially if given monthly (8). Therefore, the drug manufacturer and the European Medicines Agency (EMA) recommend that repeated treatment courses of DHA/PQP with the brand name Eurartesim 160mg/20mg film coated tablets should not be administered within two months of initial treatment (9). Unfortunately, this is unlikely to be optimally effective if DHA/PQP is given as MDA; mathematical models suggest a maximum interval of one month between treatment rounds is required to interrupt transmission (10).

The current study aims to assess the cardiac safety of three repeated monthly courses of DHA/PQP, in Lihir island, PNG, a malaria-endemic population likely to be targeted in future MDA activities.

METHODS

Participants and study setting

From Sept 21 to Dec 21, 2015 we conducted a prospective, single-arm, intervention study with healthy volunteers who resided in Lihir Island, New Ireland Province, Papua New Guinea (PNG). Eligible participants were male or female individuals aged 3 to 60 years with good general health by medical history, physical examination, baseline electrocardiographs and laboratory tests. Participants were excluded if they i) had a QT interval (adjusted using Fridericia's correction; QTcF) greater than 450 ms or clinically significant abnormalities of rhythm at screening, ii) had a known history of additional risk factors for TdP, iii) had a family history of long QT syndrome or sudden cardiac death, iv) were using concomitant

121 medications known to prolong the QT/QTc interval, or v) had a history of relevant allergic
122 reactions. All female participants in reproductive age were tested for pregnancy (urinary
123 βHCG dipstick) and excluded if they were in the first trimester.

124 The study protocol was approved by the PNG Institute of Medical Research – Institutional
125 Review Board (number 15.01), the PNG Ministry of Health - Medical Research Advisory
126 Committee (number 15.14), and the Ethics Committee of the Barcelona's Hospital Clinic, in
127 Spain (HCB/2014/0424). Written informed consent was obtained from all adult participants
128 and, for children from parents or guardians.

129 **Study procedures**

130 We recruited a non-probabilistic convenience sample of healthy volunteers through group
131 presentations by trained staff in 11 local communities, followed by one-to-one interviews.
132 The volunteers were admitted at the hospital for a period of 72 hours, to facilitate drug
133 administration under direct observation and fasting, and measurement of ECG traces.
134 Arterakine® (Pharbaco Central Pharmaceutical, Vietnam) was given daily for 3 days (i.e. at
135 time 0, 24 and 48 hours) following the dosing schedule of the PNG National Malaria
136 Treatment Protocol, DHA/PQP 2.1/17.1 mg/kg (11). Patients were fasted for the three hours
137 before and after each DHA/PQP dose.

138 Detailed physical examination, routine clinical laboratory tests, pregnancy test for women of
139 reproductive age, a rapid diagnostic test (RDT) for malaria, and a malaria blood slide were
140 performed at baseline (time 0 hours) prior to treatment. Participants were assessed for adverse
141 events (AEs) with a structured questionnaire and examination every 8 hours throughout day 3
142 (i.e. at time 48, 56, 64 and 72 hours) of each course. Examination included measurement of
143 blood pressure, pulse, respiratory rate, cardiac auscultation, respiratory auscultation and

144 abdominal palpation for all participants. Blood samples to analyse drug levels were collected
145 on 52h (4h after third dose) of each administration course.

146 **Electrocardiography and electrocardiographic endpoints**

147 Twelve-lead ECG readings were conducted using an ELI 150 Cardiograph® at 25 mm/sec
148 speed at pre-dose (at time 0 hours, in triplicate), immediately prior to administration of the
149 third dose (at time 48 hours, single trace), and 4h after the third dose (at time 52 hours, in
150 triplicate). For ECGs taken in triplicate, parameter measurements for the final analysis were
151 based on the arithmetic mean of measurements from the 3 readings. Participants were
152 discharged 24 hours after the last dose of treatment (at time 72 hours). In addition, ECGs
153 were conducted at 7 days after the start of treatment and before (at time 0 hours) the
154 following treatment course. The same procedure was repeated for the second and the third
155 monthly treatment courses. Throughout the study period ECG readings were conducted at 12
156 time-points over the 63-day follow-up period: day $0_{\text{pre-dose}}$ ($0h_{\text{course1}}$), day $2_{\text{pre-dose}}$ ($48h_{\text{course1}}$),
157 day $2_{\text{post-dose}}$ ($52h_{\text{course1}}$), day 7 ($7\ days_{\text{course1}}$), day $28_{\text{pre-dose}}$ ($0h_{\text{course2}}$), day $30_{\text{pre-dose}}$ ($48h$
158 $_{\text{course2}}$), day $30_{\text{post-dose}}$ ($52h_{\text{course2}}$), day 35 ($7\ days_{\text{course2}}$), day $56_{\text{pre-dose}}$ ($0h_{\text{course3}}$), day $58_{\text{pre-dose}}$
159 ($48h_{\text{course3}}$), day $58_{\text{post-dose}}$ ($52h_{\text{course3}}$), and day 63 ($7\ days_{\text{course3}}$).

160 The QT interval (i.e, distance from the Q wave to the end of the T wave) was corrected for
161 heart rate using Fridericia's correction formula (QTcF). This was defined as the measured QT
162 interval divided by the cube root of the RR interval. The auto-calculated QTcF measurements
163 were manually verified by the study clinician for safety purposes. All ECGs were
164 electronically transferred to a cardiac core lab (Banook/Cardiabase in France) where
165 independent and centralized interpretation of the tracings were repeated by a certified
166 cardiologist blinded to the participant's details. The following parameters were obtained: RR

(ms), HR (bpm), PR (ms), QRS (ms), QTcF (ms), and ΔHR (bpm), ΔPR (ms), ΔQRS (ms), ΔQTcF (ms) by comparison with baseline ECG (time 0 hours) for each course of treatment.

A Data Safety Monitoring Board (DSMB) was established to which the site clinician (PM) was responsible for reporting any AE classified as either "Adverse Events of Special Interest" (AESI) or "Severe Adverse Events" (SAE) according to whether they met pre-specified criteria: We defined AESI as QTcF prolongation from baseline >60 ms; QTcF at any time >450 ms; T wave morphologic changes during therapy; bundle branch block or any new arrhythmia. We defined cardiologic SAEs as QTcF >500 ms (sensitivity is 94% and specificity 97% for prediction of malignant arrhythmia in overdose schedules), any malignant ventricular arrhythmia (e.g. TdP) or any episode of sudden death (12). We established a pre-determined threshold for study cessation as the occurrence of three episodes meeting any of our criteria for a cardiologic SAE.

Statistical analysis

The primary endpoint of the study was ΔQTcF, calculated as the difference between QTcF measured at 0 h recording to 52h of each of the three courses. Secondary endpoints were i) occurrence of AESI and SAEs and ii) QTcF resolution. For analysis of the primary endpoint, we estimated the risk difference and two-sided 95% CIs in ΔQTcF between the ECG at 52h $_{\text{course 3}}$ and the ECG at 52h $_{\text{course 1}}$ using a paired T-test for comparisons. For analysis of the secondary endpoints, we estimated the risk difference and two-sided 95% CI for the difference in QTcF between the ECG at 0h $_{\text{course3}}$ and the ECG at 0h $_{\text{course1,}}$ we also looked at the difference between the ECG at 7days $_{\text{course3}}$ and ECG at 7days $_{\text{course1}}$. We counted and summarized the number of AESIs and we used the McNemar test to compare the difference in occurrence of AESIs between the third and first courses of treatment. Data analysis was performed using Stata 15.1 software (Stata Corporation, College Station, TX, USA).

We calculated that 73 individuals would be required for the primary analysis to estimate a 5 ms difference in mean QTcF prolongation between the first and third treatment course (assuming a prolongation in mean QTcF of 20 ms for the first month and 25 ms for the third month) with a confidence level of 95% and a power of 80%. Assuming that 10% of participants would be lost to follow up, the target recruitment was 82 individuals.

RESULTS

Of 110 individuals screened, 84 (76.3%) met the inclusion criteria and consented to participate in the study. Eighteen declined consent and 8 were ineligible (first trimester pregnancy, n=2; uncontrolled hypertension, n=2: asymptomatic arrhythmia, n=2; clinical heart failure, n=1; and congenital heart disease, n=1). Of the 84 participants who were initially enrolled, 69 (82.2%) completed all treatment courses and follow-up appointments (per-protocol population) and were included in subsequent analysis.

The mean age of participants in the per-protocol population was 27.4 years (SD 12.6); 7 (10%) were children (aged 3-15), and 34 (49%) were female. One female participant was pregnant (confirmed second trimester by dates and clinical examination). At baseline the mean QTcF was 397.3 ms (SD 17.2). Sixty-five (94%) participants had a normal baseline-ECG and 4 (6%) participants had minor abnormalities of no clinical relevance (i.e. 3 with non-pathological T-wave inversion and 1 with poor R-wave progression). Comparison of baseline demographic, clinical and electrocardiographic characteristics of participants that completed follow up and those that were lost to follow up were not-significantly different (Table 1).

213 The mean (SD) ΔQTcF (from 0 h to 52 h) was 19.6 ms (17.8) in course 1, 23.6 ms (15.3) in
214 course 2 [difference 4.0 ms (95% CI 0.1 to 8.0); p =0.043], and 17.1 ms (17.1) in course 3
215 [difference -2.4 (95%CI -6.9 to 2.1); p=0.285; Table 2 and Figure 1 and 2].

216 In subgroup analyses (Supplementary Table S1), the mean (SD) ΔQTcF was higher in
217 females [25.8 ms (20.5)] than in males [13.5 ms (12.2)] after course 1 (p=0.003). However,
218 there was no significant difference in ΔQTcF between males and females after the second and
219 third courses [22.7 ms (15.9) in males and 24.5 ms (14.9) in females, p=0.623 for the second
220 course; and 14.3 ms (15.5) in males and 20.1 ms (18.4) in females, p=0.158 for the third
221 course]. Mean QTcF segment and ΔQTcF did not differ significantly according to age group
222 (data not shown).

223 Figure 1 shows the evolution of QTcF at all time-points (days 0, 2, 7, 28, 30, 35, 56, 58, and
224 63). Table 3 shows the resolution in ECG parameters at 7 days after the start of each
225 DHA/PQP treatment course and prior (at 0 hours) of the following course. QTcF 0 h $_{course3}$
226 was shorter compared to QTcF 0 h $_{course1}$ (-4.0 ms, p<0.001), which represents a full
227 resolution of the QTcF prolongation 28 days after the start of treatment course 2. QTcF and
228 ΔQTcF parameters on 7d $_{course\ 2}$ and 7d $_{course\ 3}$ showed no differences compared to 7d $_{course\ 1}$
229 (Supplementary Table S2).

230 No participant had cardiac related SAEs during the study period. Table 4 shows the recording
231 of cardiac AESIs occurring any time during the 63-day study observation period. ΔQTcF >60
232 ms was observed in 3 (4.3%), 1 (1.4%, p=0.50), and 2 (2.9%, p=1.00) after the first, second
233 and third course of treatment, respectively. None of the participants had QTcF readings of
234 more than 500 ms. QTcF readings of ≥450 to <500 ms were noted in 6 (8.9%), 5 (7.2%,
235 p=1.00), and 5 (7.2%, p=1.00) after the first, second and third course of treatment,
236 respectively. Other reported cardiac AESIs included sinus bradycardia (<40 bpm) in 1 (1.4%)

participant in each treatment course, and 2 (2.9%) participants with changes in T-wave morphology after the second course of treatment. No AESIs were accompanied by clinical symptoms. Other previously defined AESIs (new bundle branch block and arrhythmia of new appearance) were not reported in any participant at any time during the study.

Non-cardiac AEs occurred in 29 (14.0%) of 207 patient-visits. All reported AEs were mild and transient, with no participant requiring specialised treatment. The most commonly reported AE was abdominal pain (n=12, 5.8%), followed by headache (3.8%), cough (2.4%), and nausea (1.9%). All RDTs and microscopy smears collected at 0 $h_{course2}$ and 0 $h_{course3}$ were negative for malaria parasitaemia.

Other ECG measurements (HR, ΔHR, PR, ΔPR, QRS and ΔQRS) along the 63 days of evolution are described in supplementary tables S3 and S4.

DISCUSSION

In this study three monthly repeated courses of DHA/PQP resulted in our primary study endpoint, change in mean post-final dose QTcF between the first and final courses, of 17.7 ms; a magnitude generally similar to those described after a single course and lying below the U.S Food and Drug Administration's 20 ms threshold of high level of concern (13). Our findings that QTcF prolongation did not increase in a cumulative manner with repeat courses was supported by the observation that QTcF interval returned to normal prior to each subsequent course of treatment. Interestingly, the QTcF difference increased slightly (23.6 ms) after the second but not the third course. This result is intriguing and will require correlation with drug concentrations once the results related to the drug's pharmacokinetics of the current study become available. In addition, it is reassuring that no individual had any

QTcF > 500 ms and that the number of cases with an absolute increase >60 ms were limited to 6 individuals, evenly spread through each of the monthly courses. This therefore does not add to concerns raised by a recent large multicentric clinical trial about the possibility of increased incidence of this event after a repeated dose (7). Our results support previous findings that QT/QTc prolongation following DHA/PQP administration is consistently lower than that caused by other commonly used antimalarial drugs such as quinine (14, 15) or chloroquine (16). Previous studies assessing the cardiotoxicity of DHA/PQP have shown minimal QTc prolongation following a single 3-day course. For example, in Cambodia the mean (corrected by Bazett's formula; QTcB) prolongation in 62 individuals was 11 ms 24 h after first dose (17) and the mean QTcF prolongation in 56 adults in Thailand it was 29 ms 52 h after the start of treatment (18). In a cohort of 1002 malaria patients from a study conducted in four African countries, only 3 (0.3%) had QTcF >500ms after standard 3-dose treatment (one-month course) and less than 10% of participants had an increase in QTcF more than 60 ms from baseline (19). A recent metanalysis of 11 studies involving repeated exposures of DHA/PQP for seasonal malaria chemoprevention or treatment of clinical malaria found no increased incidence of AEs with repeated dosing; however, only one of these (an unpublished study in 13 pregnant women) performed electrocardiographic assessments of QTc prolongation). The authors called for more studies incorporating electrocardiogram measurements (4). Recent MDA programmes using DHA/PQP in large populations have shown that this drug combination is safe to use due to the minimal occurrence of serious adverse events; however, electrocardiographic assessments were not performed to monitor cardiac side effects (20-22).

Several secondary observations are worth noting, including gender differences in the measurements of QT related parameters. The study showed an increase in QTcF among females at all time points that is consistent with previous studies (23, 24). Regarding AEs,

less than one fifth of patients reported mild abdominal pain, headache, cough or nausea. All appeared to be self-limiting and none required specific treatment or intervention. Although this trial was not designed to evaluate the efficacy of DHA/PQP, all participants remained parasite negative for the duration of the study. This lack of breakthrough infections, in an area where malaria transmission is high (5, 6, 25) and endogenous *Plasmodium vivax* relapses are common, reassuringly suggested that post-treatment prophylaxis was satisfactory.

This study, however, does present certain limitations. The first one includes the lack of a control group, that may hinder some of the interpretation of our results. Another limitation was the relatively high attrition rate that saw 18% of enrolled participants fail to complete the scheduled follow-up appointments and were therefore excluded from our per-protocol analysis. If those lost to follow-up had been prone to greater QTc prolongation, this could have been a source of bias. However, there were no statistically significant differences in baseline characteristics between participants who were lost to follow-up compared with those who completed follow-up. We also acknowledge an issue of generalizability of our findings regarding the way we managed food co-administration in this study. Administration of DHA/PQP with food, particularly fat, increases the bioavailability leading to increased drug concentrations and greater degree of QT prolongation (8). We carefully controlled this by advising all participants to avoid food intake for the 3 hours before and after drug administration. However, whilst feasible in the context of a tightly controlled research study, this level of compliance may be difficult to achieve in a real MDA campaign delivered at very large scale. Hence, it is possible that individuals not following this dietary advice could be prone to greater drug absorption, higher cumulative doses and greater QTc prolongation than seen in our study.

Our primary endpoint examined changes in mean QTcF values, consistent with US FDA and other regulatory bodies. However, in terms of population risk, the way in which values are

distributed across populations is perhaps more important than the measures of centrality reported here. It is those individuals who lie on the upper edge of the population distribution who are most important. For example, if an MDA intervention is deployed in 100,000 people, presuming that QTc distribution has a normal population distribution, 2,500 will have QTc prolongations that are 2SD above the population mean. These would be the individuals of greatest risk of a serious cardiac event. Our clinical study, like all others performed to date, did not have anywhere near the sample size required to define the population distribution accurately enough to define risk in this group. In practice, the only way this will ever be further clarified is by robust pharmacovigilance employed during large-scale MDA implementation programs.

In this study, all patient QTc measurements after three courses of DHA/PQP treatment were within approved safety margins outlined by US drug regulatory institutions. The data do not suggest that the known risk of QT prolongation increases cumulatively with repeated monthly courses out to the 3rd course. However, the observation of slightly increased QT prolongation after two courses of treatment requires further investigation. At this stage, data from this study, together with the lack of reported adverse cardiac events in repeated-course MDA intervention programs elsewhere (5, 25), suggest that DHA/PQP can be used safely as MDA delivered using conventional dosing in up to 3 monthly rounds as a tool for malaria elimination. Cautions need to be articulated in relation to the antimalarial major drawbacks. First, multidrug resistance could reduce the impact, therefore monitoring of the drug's efficacy will be required (26). Second, the three daily doses required for each round of MDA result in a major logistic burden and stretch the tolerance of the target population. Nevertheless, the absence of a single dose antimalarial with safety data for repeated dosing, support DHA/PQP as one of the best available options for MDA.

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CONFLICT OF INTEREST

QB is member of the WHO Malaria Treatment Guidelines Group. This Group produces global guidance on the treatment of malaria and this includes decisions about pyronaridine–artesunate. The views expressed by the Authors are personal opinions and do not represent the recommendations of WHO. The remaining authors of this research declare no conflicts of interest.

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Author contributions: OM and QB conceived the study and developed the analysis plan. PM and RI conducted the study including patient enrolment and data collection. HA and KP conducted the laboratory tests. PM and SS conducted the statistical analyses. OM, PM and QB prepared the first draft of the article with important intellectual input and revisions of RI, ML, LR, HK, LM, and BM. All authors approved the final version of the article.

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FIGURE LEGENDS:

Figure 1. Electrocardiographic QTcF measurements over the study period

Legend: Results are median and Interquartile Ranges (IQR) of all QTcF (QT corrected by Fridericia's correction) measurements over the 63 days of study. Arrows are the DHA/PQP doses. Day 0 corresponds to 0h value of each month, day 2 values correspond to 48h (pre-dose) and 52h (4h post-dose) measurements and Day 7 value is the value of the Day 7 measurement of each month.

Figure 2. Changes in the QTcF measurements (ΔQTcF) over the study period

Legend: Results are mean and standard deviation (SD) of the ΔQTcF (difference in QT corrected by Fridericia's correction between baseline and each point of measurement) over the 63 days of study. $0h_{course1}$ corresponds to day 0 pre-dose, $52h_{course1}$ corresponds to day 2 post-dose, $day7_{course1}$ corresponds to day 7 post-dose, $0h_{course2}$ corresponds to day 28 pre-dose, $52h_{course2}$ corresponds to day 30 post-dose, $day7_{course2}$ corresponds to day 35, $0h_{course3}$ corresponds to day 56 pre-dose, $52h_{course3}$ corresponds to day 58 post-dose and $day7_{course3}$ corresponds to day 63.

Table 1. Comparison of baseline characteristics of participants that completed follow up and participants that were lost to follow up.

Variable		Status			p-value
		Finish study	Lost to follow up	Total	
Demographic variables					
Sex	Male	35 (51%)	11 (73%)	46 (55%)	0.111
	Female	34 (49%)	4 (27%)	38 (84%)	
Age (years)		27.4 (12.6)	29.6 (12.8)	27.8 (12.6)	0.546
	<15 years old	7 (10%)	1 (7%)	8 (10%)	1.000
	≥15 years	62 (90%)	14 (93%)	76 (90%)	
Clinical variables					
Hypertension	No	68 (99%)	15 (100%)	83 (99%)	1.000
	Yes	1 (1%)	0 (1%)	1 (1%)	
Diabetes	No	69 (100%)	15 (100%)	84 (100%)	_
	Yes	0 (0%)	0 (0%)	0 (0%)	
Chronic treatment	No	68 (99%)	14 (93%)	82 (98%)	0.327
	Yes, no related with risk of QT enlargement	1 (1%)	1 (7%)	2 (2%)	
Temperature (ºC)		35.5 (0.9)	35.2 (1.1)	35.4 (0.9)	0.238
Cardiac auscultation	No alterations	64 (93%)	15 (100%)	79 (94%)	0.580
	Presence of murmur	5 (7%)	0 (0%)	5 (6%)	
Splenomegaly	No	64 (93%)	15 (100%)	79 (94%)	0.580
	Yes	5 (7%)	0 (0%)	5 (6%)	
Blood tests					
Malaria test (blood slide)	Negative	63 (91%)	15 (100%)	78 (93%)	1.000
	Positive *P.falciparum*	4 (6%)	0 (0%)	4 (5%)	
	Positive *P.vivax*	1 (1%)	0 (0%)	1 (1%)	
	Positive *P.malariae*	1 (1%)	0 (0%)	1 (1%)	
Glucose (mmol/L)		4.8 (3.7)	5.4 (1.4)	4.9 (3.4)	0.564
Creatinine (µmol/L)		70.7 (26.5)	79.6 (26.5)	70.7 (26.5)	0.370
Sodium (mmol/L)		139.9 (3.8)	141.7 (1.4)	140.2 (3.5)	0.083

Potassium (mmol/L)		4.0 (0.3)	4.3 (0.3)	4.1 (0.3)	**0.018**
Haemoglobin (g/dL)		12.5 (2.1)	13.1 (2.0)	12.6 (2.1)	0.309
ECG parameters					
Heart rate (bpm)		68.4 (12.4)	73.8 (11.6)	69.4 (12.3)	0.127
PR segment (ms)		166.9 (18.7)	165.1 (22.0)	166.6 (19.2)	0.749
QRS segment (ms)		90.9 (9.5)	92.7 (9.2)	91.2 (9.4)	0.525
QTcF segment (ms)		397.3 (17.2)	391.6 (18.8)	396.3 (17.5)	0.255
Conclusion	ECG Normal	65 (94%)	12 (80%)	77 (92%)	0.104
	Minor non-pathological abnormalities	4 (6%)	3 (20%)	7 (8%)	

Legend: For quantitative variables results are described in mean ± Standard Deviation (SD); for qualitative variables results are described in numbers and percentage (%). P-value corresponds to the results of the paired T-test comparing both groups. Abbreviations: bpm= beats per minute; ms= milliseconds. Statistically significant results are reflected in bold.

Table 2. Electrocardiographic measurements of QTcF and ΔQTcF at 52 hours post dose during the second and third monthly course with dihydroartemisinin-piperaquine compared with the first month.

ECG parameter (units)	0h $_{course1}$	52h $_{course1}$	52h $_{course2}$	p-value	Risk difference (95%CI)	52h $_{course3}$	p-value	Risk difference (95%CI)
QTcF (ms)	397.3±17.2	416.8±23.4	421.0±19.8	**0.040**	**4.1 (0.2 to 8.1)**	414.3±23.6	0.270	-2.5 (-7.1 to 2.0)
ΔQTcF (ms)	-	19.6±17.8	23.6±15.3	**0.043**	**4.0 (0.1 to 8.0)**	17.1±17.1	0.285	-2.4 (-6.9 to 2.1)

Legend: 52h readings were conducted 4h after administration of the third dose. The parameter measurements are the arithmetic mean of measurements from the triplicate reading. Results of measurements, p-values and 95%CI for the paired t-test for comparison with measurement of **52h $_{course1}$**. Measurements are described in mean ± Standard Deviation (SD). Abbreviations: ms= milliseconds. Statistically significant results are reflected in bold.

494 **Table 3. Electrocardiographic measurements of QTcF and ΔQTcF at 7 days after the start of each course of DHA/PQP and before the**

ECG parameter (units)	0h $_{course1}$	7d $_{course1}$		0h $_{course2}$		7d $_{course2}$		0h $_{course3}$		7d $_{course3}$	
		measurement	p-value	measurement	p-value	measurement	p-value	measurement	p-value	measurement	p-value
QTcF (ms)	397.3±17.2	405.7±20.9	**<0.001**	393.9±18.1	**0.043**	407.7± 22.7	**<0.001**	389.9±18.9	**<0.001**	407.7±22.4	**<0.001**
ΔQTcF (ms)	-	8.0±15.7	-	-3.7±13.3	-	10.1±16.1	-	-7.4±14.8	-	11.0±16.9	-

495 **start (at 0 hours) of the following monthly course compared with 0h measurements on day0 first course.**

496 Legend: Results of measurements and p-values for the paired t-test for comparison with baseline 0h $_{course1}$ measurements. Measurements are

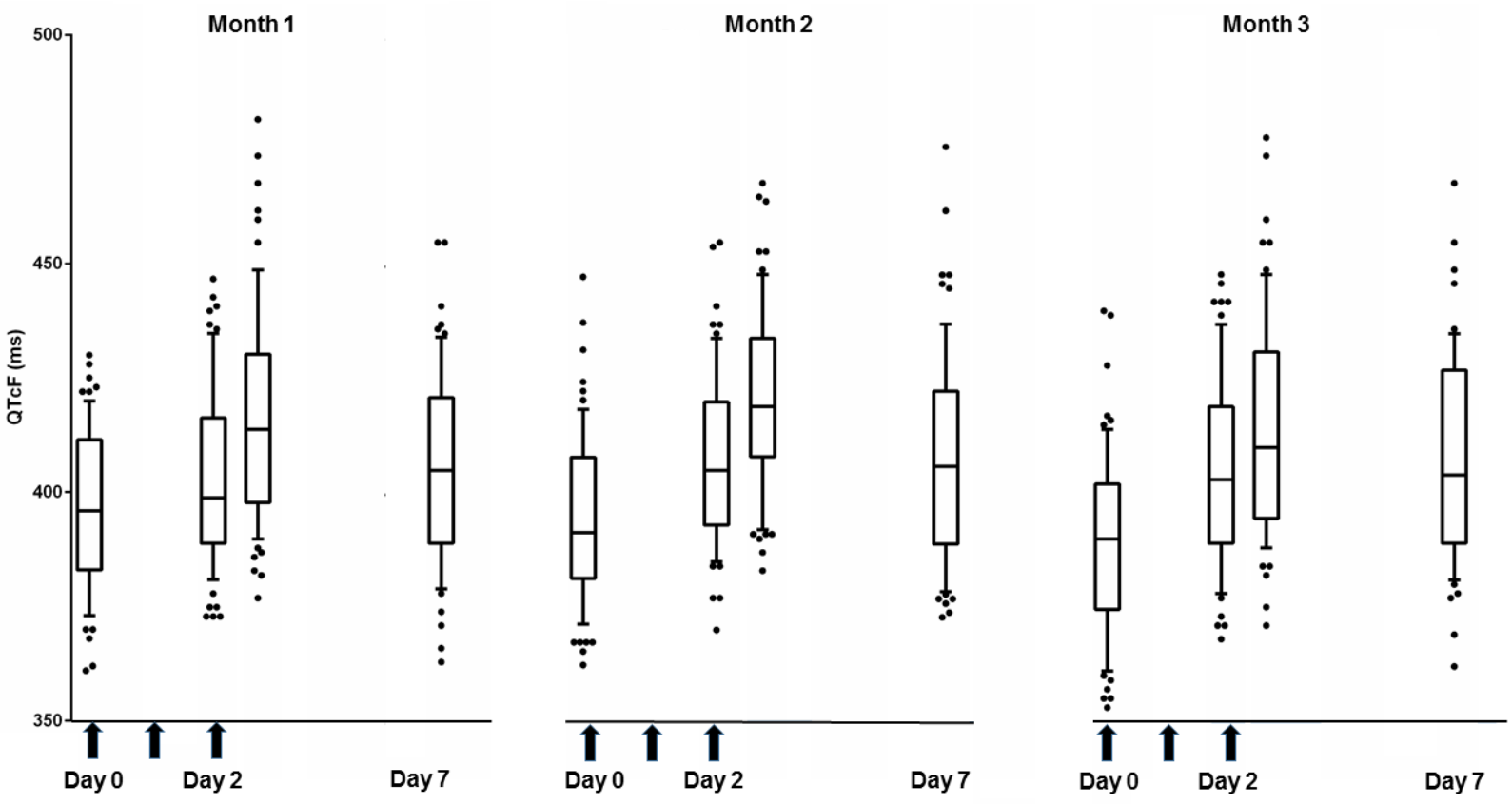
497 described in mean ± Standard Deviation (SD). Abbreviations: ms= milliseconds. Statistically significant results are reflected in bold.

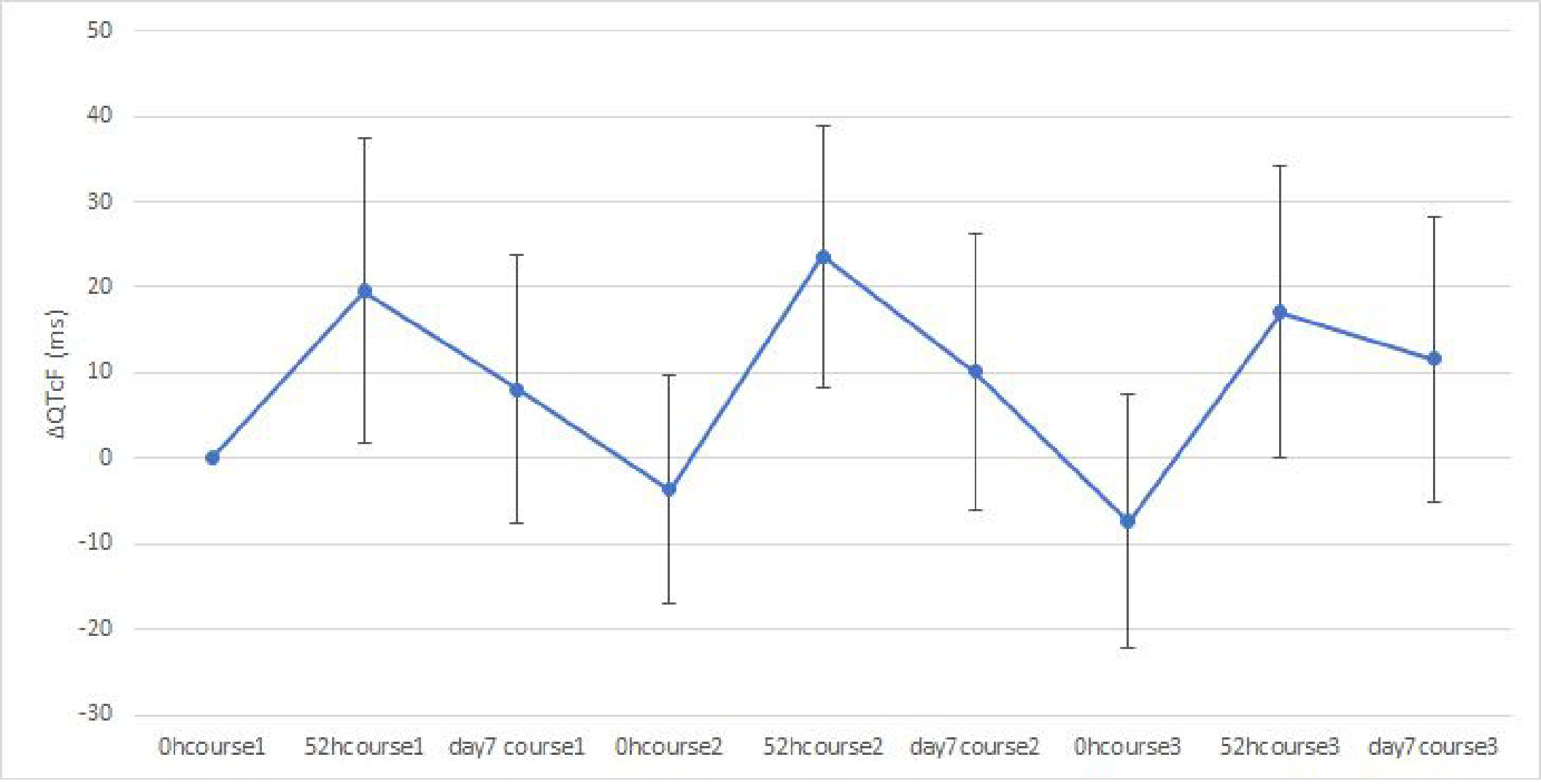
498

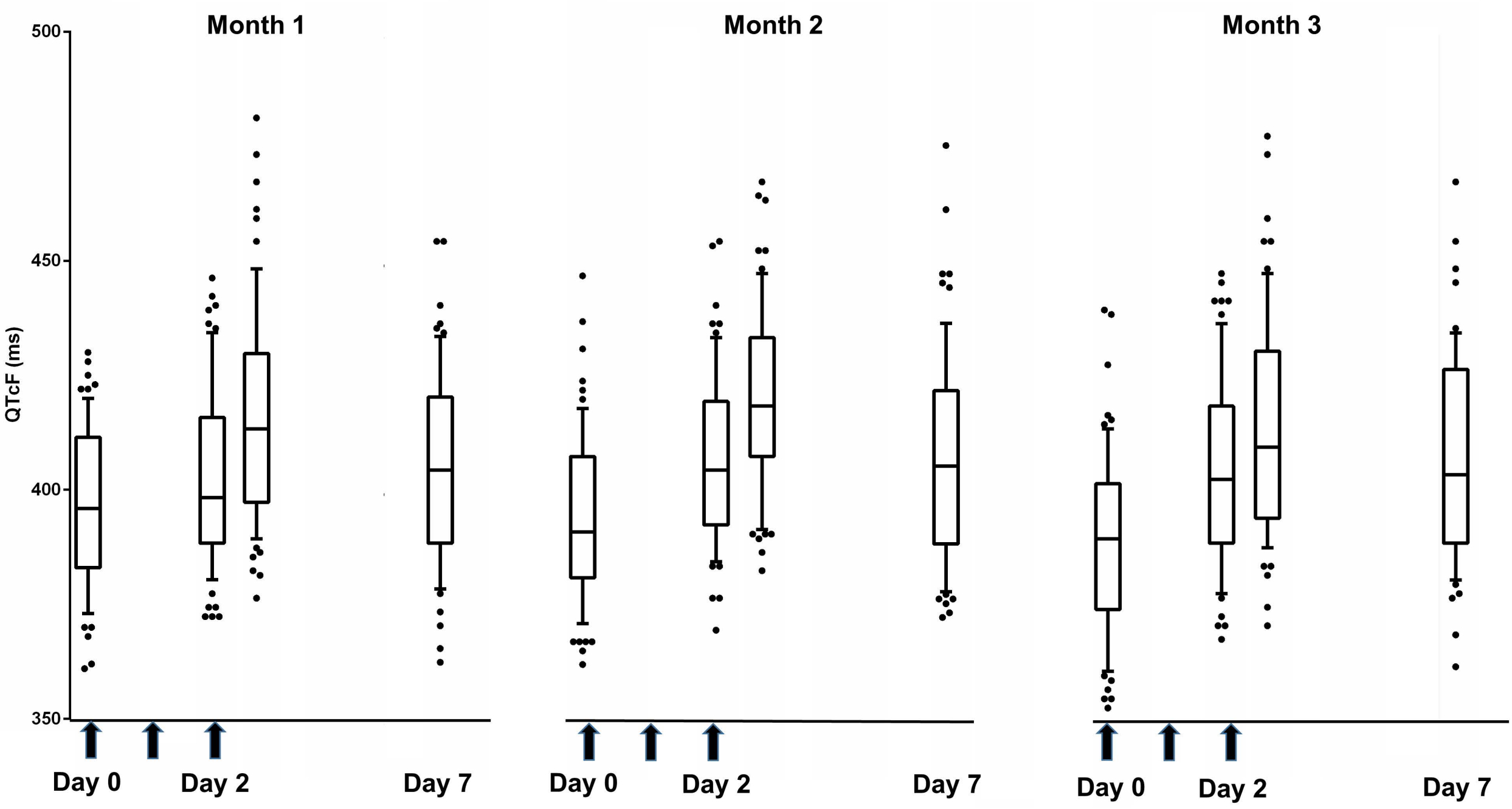
Table 4. Adverse Events of Special Interest recorded on 52h of each treatment course.

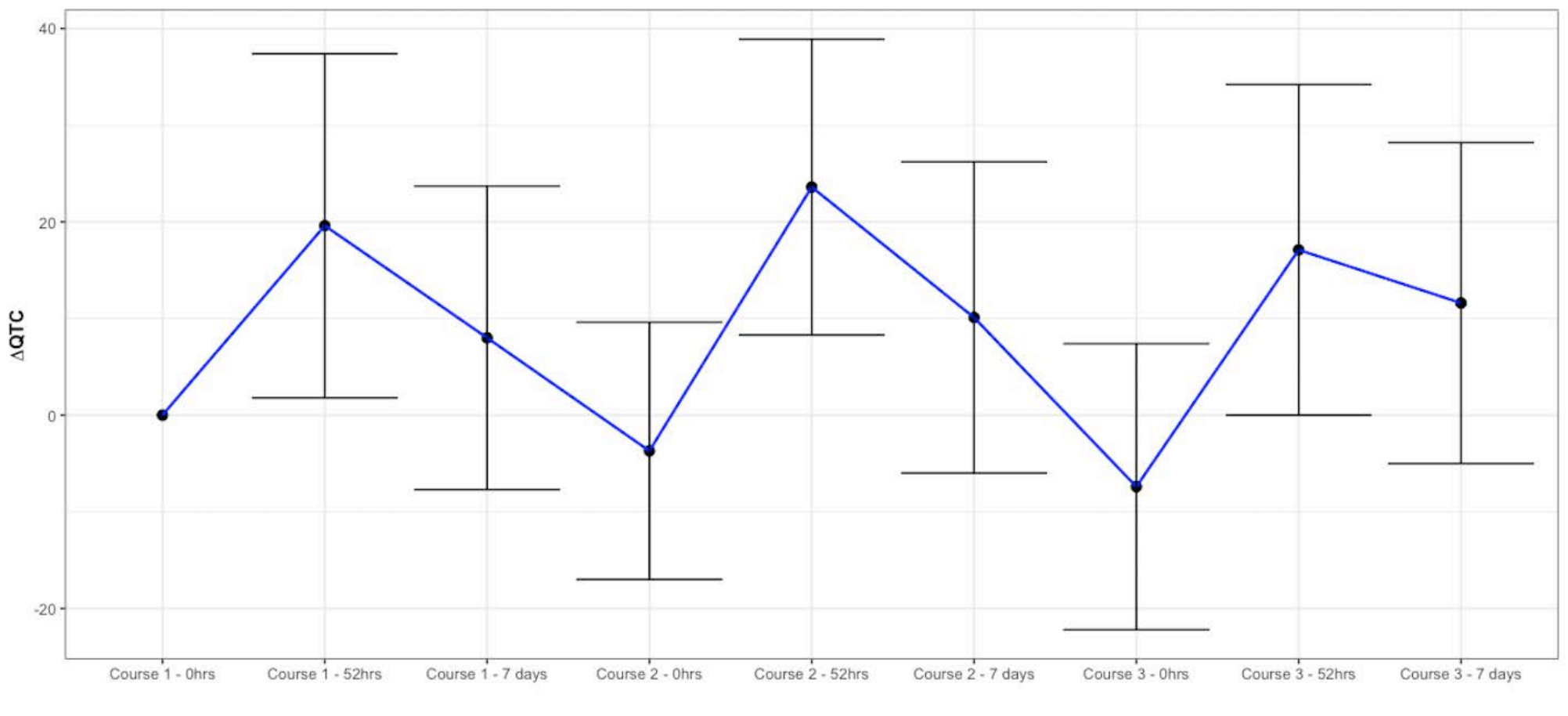
AESI description	52h $_{course1}$ N (%)	52h $_{course2}$ N (%)	p-value for the difference with course 1	52h $_{course3}$ N (%)	p-value for the difference with course 1
QTcF prolongation >60 ms	3 (4.3%)	1 (1.4%)	0.50	2 (2.9%)	1.00
QTcF ≥450 ms <500 ms	6 (8.7%)	5 (7.2%)	1.00	5 (7.2%)	1.00
Sinus bradycardia <40 bpm	1 (1.4%)	1 (1.4%)	1.00	1 (1.4%)	1.00
T wave morphologic changes	0 (0.0%)	2 (2.9%)	0.50	0 (0.0%)	-

Legend: Results are described in number of cases (N) and percentage (%) in the per-protocol study population. P-values results are shown for the exact McNemar significance probability test. AESI: Adverse Event of Special Interest. Other AESIs: none (0) cases of new bundle branch block and none (0) of arrhythmia of new appearance were described.


Month 1
Month 2
Month 3
500
450
400
350
QTcF (ms)
Day 0
Day 2
Day 7
Day 0
Day 2
Day 7
Day 0
Day 2
Day 7


50
40
30
20
10
0
-10
-20
-30
ΔQTcF (ms)
0hcourse1
52hcourse1
day7 course1
0hcourse2
52hcourse2
day7course2
0hcourse3
52hcourse3
day7course3


Month 1
Month 2
Month 3
500
450
400
350
QTcF (ms)
Day 0
Day 2
Day 7
Day 0
Day 2
Day 7
Day 0
Day 2
Day 7


40
20
0
-20
ΔQTC
Course 1 - 0hrs
Course 1 - 52hrs
Course 1 - 7 days
Course 2 - 0hrs
Course 2 - 52hrs
Course 2 - 7 days
Course 3 - 0hrs
Course 3 - 52hrs
Course 3 - 7 days