Prospective observational study of dihydroartemisinin-piperaquine treatment of vivax malaria in North Sumatera, Indonesia

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Research Article

Keywords:

Posted Date: October 31st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2198036/v1

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Abstract

Objectives. *Plasmodium vivax* malaria treated by dihydroartemisinin-piperaquine (DHA-PPQ) in Indonesia remains a challenge. *Plasmodium falciparum* resistance to DHA-PPQ was documented in Asia and it is suspected that this will also be a concern for *P. vivax* malaria. Thus it is needed to test the sensitivity of *P. vivax* on a regular basis. Parasite clearance time (PCT) and molecular markers of resistance are efficient sentinel tools for this goal.

Methods. A prospective observational study was conducted at North Labuhan Batu Regency (Sumatra). The outcome were the clinical and parasitological efficacy of the 3-day DHA-PPQ therapy corrected by PCR and the prevalence of *Pvmdr1, PvK12* and *PvPM4* molecular markers of chloroquine and DHA-PPQ resistance.

Results. During the 6-months study period, 100 patients were included and 6 were lost to follow-up. Ninety-four patients were included in the per-protocol analysis. The parasite clearance half-life increased over 18h in 8.5% of the cases while no clinical recurrence were observed during the Day-28 follow-up. None of the molecular marker of ACT resistance were detected among the samples tested.

Conclusions. This study highlighted the need for active surveillance of ACT efficacy against *P. vivax* malaria in Indonesia, using simple method such as PCT during observational studies, as it may provide a cost-effective early warning signal.

Introduction

*Plasmodium vivax* (*P. vivax*) malaria remains a public health issue in many areas of Indonesia archipelago after *Plasmodium falciparum* (*P. falciparum*) malaria was limited to few regencies [1–3] and while *Plasmodium knowlesi* may be a new threat for the future [4]. *Plasmodium vivax* resistance to chloroquine emerged more than a decade ago, mostly in south-east Asia, including Indonesia and Papua New Guinea [5–7]. High-grade chloroquine-resistance led those countries to recommend artemisinin-based combined therapies (ACTs) for all parasites species, naming dihydroartemisinin-piperaquine (DHA-PPQ) in Indonesia and artemether-lumefantrine (AL) in Papua New Guinea [8, 9]. But in the meantime, *P. falciparum* resistance to DHA-PPQ was documented in Cambodia and it must be anticipated that DHA-PPQ resistance can become a concern for *P. vivax* malaria in the future. In this context, it is of utmost importance to detect a decreased sensitivity of *P. vivax* to DHA-PPQ before significant therapeutic failures occurred. For that purpose, systematic survey of parasite clearance time (PCT) and molecular markers of resistance are useful tools. Delays in PCT were indeed the first observations in south-east Asia reporting a decrease efficacy of artemisins against *P. falciparum* [10, 11]. Delayed PCT was clearly identified as a risk factor of recurrence at day 28 for chloroquine treatments during *P. vivax* malaria [8, 12]. The parasite clearance time should be measured with accurate and reproducible methods to allow an effective survey of early events. These methods were tested during the last decade [13] leading to recommendations from the WorldWide Antimalarial Resistance Network (WWARN) (https://www.wwarn.org/parasite-clearance-
However, the recommended blood sampling every 6 hours until two consecutive negative parasite counts is difficult to implement in standard clinical care facilities and activities. Most of the data on delayed PCT obtained so far came from *falciparum* malaria studies, while there is no consensus on the threshold between normal and delayed PCT due to the multivariable nature of this data. The *P. vivax* parasite clearance time is far less documented and the model used for *P. falciparum* may be less relevant to discriminate between drug efficacy or resistance based on normal or delayed PCT. During a study conducted in 2001–2002 in Cambodia, parasites clearance time after DHA-PPQ treatment of uncomplicated *P. vivax* malaria was 12h [12–18] confirming a rapid efficacy [14]. More recently, during a study conducted in Vietnam, the *P. vivax* PCT was in the same range (18h [12–18]) [15].

Molecular markers of *P. vivax* drug resistance are less discriminant than those of *P. falciparum* [16, 17]. Three main genes (*Pvmdr1, PvK12* and *PvPM4*) presenting single nucleotide polymorphisms were associated with *P. vivax* resistance to chloroquine, artemisinin and piperaquine, respectively. In 2005, Brega *et al.* were the first to describe the role of *P. vivax mdr1* gene (*Pvmdr1*) in chloroquine resistance [18]. This marker was tested in different areas [6, 19–25]. These studies did not show a definitive relationship between the presence of mutations of this gene and sensitivity of the parasite to quinolone. Studies conducted in south-east Asia helped to clarify the role of *Pvmdr1* in antimalarial drug resistance [26–28]. Recently, a potential molecular marker or artemisinin resistance was identified in the gene *Plasmodium vivax k12*, ortholog of the *P. falciparum Pfk13*. It was observed that non-synonymous mutations in this gene were circulating at very low frequencies in Cambodia [29]. Studies conducted later in south-east Asia confirmed the limited polymorphism of *Pvk12* making the role of this gene in artemisinin resistance unclear [30, 31]. Lastly, resistance to piperaquine was suspected to be associated with I165V variant or increased copy number of *Plasmepsin IV* gene (*PvPM4*), an orthologue of *P. falciparum plasmepsin II* [32].

Dihydroartemisinin-piperaquine is used as first-line therapy in Indonesia since a decade but evaluation whether this drug is still effective against *P. vivax* was seldomly conducted. In this context, sentinel surveillance of *P. vivax* sensitivity to antimalarials is needed [26]. Thus, a prospective observational study was conducted at North Labuhan Batu Regency (Sumatera, Indonesia) where *P. vivax* is predominant [2] and treated with DHA-PPQ.

The primary outcome of this study was the clinical and parasitological efficacy of the 3-day DHA-PPQ therapy corrected by PCR and the prevalence of *Pvmdr1, PvK12* and *PvPM4* molecular makers of chloroquine and DHA-PPQ resistance.

**Material And Methods**

This was an observational study conducted at Tanjung Leidong Health Centre in Kualuh Leidong District, North Labuhan Batu Regency, from July to December 2018. The district is a coastal area which covers an area of 394 sq km with a population of more than 29,552. *Plasmodium vivax* is the predominant species in this location. Tanjung Leidong Health Centre offers malaria diagnosis using microscopy and treatment
was free for the people. Dihydroartemisinin-piperaquine has been used as drug of choice for *P. vivax* malaria in Indonesia since 2010. Any person with a fever or a history of fever within 48h who visited the health centre and did not took anti-malarial drugs within the four weeks prior to the study, was selected as a study participant. Individuals who refused to participate were not included. Individuals who had a *P. falciparum* infection or where unable to follow-up, were excluded. Antimalaria treatments were provided by Ministry of Health. Dihydroartemisinin–piperaquine (containing 40 mg dihydroartemisinin and 320 mg piperaquine) was administered once daily for 3 days following the National guidelines. Dihydroartemisinin–piperaquine was taken in front of the healthcare staff for 3 consecutive days. In addition, patients with *P. vivax* malaria were prescribed primaquine (0.25 mg/kg/day for 14 days). All patients were followed up to day 28.

**Microscopy for malaria diagnosis**

Thick and thin blood smears were prepared on the same slide from samples obtained from each patient with acute febrile illness; Each slide was stained with a 3% Giemsa solution for 45 min. All sample slides included in this study were examined by a single field microscopist. For positive smears, the numbers of parasites were counted against 200 white blood cells (WBCs) in thick smears or 500 WBCs for low-density infections. Parasite density was calculated assuming 8000 WBCs per μl. The thin smears for the positive samples were examined for species identification. All slides positive at inclusion were sent to Eijkman Institute, Jakarta, for quality control.

**Parasite clearance half-life**

The parasite clearance half-life (*PC*$_{1/2}$) was determined using $T_{1/2} = \log_e(2)/K$ derived from the linear segment of the log parasitemia–time curve. The Worldwide Antimalarial Resistance Network (WWARN) Parasite Clearance Estimator (PCE) was not used because the 6-hourly blood sampling required was not possible during this study. However, the *PC$_{1/2}$* at admission was calculated from the first three samples during the log-phase at H0, H24 and H48. We defined the threshold for *PC$_{1/2}$* as 12-18 hours (<12H: normal, 12-18: suspect, > 18: increased).

**Molecular analysis**

DNA was extracted from blood-spot samples on filter paper according to the spin-column method using the QIAamp DNA Mini Kit, according to the manufacturer’s instructions (Qiagen, Germany) and eluted in a total volume of 150 μl for each sample. The identification of species was confirmed by real-time PCR using species-specific primers [33]. All amplification reactions were carried out in a total volume of 20 μl and the presence of 250 nM of each oligonucleotide primers and 2.0 μl of Light Cycler Fast Start DNA Master SYBR Green 1 reaction mix. Primary amplification reactions were initiated with 5.0 μl of the template genomic DNA, and 1.0 μl of the product of these reactions was used to initiate the secondary amplification reactions.

**Genotyping of Pvcsp, and Pvmsp1**
Two genes \((Pvcsp, Pvmsp1)\) were used to compare the sequences on admission and on recurrence at D7, D21 and D28. PCR reactions were performed in a total volume of 30 µl containing 1 µM of each primer, Mix Hotstart 5X (Solis Biodyne), 12.5 mM MgCl\(_2\) and 2µL of genomic DNA. Before sequencing, all PCR products were purified using ExoSap-it (Thermofisher). Sequencing was carried out by Biofidal-MicroSynth (Lyon, France) using BigDye V3.1 Terminator Cycle Sequencing kit (Thermofischer), purification by BigDye-X- Terminator, on ABI-3730XL sequencer.

*Plasmodium vivax* circumsporozoite \((Pvcsp)\) gene comprises a central repetitive domain of a 27 bp element repeated a variable number of times \([34]\). Two types of repeats have been described, VK210 (type I: GDRADGQPA) and VK247 (type II: ANGAGNQPG). The confirmation of sequences was performed by BLAST and nucleotide sequences were translated into amino acid sequences. BioEdit version 7.2.5 was used to analyze and control the DNA sequences. MUSCLE (MUltiple Sequence Comparison by Log-Expectation) was used for sequence alignment.

**PCR amplification of \(Pvmdr1, PvK12\) and \(PvPM4\) genes**

PCR reactions were performed in a total volume of 30 µl containing 1 µmol/L of each primer, Mix Hotstart 5X (Solis Biodyne) - 12.5 mmol/L MgCl\(_2\), 10X GC Enhancer (Solis Byodine), and 2µL of genomic DNA. Before sequencing, all PCR products were purified using ExoSap-it (Thermofisher). Sequencing was carried out by Biofidal-MicroSynth (Lyon, France) using BigDye V3.1 Terminator Cycle Sequencing kit (Thermofischer), purification by BigDye-X- Terminator, on ABI-3730XL sequencer. Consensus sequences were obtained using Chromas Pro (Technelysium Pty). Primers sequences and PCR program are presented in Table 1.

**Table 1: Primers used for the amplification of \(pvcsp, pvmsp1, pvmdr1, pvk12, and pvpm4\)**
<table>
<thead>
<tr>
<th>Genes</th>
<th>Primers</th>
<th>Sequences 5' – 3'</th>
<th>PCR cycling</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PvCSP</strong></td>
<td><strong>PvCSP-Ofidt</strong></td>
<td>GACGAGGAAGGAGATGCTAAA</td>
<td>96°C 12 min (96°C 20 sec, 52°C 20 sec, 72°C 2 min) 35 cycles, 72°C 10 min</td>
<td>1087</td>
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<tr>
<td></td>
<td><strong>PvCSP-Oridt</strong></td>
<td>GTACATACAGTTAGTGAAAC</td>
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<tr>
<td><strong>PvMSP1</strong></td>
<td><strong>PvMSP1-icb-F1</strong></td>
<td>CCCTACTACTGTGGGTCCTC</td>
<td>96°C 12 min (96°C 20 sec, 52°C 20 sec, 72°C 2 min) 35 cycles, 72°C 10 min</td>
<td>1827</td>
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<tr>
<td></td>
<td><strong>PvMSP1 F3-O3R</strong></td>
<td>GTTTGTACTTGCTTCTCCTCCC</td>
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<td><strong>PvMSP1-NestF2</strong></td>
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<td><strong>PvMSP1-N3R</strong></td>
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<td>ACGTTTGGCTGAGCAGTAT</td>
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<td><strong>PvMDR1-R1</strong></td>
<td>ATAGTCATGCCCGAGGATTG</td>
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<td><strong>PvK12</strong></td>
<td><strong>PvK12_F1-F</strong></td>
<td>CCATACGTAAAACGCTGAAAT</td>
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<td><strong>PvK12-c2216R</strong></td>
<td>ACGCAACACCGCAGATAA</td>
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<td><strong>PvK12_F1-idt</strong></td>
<td>GTTGTAGGGGTGGCCTAGAAG</td>
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<td>CTACACTAAGCTTGAGACAGGAA</td>
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<td><strong>PvK12_F3-idt</strong></td>
<td>TCAAAAGGAGTACAGAAGCATCAA</td>
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<tr>
<td><strong>PvPM4</strong></td>
<td><strong>PvPM4F1_F</strong></td>
<td>TGTTCTAATTACAGCAGGACTTAG</td>
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<tr>
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<td><strong>PvPM4F2_R</strong></td>
<td>ATGGGTTCTAATCATCAGTGTC</td>
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<tr>
<td></td>
<td><strong>PV_PM4F1_F</strong></td>
<td>ATGGGTTCTAATCATCAGTGCA</td>
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<tr>
<td></td>
<td><strong>PV_PM4F2_F</strong></td>
<td>GATGACAGCATATAATACTGTACG</td>
<td>96°C 12 min (96°C 20 sec, 52°C 20 sec, 72°C 2 min) 35 cycles, 72°C 10 min</td>
<td>1086</td>
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<tr>
<td></td>
<td><strong>PV_PM4F1_R</strong></td>
<td>ATGGGTTCTAATCATCAGTGCA</td>
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<tr>
<td></td>
<td><strong>PV_PM4F2_F</strong></td>
<td>GATGACAGCATATAATACTGTACG</td>
<td></td>
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</tr>
</tbody>
</table>
cycles, 72° 10 min

96°C 12 min
(96°C 20 sec, 52°C 20 sec, 72°C 2min) 35 cycles, 72° 10 min

96°C 12 min
(96°C 20 sec, 52°C 20 sec, 72°C 2min) 35 cycles, 72° 10 min

95 °C 10 min
(95 °C 30s, 62°C 30s, 72 °C 150s) 40 cycles

95 °C 10 min
(95 °C 30s, 62°C 30s, 72 °C 150s) 30 cycles


**Ethical clearance**

Explanations regarding the study were given to the participants before the samples were collected. The Ethical Committee of the Medical Faculty Universitas Sumatera Utara/Adam Malik General Hospital (No. 588/TGL/KEPK FK USU-RSUP HAM /2018) approved the study. Written informed consent was obtained
from each of the study participants, or from the parents or guardians of the children who were included. Material Transfer Agreement between Universitas Sumatera Utara and Lyon University was provided before the samples shipment. The samples shipment was made in agreement with the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization.

**Results**

**Population description and clinical/parasitological outcomes**

During the 6-months study period, 134 patients were eligible for inclusion (Figure 1). Thirty-four patients (25%) were not included due to *P. falciparum* infection detected by microscopy (14), inability to follow-up (12), or no consent given (8). Among the 100 patients included, 6 were lost to follow-up at day 21 (2) and day 28 (4). Ninety-four patients were included in the per-protocol analysis. The demographic information of included patients for the intention-to-treat analysis (ITT) (n=100) were: age: mean=23.5 and the sex ratio (M/F) = 1.6 (61/39). All the patients presented simple or mild malaria and none experienced severe disease. Most of the symptoms were fever, headache, chills sweating and myalgia. These symptoms resolved at day 1 or day 2 after treatment without significant side effect. None of the patient experienced a clinical recurrence during the day 28 follow-up.

**Parasite clearance using microscopy**

The mean parasitemia at inclusion (D1) was $m = 5180 \ [10 - 49600]$ parasites/µL. Blood samples were collected during the dihydroartemisinin-piperaquine treatment immediately before drug administration at H24 and H28 (± 3 hours) to estimate the parasite clearance time. At H24 and H48 following treatment, 89/94 patients (94.7%) and 53/94 (56.4%) had detectable parasitemia, respectively. Surprisingly, 10/94 (10.7%) had a residual parasitemia at day 7 post-treatment, 1 patient at day 21 and 5 patients (5.3%) at day 28. None of these residual parasitemia observed by microscope was associated with fever or symptoms and none of these patients received salvage treatment.

Almost half of the patients (44/94 ; 46.8%) carried sexual forms of the parasite at admission. During the follow-up, the decrease in asexual and sexual forms evolved in parallel.

**Parasite clearance half-life**

The $PC_{1/2}$ was calculated using the first three samples (H0, H24 and H48) for the 53 patients still presenting a positive parasitemia at day 2. No lag phase can be detected due to the lack of sampling between admission and H24. Based on this subgroup, 39/53 patients (73.5%) showed a parasite clearance time between 6 and 12 hours which was considered as normal efficacy. There was no correlation between the initial parasitemia at inclusion and the parasite clearance time ($R = 0.22$).
Surprisingly, 6 patients had a parasite clearance considered as suspect (12< PC$_{1/2}$ <18h), and 8 patients (8/94; 8.5%) had a significantly increased parasite clearance time using microscopy: > 18 hours (26.3 [18 - 45]).

**Parasite clearance by RT-PCR**

Molecular data were obtained from 85 out of 94 samples. Using real-time PCR data from filter papers, the percentage of patients with residual parasitemia at H24 and H48 were 50 and 44.6%, respectively. Five patients presented a positive microscopy at day 28 and this was confirmed by a positive PCR for 2 of them. The PC$_{1/2}$ was not increased for these cases.

**Distinguishing between treatment failures and new infections or relapses**

Parasite samples collected at admission were genotyped and compared to parasite samples collected at D7, D14, D21 or D28 when a residual parasitemia was detected.

Sixteen samples collected at admission were compared to 10 samples collected at D7, 1 at D21 and 5 at day 28. All the samples collected at admission displayed the Pvcsp VK210 type (Type 1: GDRADGQPA) with two haplotypes (8 repeats of GDRADGQPA / 9 repeats of GDRAAGQPA and 4 repeats of GDGAAGQPA / 13 repeats of GDRAAGQPA). Similar patterns were obtained with samples collected during the follow-up at the different time points. Correct sequences of the F1 fragment of Pvmsp1 from samples at admission and 13 samples out of 16 collected during the follow-up were obtained, given a single haplotype of five short tandem repeats (tripeptides) for all samples.

**Molecular markers of drug resistance:**

We searched for single nucleotide polymorphisms known to be associated with drug resistance in Pvmdr1, PvK12 and PvPM4 genes from samples collected at admission.

We obtained correct pvmdr1 sequencing for 59 samples out of 85 collected at D0. The missing sequences were due to poor quality of extracted DNA. All these samples displayed the 958 M/ 976 F/ 1076 L associated with chloroquine resistance. No polymorphism in the pvkelch12 V552I or in the Pvpmp4 V165I was detected among the samples collected at D0 and during the follow-up at D7, D21 or D28.

**Discussion**

Dihydroartemisinine-piperaquine is the first line drug for *P. vivax* malaria in Indonesia since 2010. This study confirmed its excellent clinical efficacy. There is no doubt that this ACT regimen should be maintained in the near future. However, artemisinin-derivatives resistance of *P. falciparum* was detected a decade ago in south-east Asia and huge efforts were needed to contain the spread of this resistance. A similar scenario should be considered if the ACTs used to treat *P. vivax* malaria in the same area start to face decreased efficacy. Thus, it is needed to conduct observational studies to allow a very early detection of any signs of decreased efficacy. Small scale observational studies are easier and cheaper.
than drug efficacy blinded clinical trials and thus it can be reiterated at a country level to provide early signals of decreased efficacy. Such observational studies can confirm the efficacy of a regimen or detect signals justifying larger studies to document the trend toward resistance.

Thus, an observational study was conducted in Kualuh Leidong District, the coastal area of North Labuhan Batu Regency, located in North Sumatera, Indonesia. Patients presenting *P. vivax* malaria and treated with DHA-PPQ were included and follow-up until day 28. Blood samples were collected during the follow-up for microscopy and molecular tests were performed after the end of the clinical study. Two main parameters were used to evaluate the overall drug efficacy against *P. vivax*: the parasite clearance half-life and a panel of suspected molecular markers of drug resistance (*Pvmdr1*, *PvK12* and *PvPM4*).

Early markers of drug decreased efficacy are delayed parasite clearance time and molecular markers, while both remain unclear in the case of *P. vivax*. However, clinical and parasitological treatment failure rate may be increased with an unpredictable delay after early signals of decreased efficacy, and most of the time it may be too late to contain the resistance to the drug. Thus sentinel observational studies of DHA-PPQ effect on vivax malaria are worth conducting.

During this study, none of the included patient presented a clinical therapeutic failure. However, 56.4% of patients had detectable parasitemia at H48 post-treatment. Moreover, 10.7% had a residual parasitemia at day 7 post-treatment and 5 patients (5.3%) at day 28. Due to the status of observational study in standard clinical care facilities and activities, it was not possible to follow the WWARN recommendation of 6-hourly blood sampling until two consecutive negative parasite counts to measure the parasite clearance time.

Moreover, those results may be affected by the heterogeneity of clinical care, laboratory experience and sample time collection [35]. Then the PC_{1/2} was used in this study to overcome parasite clearance time difficulties and to provide an experimental early warning marker of decreased efficacy.

Using DHA-PPQ to treat *P. vivax* malaria cases in this study, it was considered that a normal parasite clearance half-life should be below 12 hours as previously reported [15]. We defined the threshold for PC_{1/2} as suspect for 12–18 hours (“grey zone”) and delayed > 18h (“red zone”). While the majority of the patients (80/94 ; 85%) had a PC_{1/2} between 6 to 12 hours, it was increased over 12H for 14 patients, among those 8 had an increase over 18H (26.3 [18–54]). This delay was not associated with initial high parasitemias which may be a confounding factor and there was no correlation between initial parasitemia and parasite clearance time for the whole population. None of the patient with delayed parasite clearance experienced a clinical failure or an increase in body temperature during the 28-day follow-up.

This delayed parasite clearance time may be a relevant signal of decreased drug efficacy since genotype-adjusted estimates of failure rate are greatly impaired by the impossibility to clearly discriminate between recrudescence, relapse or reinfection [26]. In this study, an attempt to detect potential reinfection among patients presenting residual parasitemia during the follow-up was made using *Pvcsp* and *Pvmsp1*
genotyping. No polymorphism was detected for both markers between parasite at admission and during the follow-up, precluding any definitive conclusion. In the context of the lack of clear method to discriminate between recrudescence or reinfection during \textit{P. vivax} malaria, no more evidence can be obtain from these data [26, 36].

None of the molecular marker of ACT resistance were detected among the samples tested, but all of them displayed a mutation associated with chloroquine resistance, which drove the change of first line treatment to ACT in the area a decade ago.

This study highlighted the need for active surveillance of ACT decreased efficacy using PCT or PC$_{1/2}$ during observational studies, as it may be a warning signal before resistance detection through clinical therapeutic failure.

One of the limitation of this study is the absence of information on drug absorption and metabolism and absence of therapeutic monitoring of antimalarials. This is inherent of such observational study in routine clinical care according to local facilities, staff available, and resources. The interest of such study is not to provide a definitive and well-documented evidence of therapeutic failure in a specific area, but to act as a sentinel for early detection of an incubating fire.

**Declarations**

**Authors' contributions**

APP and SP conceived and planned the experiments. APP, ISN, AL, FF conducted the clinical trial. IBS, GB, ALB and SP conducted the biological and molecular analysis. APP, IBS, ALB and SP, contributed to interpretation of the results. APP, IBS, ALB and SP wrote the manuscript. All authors provided critical feedback and helped shape the research manuscript.

**Acknowledgements**

The authors thank the Institut Français d'Indonésie (IFI) and the Institut National des Sciences Appliquées (INSA) de Lyon.

- **Availability of data and materials**

All datasets can be requested upon reasonable request

- **Ethics approval and consent to participate**

Explanations regarding the study were given to the participants before the samples were collected. The Ethical Committee of the Medical Faculty Universitas Sumatera Utara/Adam Malik General Hospital No. 588/TGL/KEPK FK USU-RSUP HAM /2018, approved the study. Written informed consent was obtained
from each of the study participants, including from the parents and guardians of the children who were included.

- **Competing interests**

The authors declare that they have no competing interests

- **Funding**

IBS was supported by a grant from Indonesian Ministry of Religious Affairs (MORA) Scholarship 5000 Doktor and supported by Kediri State Islamic Institute (IAIN) Indonesian Republic. APP was supported by a grant from the Directorate General of Research and Development Strengthening, the Ministry of Research, the Technology and Higher Education of The Republic of Indonesia (No:135/UN5.2.3.1/PPM/KP-DRPM/2018) for conducting the field study. This study was supported by grant PHC NUSANTARA 2018 n° 38927UF from French Ministry of Europe and Foreign Affairs (MEAE) and French Ministry of Higher Education, Research and Innovation (MESRI).

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Figures

Figure 1

Study flow-chart
Figure 2

Evolution of parasitemia at inclusion (D0) and during the patient’s follow-up (D1, D2, D7 or 14, D21, D28). The parasitemia is expressed as the number of parasites per µL of blood, observed by trained microscopist.
Figure 3

Distribution of parasite clearance time among patients with positive parasitemia at day 2.