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Risk factors for *Plasmodium falciparum* and *Plasmodium vivax* gametocyte carriage in Papua New Guinean children with uncomplicated malaria

Stephan Karl[a,b,1], Moses Laman[c,1], Brioni R. Moore[d], John M. Benjamin[c], Mary Salib[c], Lina Lorry[c], Samuel Maripal[c], Peter Siba[c], Leanne J. Robinson[a,b,c], Ivo Mueller[a,b], Timothy M.E. Davis[d,*]

[a] *Population Health and Immunity Division, Walter and Eliza Hall Institute, Parkville, Victoria, Australia*
[b] *Department of Medical Biology, University of Melbourne, Melbourne, Victoria, Australia*
[c] *Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea*
[d] *School of Medicine and Pharmacology, University of Western Australia, Perth, Western Australia, Australia*

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ABSTRACT

There are limited data on gametocytaemia risk factors before/after treatment with artemisinin combination therapy in children from areas with transmission of multiple *Plasmodium* species. We utilised data from a randomised trial comparing artemether-lumefantrine (AL) and artemisinin-naphthoquine (AN) in 230 Papua New Guinean children aged 0.5–5 years with uncomplicated malaria in whom determinants of gametocytaemia by light microscopy were assessed at baseline using logistic regression and during follow-up using multilevel mixed effects modelling. Seventy-four (32%) and 18 (8%) children presented with *P. falciparum* and *P. vivax* gametocytaemia, respectively. Baseline *P. falciparum* gametocytaemia was associated with Hackett spleen grade 1 (odds ratio (95% CI) 4.01 (1.60–10.05) vs grade 0; $P<0.001$) and haemoglobin (0.95 (0.92–0.97) per 1 g/L increase; $P<0.001$), and *P. falciparum* asexual parasitaemia in slide-positive cases (0.36 (0.19–0.68) for a 10-fold increase; $P=0.002$). Baseline *P. vivax* gametocytaemia was associated with Hackett grade 2 (12.66 (1.31–122.56); $P=0.028$), mixed *P. falciparum/vivax* infection (0.16 (0.03–1.00); $P=0.050$), *P. vivax* asexual parasitaemia (5.68 (0.98–33.04); $P=0.053$) and haemoglobin (0.94 (0.88–1.00); $P=0.056$). For post-treatment *P. falciparum* gametocytaemia, independent predictors were AN vs AL treatment (4.09 (1.43–11.65)), haemoglobin (0.95 (0.93–0.97)), presence/absence of *P. falciparum* asexual forms (3.40 (1.66–0.68)) and day post-treatment (0.086 (0.82–0.90)) ($P<0.001$). Post-treatment *P. vivax* gametocytaemia was predicted by presence of *P. vivax* asexual forms (596 (12–28,433); $P<0.001$). Consistent with slow *P. falciparum* gametocyte maturation, low haemoglobin, low asexual parasite density and higher spleen grading, markers of increased prior infection exposure/immunity, were strong associates of pre-treatment gametocyte positivity. The persistent inverse association between *P. falciparum* gametocytaemia and haemoglobin during follow-up suggests an important role for bone marrow modulation of gametocytogenesis. In *P. vivax* infections, baseline and post-treatment gametocyte carriage was positively related to the acute parasite burden, reflecting the close association between the development of asexual and sexual forms.

1. Introduction

Gametocytes are *Plasmodium* blood stages that propagate the infection from the human host to the mosquito vector. The two main species of malaria parasites, *P. falciparum* and *P. vivax* have evolved to produce gametocytes with different characteristics (Bousema and Drakeley, 2011). *P. falciparum* gametocytes sequester in bone marrow (Abdulsalam et al., 2010; Aguilar et al., 2014) and mature over 10–12 days after commitment to gametocytogenesis (Josling and Llinas, 2015). Following release from the marrow, they circulate for extended periods particularly in untreated patients (Karl et al., 2008; Smalley and Sinden, 1977). Mature *P. falciparum* gametocytes have an arrested metabolism and a reduced susceptibility to antimalarial drugs (Bousema and Drakeley, 2011; Eichner

* Corresponding author at: School of Medicine and Pharmacology, University of Western Australia, Fremantle Hospital, P.O. Box 480, Fremantle, Western Australia 6959, Australia.
E-mail address: tim.davis@uwa.edu.au (T.M.E. Davis).
[1] These authors have contributed equally.

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et al., 2001). Relative to *P. falciparum* gametocytes, the development and clearance of *P. vivax* gametocytes more closely resembles that of asexual stages, with minimal or no delay between asexual and gametocyte stage development (Josling and Llinas, 2015). *P. vivax* gametocytes are also more vulnerable to drug treatment (Bousema and Drakeley, 2011).

The proportion of parasites formed by gametocytes is usually smaller in *P. falciparum* than *P. vivax* infections but the densities are similar as the overall *P. falciparum* burden is usually higher than that of *P. vivax* (Bousema and Drakeley, 2011). Not all patients carry gametocytes, especially in *P. falciparum* infections, which is likely due to early treatment (Bousema and Drakeley, 2011; Karl et al., 2008). In addition, confirmation of gametocytaemia is often difficult due to low peripheral blood densities, especially when light microscopy is used (Karl et al., 2008, 2009; Koepfli et al., 2015; Okell et al., 2009). Better sensitivity is achieved by molecular techniques that mostly target the gametocyte markers *Pfs25* and *Pvs25* (Mens et al., 2008; Wampfler et al., 2013). Nevertheless, the specificity of *Pfs25* and *Pvs25* detection by PCR remains problematic (Buates et al., 2010; Karl et al., 2009).

Independent risk factors for *P. falciparum* gametocyte carriage include younger age (Bousema et al., 2004; Karl et al., 2008; Koepfli et al., 2015; Molineaux and Gramiccia, 1980), male sex (Sowunmi et al., 2004), high (Nacher et al., 2002; von Seidlein et al., 2001) and low (Price et al., 1999; Sowunmi et al., 2004) asexual parasite densities, low haemoglobin (Nacher et al., 2002; Price et al., 1999), longer duration of infection (Sowunmi et al., 2004), fever (Sowunmi et al., 2004; von Seidlein et al., 2001), haemoglobin E trait (Nacher et al., 2002) and blood groups A, B and O (Grange et al., 2015). Few studies have investigated risk factors for *P. vivax* gametocyte carriage but available evidence suggests that they include younger age, an inconsistent relationship with the degree of anaemia (Koepfli et al., 2015; Nacher et al., 2004). However, while post-treatment *P. falciparum* gametocyte carriage is strongly dependent on the antimalarial drugs administered (Bousema and Drakeley, 2011; Bousema et al., 2010; Karl et al., 2015; Shekalaghe et al., 2011), treatment is much less important for *P. vivax* gametocyte carriage as they are cleared within the same time-frame as asexual stages (Karl et al., 2015).

Treatment with conventional drugs such as chloroquine, amodiaquine, sulfadoxine, pyrimethamine, and combinations of these, is associated with persistence of *P. falciparum* gametocytaemia for weeks to months. Some studies have found increased gametocyte density and prevalence after treatment with these drugs, particularly when there is parasite resistance (Barnes et al., 2008). Artemisinin combination therapy (ACT) regimens clear gametocytes faster, within 4–6 weeks in most patients as detected by PCR (Bousema and Drakeley, 2011). Primaquine-containing ACT regimens are the most effective against *P. falciparum* gametocytes, reducing carriage to <2 weeks post-treatment in most individuals (Bousema et al., 2010; Sutanto et al., 2013), although it is currently debated as to whether a dose of primaquine should be given routinely to reduce *P. falciparum* transmission due largely to potential toxicity (Eziefula et al., 2014). In addition, there are questions regarding the viability of *P. falciparum* gametocytes post-treatment, especially since some drugs seem to flush out immature sequestered forms which may have a compromised potential to cause infection in the mosquito (Beavogui et al., 2010; Kone et al., 2010). In the case of *P. vivax*, the prophylactic effect of non-primaquine containing treatment regimens is important in determining the timing of post-treatment relapses and associated *P. vivax* gametocytaemia.

We have previously examined the treatment-dependent effects on gametocyte carriage determined by light microscopy during follow-up of children living in an area of decreasing transmission in Papua New Guinea (PNG) who participated in a randomised trial of artemether-lumefantrine (AL) or artemisinin-naphthoquine (AN) ACT for uncomplicated *P. falciparum* and/or *P. vivax* malaria (Laman et al., 2014b). In addition, we have compared the kinetics of gametocyte clearance by allocated therapy as assessed by sensitive quantitative magnetic fractionation and a mathematical model in a subset (30%) of these children (Karl et al., 2015). In the present study, we have extended these analyses by assessing a range of candidate risk factors for gametocyte carriage before treatment and for persistent gametocytaemia by light microscopy in the total sample of children from the same intervention trial (Laman et al., 2014b).

2. Methods

2.1. Study site, patients, design and approvals

The present open-label, parallel group trial was conducted between March 2011 and April 2013 in Mugil and Alexishafen Health Centres in Madang Province, PNG (Laman et al., 2014b). Children aged 0.5–5 years with fever (axillary temperature >37.5 °C) or a history of fever in the last 24 h and light microscopy parasite densities of >1000 parasites/μL (*P. falciparum*) or >250/μL (*P. vivax*) were recruited. Exclusion criteria comprised severe malaria or other illness, recent treatment with study drugs, allergies to study drugs or evidence of comorbidities. The trial was registered in the Australia New Zealand Clinical Trial Registry (ACTRN12610000913077) and was approved by the PNG Institute of Medical Research Advisory Committee of PNG (MRAC10.39) and the University of Western Australia Human Research Ethics Committee. Written informed consent was sought from the parents/guardians of all children before recruitment.

2.2. Clinical and laboratory procedures

An initial standardised clinical assessment was performed after enrolment and blood was drawn for measurement of haemoglobin and blood glucose (Hemocue 201+, Hemocue, Ängelholm, Sweden). Children were assigned to receive either AL (1.7 mg/kg artemether plus 10 mg/kg lumefantrine twice daily for three days) or AN (20 mg/kg artemisinin plus 8 mg/kg naphthoquine daily for three days) based on computer-generated block randomization. As per manufacturer's recommendation, AL (1–3 tablets per dose) was administered twice daily with milk (250 mL) and AN (1–4 tablets per dose) once daily with water.

Standardized clinical assessment, including axillary temperature measurement and thick and thin blood film light microscopy was performed on Days 0–3, 7, 14, 28 and 42, while a subset of study participants were reviewed over six months post-treatment to document clinical episodes of malaria (Laman et al., 2015). Parasite density was determined by counting the parasites present in 500 white blood cells on the thick film (Laman et al., 2014a). All slides were assessed in the field and then re-examined by two skilled microscopists. Differences in speciation and density (>3× difference) were adjudicated by a senior microscopist. Gametocyte positivity/negativity and density were documented. Venous blood was drawn for determination of plasma concentrations of lumefantrine and naphthoquine on Days 7 and 28 using validated high-performance liquid chromatography tandem mass spectrometry or high-performance liquid chromatography assays (Batty et al., 2012; Salman et al., 2011).

2.3. Statistical analysis

Statistical analyses were conducted in Stata 12 (StataCorp, College Station, TX, USA) and Mathematica 9 (Wolfram Research, Champaign, IL, USA). The risk of gametocyte carriage before treatment (Day 0) was assessed using logistic regression and analysis of