



Morbidity and mortality due to
***Plasmodium vivax* malaria in**
Papua, Indonesia and its control
using antimalarial drugs

A thesis submitted for the degree of *Doctor of Philosophy*

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St Catherine's College, University of Oxford

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Abstract

Plasmodium vivax malaria threatens nearly half the world's population. This relapsing disease may be more severe than previously recognised and is proving refractory to current malaria control measures. This thesis aimed to describe the burden of anaemia and mortality attributable to vivax malaria in Southern Papua, Indonesia, an area endemic for multidrug-resistant *P. vivax* and *P. falciparum*, and to determine the potential of currently available antimalarial drugs to reduce transmission of *P. vivax* in co-endemic regions.

Approximately 0.5 million uniquely identified clinical records from patients presenting to Mitra Masyarakat Hospital between April 2004 and May 2009 were matched with corresponding laboratory and pharmacy data in order to determine the burden of anaemia in the hospital setting and the effectiveness of primaquine prescription for preventing *P. vivax* relapses. Clinical information extracted from patient notes was used to clarify the contribution of *P. vivax* malaria to a series of deaths detected by an active hospital-based surveillance system. Additional secondary sources of data used in this thesis included a large house-to-house survey and multiple clinical trials of antimalarial therapy from both Southern Papua and Northwestern Thailand.

In Southern Papua, *P. vivax* malaria is an important cause of haematological morbidity both in the hospital and community setting. This morbidity is most significant in the first year of life when *P. vivax* infection accounts for 23% of all severe anaemia (haemoglobin <5g/dL) in the hospital and approximately 28% of all moderate-to-severe anaemia (haemoglobin <7g/dL) in the community. In this region concomitant *P. vivax* infection accentuates haematological impairment associated with *P. falciparum* malaria. *Plasmodium vivax* in Southern Papua rarely causes death directly but rather indirectly contributes to mortality through exacerbation of comorbid conditions. In Northwestern Thailand, 53.8% of patients with falciparum malaria who were treated with a rapidly eliminated drug between 1991 and 2005 had a recurrence of vivax malaria within two months making *P. vivax* infection the most common cause of parasitological failure in these individuals. Slowly eliminated artemisinin combination therapies (ACT) provided the greatest protection against recurrent *P. vivax* parasitaemia during 63 days of follow-up. In three randomised controlled trials from Papua and Thailand, *P. vivax* gametocytaemia was shown to mirror asexual parasitaemia closely and to have the same characteristics in acute and recurrent infections. This emphasises that the most important chemotherapeutic means of blocking *P. vivax* transmission is prevention of future relapse. Primaquine is recommended for this purpose but analyses in this thesis suggest that in Southern Papua, unsupervised primaquine at a dose of 0.5mg/kg/day for 14 days, does not reduce the risk of subsequent relapse (Adjusted Hazard Ratio = 1.01 [95% confidence interval 0.95-1.07]).

Plasmodium vivax malaria should not be neglected. High priority must be given to new hypnozoitocidal drug discovery. In the interim, optimising the safety and effectiveness of primaquine and adoption of a unified ACT-based blood schizontocidal treatment strategy for malaria of any parasitological cause in co-endemic regions will be crucial for controlling *P. vivax* malaria.

Statement of contribution

This thesis uses data from a wide range of sources. Chapters 5, 7 and 10 are based on prospective malariometric surveillance data from Southern Papua collected since 2004 by staff of Mitra Masyarakat Hospital, the Menzies School of Health Research (MSHR)-National Institute of Health Research and Development (NIHRD) collaboration, the Malaria Control division of Freeport Mine and various community clinic staff. These data were compiled, cross-validated and cleaned by myself and Professor Ric Price. Chapter 6 uses data from a large house-to-house survey conducted in 2005 by staff and students of the MSHR-NIHRD collaboration [1]. Chapter 8 uses data from two large clinical trials carried out by the MSHR-NIHRD collaboration between 2005 and 2006 [2,3] as well as a trial conducted by staff of the Shoklo Malaria Research Unit (SMRU) on the Thai-Myanmar border between 2007 and 2008 [4]. Chapter 9 uses data from many antimalarial trials done between 1991 and 2005 by staff and students of SMRU.

My project began in August 2008. I have been responsible for formulating all of the research questions and review topics in this thesis. I conducted the literature reviews and data extraction for the two review chapters, aside from data on *P. vivax* chloroquine resistance prior to 2007 which were gathered and extracted by Professor Ric Price. I designed the analytical plans for each of the research chapters. For Chapter 7, I designed the death audit form, extracted data from the patients' notes (with assistance from Dr Daniel Lampah and Dr Gysje Pontororing), entered the data and adjudicated the panel decisions on the contribution of vivax malaria to the deaths. I merged surveillance data from various sources at Mitra Masyarakat Hospital. Data from the various Thai studies were cleaned and merged by

Professor Price and myself. I conducted all of the statistical analyses in this thesis with advice from Dr Julie Simpson. All of the chapters in this thesis, aside from the Introduction, General Discussion and Conclusions are modified from manuscripts that have been published, submitted or are soon to be submitted to peer-reviewed journals. I wrote the first draft and am the first (or equal first) author on all of these papers. I take ultimate responsibility for their contents. I received editorial comments from co-authors and these have been incorporated into the thesis chapters. The co-authors are gratefully acknowledged at the end of each chapter. Permission from both the primary author and the publisher has been sought to reproduce all previously-published tables and figures appearing in this thesis.

During my time in Darwin and Timika I have been responsible for liaising with various different research partners and for drafting and obtaining ethical approval for two large scientific research proposals – “The Timika Malaria Surveillance Study” and “The Timika Household Survey” (planned for late 2011).

Note: I often use the possessive pronoun “our” when referring to the individual studies in this thesis (for example, “our study shows...”). This is an attempt to avoid ambiguity and an acknowledgement of prior work by other people.

Published articles

1. Price RN, Douglas NM, Anstey NM. New developments in *Plasmodium vivax* malaria: severe disease and the rise of chloroquine resistance. *Curr Opin Infect Dis* 2009; **22**: 430-5.
2. Price RN, Douglas NM. Artemisinin combination therapy for malaria: beyond good efficacy. *Clin Infect Dis* 2009; **49**: 1638-40 [commentary].
3. Douglas NM, Anstey NM, Angus BJ, Nosten F, Price RN. Artemisinin combination therapy for vivax malaria. *Lancet Infect Dis* 2010; **10**: 405-16. *A modified version of this article appears in Chapter 3 of this thesis.*
4. Price RN, Douglas NM. Maximising the public health benefit of antimalarials. *Lancet Infect Dis* 2010; **10**: 654-5 [commentary].
5. Douglas NM, Nosten F, Ashley EA, Phaiphun L, van Vugt M, Singhasivanon P, White NJ, Price RN. *Plasmodium vivax* recurrence following falciparum and mixed species malaria: risk factors and effect of antimalarial kinetics. *Clin Infect Dis* 2011; **52**: 612-20. *A modified version of this article appears in Chapter 9 of this thesis.*
6. Andersen F, Douglas NM, Bustos D, Galappaththy G, Qi G, Hsiang MS, Kusriastuti R, Mendis K, Taleo G, Whittaker M, Price RN, von Seidlein L. Trends in malaria research in 11 Asian Pacific countries: an analysis of peer-reviewed publications over two decades. *Malar J* 2011; **10**: 131. *This article is not directly relevant to the content of this thesis.*
7. Price RN, Douglas NM, Anstey NM, von Seidlein L. *Plasmodium vivax* treatments: what are we looking for? *Curr Opin Infect Dis* 2011; **24**: 578-85.

8. Douglas NM, Anstey NM, Buffet PA, Poespoprodjo JR, Yeo TW, White NJ, Price RN.

The anaemia of *Plasmodium vivax* malaria. *Malar J* 2012; **11**: 135. *A modified version of this article appears in Chapter 2 of this thesis.*

Articles in submission

1. Douglas NM, Simpson JA, Phyo AP, Kenangalem E, Poespoprodjo JR, Singhasivanon P, Anstey NM, White NJ, Nosten F, Price RN. Gametocyte dynamics and the role of drugs in reducing the transmission potential of *Plasmodium vivax*. *J Infect Dis*. *A modified version of this article appears in Chapter 8 of this thesis.*

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Abbreviations

95% CI	95% confidence interval
α -HBDH	Alpha-hydroxybutyrate dehydrogenase
AAAS	American Association for the Advancement of Science
ACT	Artemisinin combination therapy
AHR	Adjusted hazard ratio
AIDS	Acquired immunodeficiency syndrome
AM	Artemether
AOR	Adjusted odds ratio
aPAF	Adjusted population attributable fraction
AQ	Amodiaquine
ARDS	Acute respiratory distress syndrome
AS	Artesunate
AV	Atovaquone
CD35	Complement receptor 1
CD55	Decay accelerating factor
Coef	Coefficient
CQ	Chloroquine
CQR	Chloroquine-resistant
CXR	Chest X-ray
D	Denominator
DDT	Dichlorodiphenyltrichloroethane
DHA	Dihydroartemisinin
ELISA	Enzyme-linked immunosorbent assay

EPO	Erythropoietin
ETT	Endotracheal tube
FCT	Fever clearance time
G6PD	Glucose-6-phosphate dehydrogenase
GI	Gastrointestinal
Hb	Haemoglobin
Hct	Haematocrit
HIV	Human immunodeficiency virus
HR	Hazard ratio
HRP2	Histidine rich protein 2
Int	Intermediate
IP	Inpatient
IQR	Interquartile range
ITN	Insecticide-treated net
L	Left
LDH	Lactate dehydrogenase
LLL	Left lower lobe
LUM	Lumefantrine
MSHR	Menzies School of Health Research
MQ	Mefloquine
n	Number
NIHRD	National Institute of Health Research and Development
NSAID	Non-steroidal anti-inflammatory drug
OP	Outpatient
OR	Odds ratio
OXTREC	Oxford Tropical Research Ethics Committee

PCT	Parasite clearance time
Pf	<i>Plasmodium falciparum</i>
PG	Proguanil
PIP	Piperaquine
PK	Pharmacokinetics
Pm	<i>Plasmodium malariae</i>
PNG	Papua New Guinea
Po	<i>Plasmodium ovale</i>
PQ	Primaquine
Pv	<i>Plasmodium vivax</i>
<i>pvdhfr</i>	<i>Plasmodium vivax</i> dihydrofolate reductase gene
<i>pvdhps</i>	<i>Plasmodium vivax</i> dihydropteroate synthase gene
<i>pvm-dr1</i>	<i>Plasmodium vivax</i> multidrug resistance gene
Q	Quinine
R	Right
RAP-2	Rhoptry-associated protein-2
RBC	Red blood cell
Ref	Reference category
RLL	Right lower lobe
RML	Right middle lobe
RSMM	Rumah Sakit Mitra Masyarakat
RSP-2	Ring surface protein-2
RUL	Right upper lobe
SE	Standard error
SOS	Save our souls
SP	Sulfadoxine+pyrimethamine

Std dev	Standard deviation
$t_{1/2}$	Elimination half-life
vs	Versus
WBC	White blood cell
WHO	World Health Organization

1. Introduction

Malaria is a mosquito-borne disease of vertebrates caused by protozoal parasites of the genus *Plasmodium*. It has afflicted human populations ever since we evolved from our ape ancestors some 5 million years ago [5,6]. Ancient Chinese texts refer to paroxysmal fevers, discernibly due to malaria, as far back as 4700 BC and widespread human suffering associated with the disease features prominently in early Greek, Roman, Assyrian, Indian and Arab literature [6-9]. Hippocrates published the first detailed account of the symptoms and signs of malaria in the 5th century BC. He also observed that the disease was most common in low-lying marshy regions leading to the popularly held belief that malaria was caused by miasmata “exhaled” from swamps (the word malaria is derived from the Italian *mala* and *aria* meaning bad air) [10]. It was not until 1880, when French military surgeon Alphonse Laveran demonstrated pigmented forms in the blood of a soldier suffering from malaria, that the parasitic cause for the disease was revealed. Ronald Ross and Battista Grassi subsequently proved that *Plasmodium* species were transmitted by anopheline mosquitoes in 1897-8 [11-13].

Today five *Plasmodium* species are known to cause malaria in humans: *P. vivax*, *P. falciparum*, *P. malariae*, *P. ovale* (recently shown to be two nonrecombining sympatric species, *P. ovale wallikeri* and *P. ovale curtisi* [14]) and *P. knowlesi*; a monkey species that was first demonstrated to be transmissible to humans in 1932 but has only recently been shown to be endemic in human populations [15-17]. As of April 2011, there were 464 named

Anopheles mosquito species of which 70 had been shown to be competent vectors of human plasmodia [18,19].

1.1 The life cycle and pathogenesis of *Plasmodium* species

Although each of the human *Plasmodium* species has biological and epidemiological peculiarities, the life cycle of these parasites is similar (Figure 1.1). Haploid sporozoites are injected into subcutaneous tissue from the salivary glands of an infected, pregnant *Anopheles* mosquito during a blood meal. These motile forms travel rapidly to the liver where they invade hepatocytes and begin a process of asexual replication that typically lasts between 5 and 15 days, depending on the infecting species [20]. Engorged hepatic schizonts eventually rupture, each releasing thousands of pathogenic merozoites into the blood stream. These merozoites begin a process of red blood cell invasion, intracellular asexual multiplication and red blood cell rupture which occurs in cycles lasting 48-72 hours and results in exponential growth of the parasite biomass. Immediately after release from the liver (in the case of *P. vivax*, *P. malariae* and *P. ovale*) and after a certain number of asexual cycles (in the case of *P. falciparum*) a proportion of merozoites will differentiate into non-pathogenic male and female sexual forms that are capable of survival in mosquitoes [20]. After ingestion by a competent *Anopheles* vector, the male gametocyte fertilises the female form in the mosquito gut cavity forming a motile, diploid ookinete which implants itself in the outer layers of the gut lining and subsequently produces thousands of sporozoites. Concentration of these sporozoites in the insect's salivary glands facilitates delivery to the human host when the mosquito next takes a blood meal.

A fundamental feature of *P. vivax* and *P. ovale* infections is that a proportion of hepatic forms will differentiate to dormant hypnozoites with the potential to reactivate and initiate recurrent blood stage infection on one or more future occasions (Figure 1.1). These relapses

are critically important for ensuring transmission of the parasite in environments that are hostile to mosquito vectors for periods of the year.

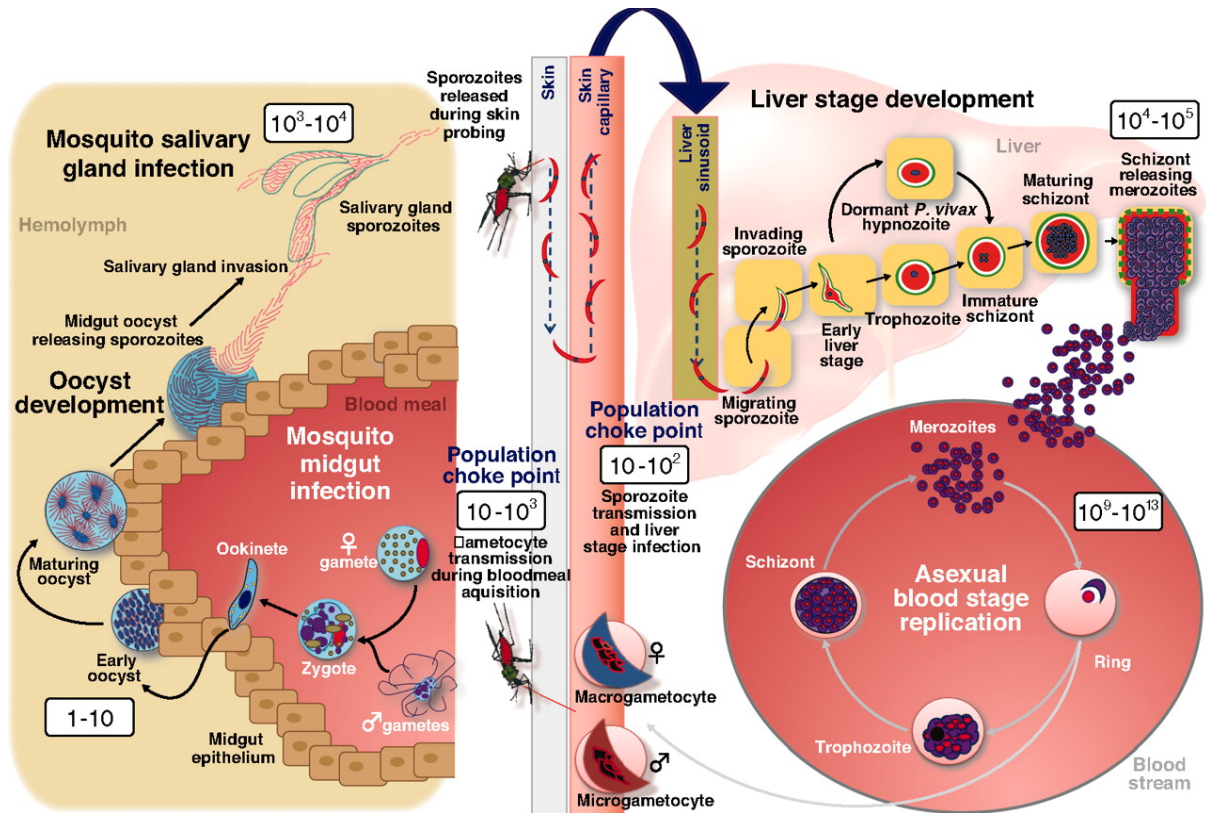


Figure 1.1 Depiction of the *Plasmodium* life cycle. From Kappe SHI, et al. That was then but this is now: malaria research in the time of an eradication agenda. *Science* 2010; **328**: 862-6. Reprinted with permission from AAAS. [21]

Malarial disease in humans is caused by parasitic invasion and destruction of red blood cells and by the host's immune response to this process. Symptoms are non-specific and may include paroxysmal fever, headache, muscle aches, arthralgia, abdominal discomfort and diarrhoea. In non-immune individuals, they develop 1-3 weeks after inoculation of *P. falciparum*, *P. vivax* and *P. ovale* [22-24] and 4-7 weeks after inoculation of the slow-growing parasite *P. malariae* [25], corresponding roughly to the time taken for asexual blood stage parasitaemia to reach ~50/μL [20]. Prolonged *Plasmodium* infection leads to splenic

and, to a lesser extent, hepatic enlargement. Uncomplicated malaria is associated with a diverse range of adverse outcomes including haematological impairment, manifest as anaemia and / or thrombocytopaenia [26-29], adverse pregnancy outcomes (including increased risk of infant mortality [30], stillbirth [31], premature delivery [31,32] and low birth weight [33-36]), impairment of mentation (described in [6]) and malnutrition (particularly following prolonged infections) [37-39]. It also causes significant social, developmental and economic disruption for individuals and societies [40].

Inadequate or delayed treatment of *Plasmodium* infections can lead to life-threatening complications (collectively referred to as manifestations of 'severe malaria'), the best known of which are cerebral malaria, severe anaemia, respiratory distress and metabolic acidosis [41]. The pathophysiological criteria for severe malaria were established in patients with falciparum infections and indeed severe malaria due to the other species (with the exception of *P. knowlesi* [16]) has been regarded as rare. The reasons for the greater pathogenicity of *P. falciparum* are complex but relate at least partially to the propensity of schizonts to adhere to endothelial cells causing major sequestration in the deep microvasculature [42,43]. In Africa as a whole, an estimated 1-2% of all *P. falciparum* infections in children under 5 years of age meet criteria for severe malaria [44].

Development of anti-disease immunity to local strains of *Plasmodium* requires extensive parasite exposure and is preceded by a period of 'tolerance' to parasitaemia [45,46]. The rapidity with which immunity develops depends on the intensity of parasite exposure which in turn is influenced by local entomological inoculation rates, access to effective antimalarial treatment and, in the case of vivax malaria, local relapse patterns. Where transmission of malaria is intense and perennial, symptomatic and severe disease tends to be limited to

children [47,48]. In regions where transmission is of low intensity or seasonal, symptomatic and severe disease may be seen throughout life [49-51].

1.2 Evolution of the burden of malaria with time

The burden of malaria in humans and the proportion of disease attributable to the different *Plasmodium* species has waxed and waned over millennia in response to both natural factors and human intervention [6,52]. Genetically distinct ancestral forms of *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* were already present at the dawn of human existence [6]. The presence of haemoglobin and red blood cell variants that protect against *Plasmodium* infection and / or severe malarial disease provides a rough genetic trail map of the subsequent spread of these species from Old World to New. Between 50 and 100% of Africans living in the tropical belt do not express the Duffy red blood cell surface antigen (an evolutionary adaptation that prevents *P. vivax* red blood cell attachment and invasion) and are therefore almost completely refractory to infection by *P. vivax* [53-58]. Outside of Africa, Duffy negativity does not even reach moderate prevalence [6,56]. By far the longest burden of vivax malaria has therefore occurred in Africa (though there is some suggestion that there may have been isolated, low-level transmission of *P. vivax* in the Mediterranean at least as early as 50,000 years ago) [6,8]. *Plasmodium vivax* is only estimated to have reached Asia and the Americas in the last 5,000 to 30,000 years [6,8]. In part due to its resilience to wide temperature variations, *P. vivax* has subsequently spread the most prodigiously of all human *Plasmodium* species [55,59].

Plasmodium falciparum, in its present form, is the least competitive of the human malaria parasites and is thought to have been selected from an ancestral form as recently as 4,000 to 5,000 years ago when the African agricultural revolution brought humans together in stable, communal settlements [6]. The lethality of this species has resulted in rapid selection of

individuals heterozygous for haemoglobin S (the sickle cell gene) which provides 90% protection against severe falciparum malaria and now reaches a prevalence of 10% in Africa as a whole despite a loss of fitness in the homozygous form of close to 100% [60,61]. Many other haemoglobin and red blood cell variants have arisen in response to the presence of *P. falciparum* and / or *P. vivax* malaria, the deleterious health effects of which can ultimately be added to the already colossal tally of human suffering caused by *Plasmodium* species.

Initially human *Plasmodium* species spread throughout the New World checked only by the extent of human migration, the presence of competent *Anopheles* mosquito vectors, climate and altitude. Humans were not *knowingly* able to significantly reduce transmission of *Plasmodium* species until Ross and Grassi's discovery of the intermediate mosquito vector in the late 19th century. In 1911, Ross wrote '*malaria can be completely extirpated in a locality by the complete adoption of any one of the three great preventive measures, namely personal protection, mosquito reduction, and treatment*' [62]. Early targeted malaria control efforts in the 20th century focused mainly on mosquito reduction through drainage of swampy mosquito breeding areas and use of petroleum oil and Paris-Green dust larvicides [52,63]. The long-lasting insecticidal properties of DDT (dichlorodiphenyltrichloroethane) were discovered in 1939 and used extensively to further reduce interaction between mosquitoes and humans during the World Health Organization's global malaria eradication programme initiated in 1955 [52,63,64].

Well before the parasitic cause for malaria had been discovered, various naturally occurring substances were being used for their anti-fever properties. Ancient Peruvian populations chewed the bark of the indigenous Cinchona tree to ameliorate malarial fever while Chinese populations used *Artemisia annua* (now known to contain artemisinin derivatives) at least as far back as 340 AD [65,66]. By the time of Ross' aforementioned proclamation, quinine had

been ‘discovered’ and purified from Cinchona bark and was being produced in very large quantities, mostly from Cinchona tree plantations in the Dutch East Indies [67]. One of the widest uses for quinine at this time was as a prophylactic for Western military troops operating in malarious areas. Although effective, quinine was less than ideal for this purpose because of its short duration of action, bitter taste and propensity to cause “cinchonism”. Military action in the First and Second World Wars prompted an extensive search by both German and Allied scientists for alternative synthetic antimalarial medications. Thousands of potential compounds were screened during the 1910s and 1920s, eventually resulting in the development of pamaquine, atebrin and the widely-used 4-aminoquinoline chloroquine [63,67]. A second wave of screening in the 1940s produced amodiaquine, primaquine (still the only licensed agent capable of killing *P. vivax* hypnozoites), proguanil and pyrimethamine [63]. Mefloquine and halofantrine were not developed until the 1970s and 1980s, the former became a particularly important prophylactic drug for the United States military [67].

Antimalarial drugs were given a large role in reducing malaria transmission in the latter stages of the WHO eradication campaign. This followed the detection of mosquitoes resistant to DDT and increasing public discontent about possible adverse health effects of the insecticide [63,68]. Chloroquine and pyrimethamine were used particularly widely both for the treatment of symptomatic disease and also as mass prophylactics, sometimes incorporated into foodstuffs such as salt [68].

Malaria is believed to have reached its peak geographic distribution some time during the late 19th or early 20th century [6,52,69]. In 1900, 53% of the earth’s land surface area is estimated to have sustained malaria transmission and 77% of the world’s population was at risk of infection (corresponding to 0.9 billion people) (see Figure 1.2) [52]. The measures employed

in the 1955-1969 WHO eradication campaign resulted in significant contraction in the geographic distribution of malaria, particularly in areas with low intensity transmission to begin with [52]. Ultimately, the campaign failed because of the world's inability to sustain the gargantuan efforts required as well as the inexorable spread of *P. falciparum* resistance to antimalarial drugs and mosquito resistance to DDT. By the 1970s progress against malaria had largely stagnated [52]. At the turn of the millennium, 27% of the earth's surface was estimated to sustain malaria transmission and 48% of the global population was at risk of infection (corresponding to 3.4 billion people) [52]. Despite significant progress in controlling malaria, between 150 and 300 million people died from the disease during the 20th century alone [6].

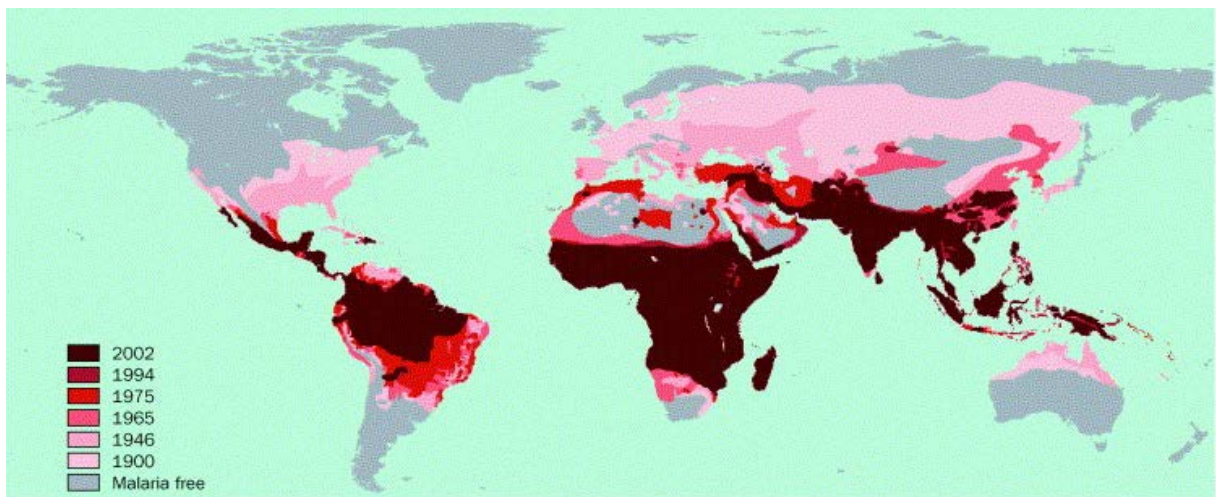


Figure 1.2 The global distribution of malaria since 1900. Reproduced with permission from Hay et al. The global distribution and population at risk of malaria: past, present, and future. *Lancet Infect Dis* 2004; 4: 327-36. [52]

1.3 Current burden and control of malaria

Over the last decade there has been a resurgence of effort in the global fight against malaria, catalysed in part by the declaration of the Millennium Development Goal to “halt and have

begun to reverse the incidence of malaria by 2015” (as compared with 1990 figures) and by the Gates Foundation’s (re)commitment to global malaria eradication. Integrated vector control and early diagnosis and treatment remain the basic tenets of control and eradication efforts but many of the tools and techniques have changed [70].

Distribution of nets impregnated with long-lasting insecticides is now a key vector control strategy though increasing mosquito resistance to these insecticides remains a problem [71]. Rapid diagnostic tests (RDTs) have been developed and are now recommended for confirmation of malaria where quality-assured light microscopy is unavailable [72]. Some of these tests have very good sensitivity and specificity for *P. falciparum* infections but their performance at correctly identifying non-falciparum infections remains relatively poor [72,73].

Perhaps the biggest change has been the adoption of the highly potent and rapidly eliminated artemisinin derivatives in combination with slowly eliminated partner drugs for the treatment of uncomplicated falciparum malaria. Aside from being active against multidrug-resistant parasite strains, these artemisinin combination therapies (ACTs) have several theoretical advantages over standard antimalarial monotherapies (referred to in detail in Chapter 3 of this thesis) [74,75]. The first fixed-dose ACT, artemether+lumefantrine, was prequalified by the World Health Organization in 2001 and recommended as first-line treatment of falciparum malaria in the same year [76,77]. Five ACTs are now recommended for falciparum malaria [78], four of which are available as coformulations [79]. Although the artemisinin derivatives are highly potent against *P. vivax* [80], first-line treatment policy for vivax malaria remains a three day course of chloroquine plus a 14-day course of primaquine for eradication of hypnozoites in all but a very few regions [78].

According to the most recent estimates, *P. falciparum* currently threatens 2.37 billion people and causes 451 million (95% credible interval 349-552 million) infections per year [81,82]. *Plasmodium vivax* threatens 2.85 billion people (91% of whom live in Central and Southeast Asia, 5.5% in the Americas and 3.5% in the Africa region) and infects anywhere between 130 and 435 million people per year (Figure 1.3) [55,83,84]. Altogether, malaria is estimated to have caused 781,000 deaths in 2009 (95% confidence interval [CI] 628,000-968,000), 85% of which were in children under the age of 5 years and 91% of which were estimated to have occurred in Africa [85].

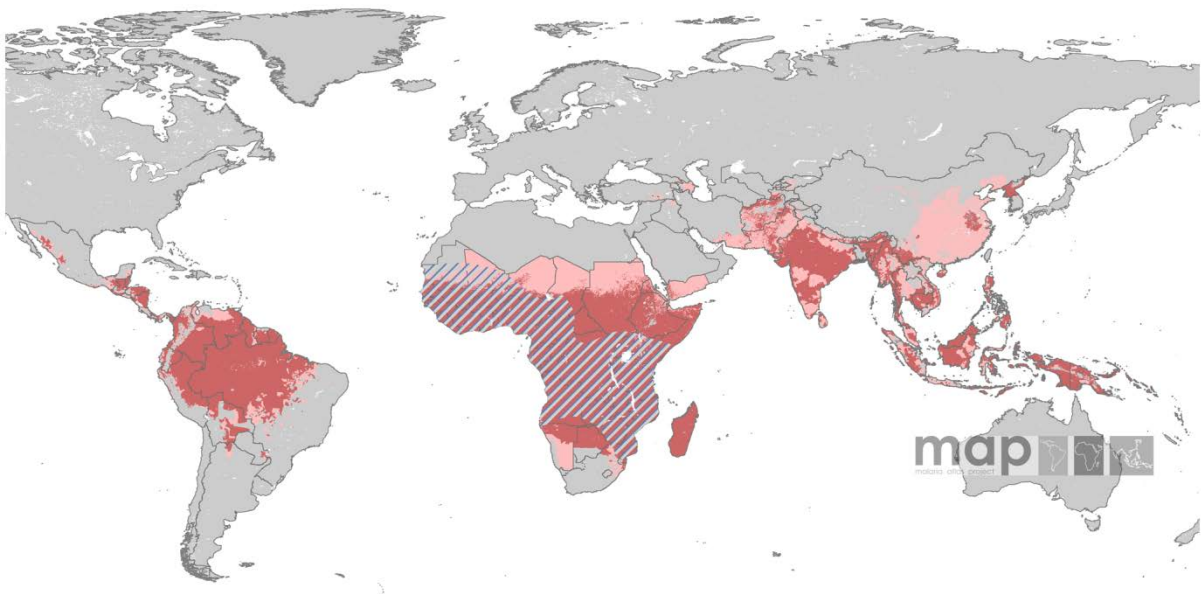


Figure 1.3 The global spatial limits of *Plasmodium vivax* malaria transmission in 2009. Areas where Duffy negativity prevalence was estimated as $\geq 90\%$ are hatched. Transmission is defined as stable (red areas, where *P. vivax* annual parasite incidence ≥ 0.1 per 1,000 people per year), unstable (pink areas, where *P. vivax* annual parasite incidence < 0.1 per 1,000 per year) and no risk (grey areas). Reproduced with permission from Guerra CA, et al. The international limits and population at risk of *Plasmodium vivax* transmission in 2009. *PLoS Negl Trop Dis* 2010; **4**: e774. [55]

Of the estimated 99 countries that are endemic for one or more human *Plasmodium* species (a figure of 106 countries has also been quoted [70]) 67 are currently “controlling” malaria and 32 are pursuing malaria elimination (Figure 1.4) [86]. Twenty five of the countries in the elimination phase have a mix of *P. vivax* and *P. falciparum* endemicity or sole *P. vivax* endemicity (Figure 1.5) [86]. In accord with predictions based on various biological features of *P. vivax* (referred to in detail throughout this thesis) [83,87], current malaria control measures are resulting in a faster reduction in the prevalence of *P. falciparum* than *P. vivax* and thus the proportion of malaria due to *P. vivax* is increasing [86,88,89].

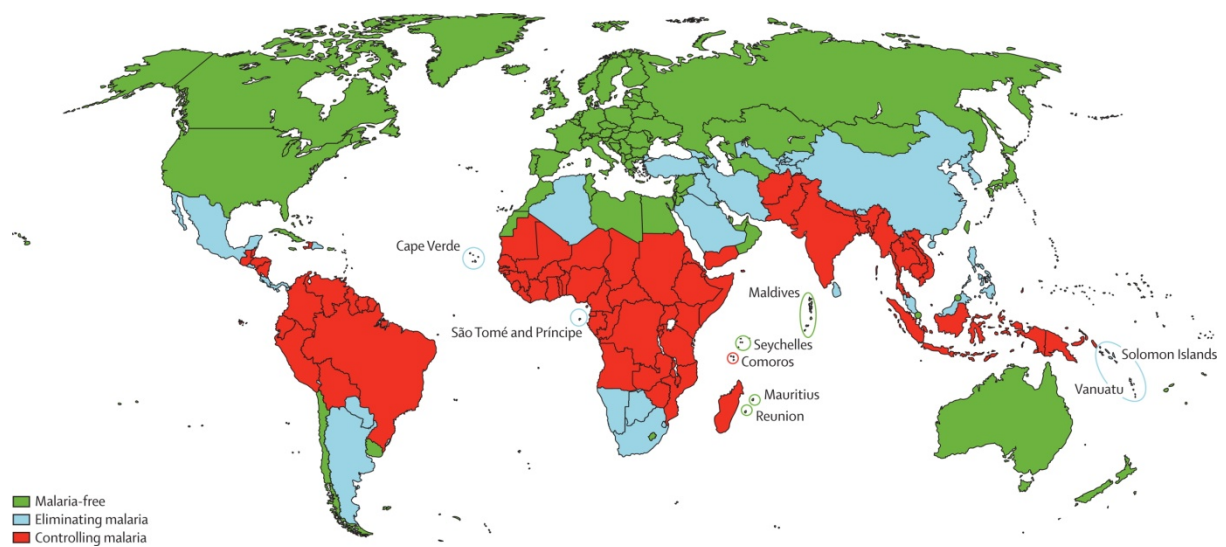


Figure 1.4 Categorisation of countries as malaria free, eliminating malaria, or controlling malaria, 2010. Reproduced with permission from Feachem RGA, et al. Shrinking the malaria map: progress and prospects. *Lancet* 2010; **376**: 1566-78. [86]

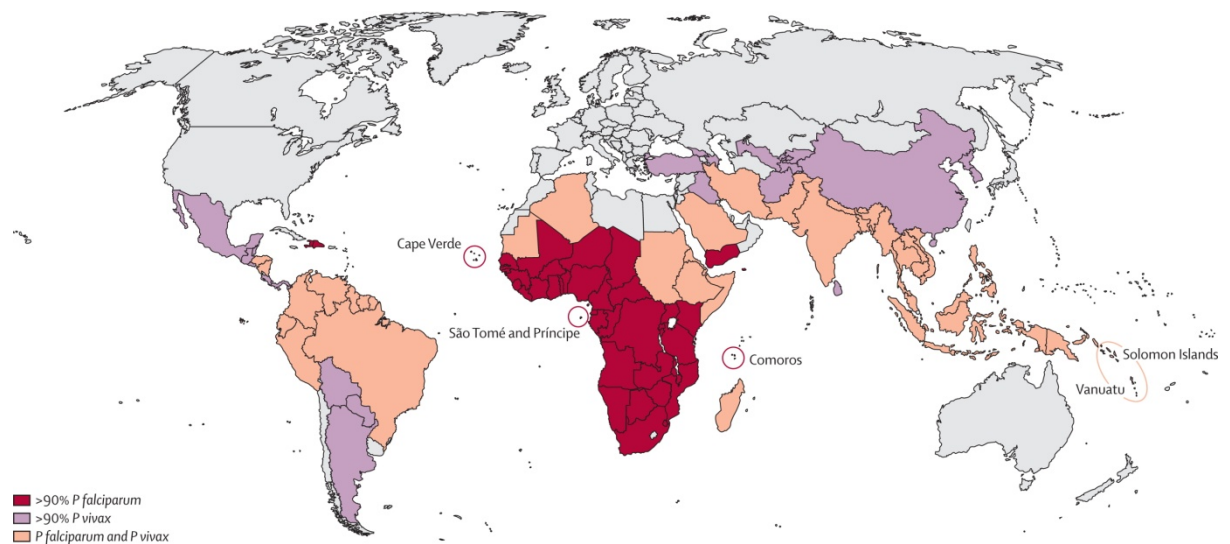


Figure 1.5 Categorisation of countries according to whether human malaria is predominantly caused by *P. falciparum*, *P. vivax*, or both *P. falciparum* and *P. vivax*, 2010. Reproduced with permission from Feachem RGA, et al. Shrinking the malaria map: progress and prospects. *Lancet* 2010; **376**: 1566-78. [86]

1.4 An introduction to the challenge of *Plasmodium vivax*

Long before the discovery of *P. vivax*, disease caused by this parasite was referred to as ‘benign tertian’ fever because of its characteristic 48 hour periodicity and apparent lack of association with life-threatening complications [6]. This benign reputation won *P. vivax* the favour of common use in the historical practice of malariatherapy for patients with neurosyphilis [90]. Between 1917 (when this mode of therapy was introduced) and 1943 when penicillin was first used for syphilis, thousands of patients were treated with induced *P. vivax* infections either through mosquito inoculation of sporozoites or intravenous administration of blood stage parasites [91,92]. Careful studies of these patients revealed important aspects of the epidemiology and pathogenesis of *P. vivax* infections. Many neurosyphilitics (as well as ‘volunteers’ from penitentiaries) also became the subjects of antimalarial drug trials – the motivation for which often came from the need of Western nations to send non-immune military personnel into malarious areas.

By the 1950s, military organizations in developed economies had access to chloroquine as a reliable prophylactic and blood schizontocidal treatment for *P. vivax*. Primaquine was available for preventing future relapses in troops returning from malarious combat zones and the era of malariatherapy had come to an end. The impetus for research related to ‘benign tertian’ malaria had waned in the nations that had the greatest financial means to carry it out. This situation remained largely unchanged for the next 50 years leading one prominent malariologist to lament that in the last few decades “we have forgotten more than we have learnt” about *P. vivax* (Prof Nicholas J. White, personal communication, 2010).

As a result of this research lethargy, relatively few new tools for the control of this species have been brought to trial over the last 50 years. Development of *P. vivax* vaccines lags way behind that for *P. falciparum* [93]. Highly accurate rapid diagnostic tests for vivax malaria have yet to be developed. Treatment of *P. vivax* hypnozoites is still reliant upon primaquine, a drug that is contraindicated in infants and pregnant women and can cause life-threatening haemolysis in those with glucose-6-phosphate dehydrogenase (G6PD) deficiency [94]. No bedside test for G6PD deficiency is publicly available and the pipeline for new, safer hypnozoitocidal alternatives is virtually bare with just one drug, tafenoquine (which has the same potential to cause haemolysis in those with G6PD deficiency), in clinical trials and one further drug, NPC-1161-B, in preclinical studies [95].

Over the last decade, the relative neglect of *P. vivax* has become increasingly untenable. There are two main reasons for this. First, as previously mentioned, outside of Africa *P. vivax* is becoming a proportionately more common cause of malaria as current control strategies ‘preferentially’ reduce the burden of falciparum malaria. Second, recent reports of severe disease associated with *P. vivax* infection, particularly from New Guinea and the Indian subcontinent, challenge the dogma that *P. vivax* is invariably a benign disease and suggest

that this parasite may be causing greater morbidity and mortality than previously thought [49,50,96-105]. The range of severe manifestations attributed to this species is exceedingly broad including severe anaemia, thrombocytopaenia, coma, respiratory distress and pulmonary oedema, metabolic acidosis, liver and renal dysfunction, shock and splenic rupture [106]. Of these, severe anaemia is the least subjective in its assessment and at least in Papua, Indonesia, is easily the most common [49,50].

1.5 Aims of this thesis and description of chapters

Plasmodium vivax may be more virulent than previously recognised and is proving refractory to current malaria control measures. There are few promising new interventions for this species on the horizon. Keeping this, rather bleak, situation in mind, this thesis has the following two overarching aims:

1. To describe the burden of anaemia and direct and indirect mortality attributable to vivax malaria in Southern Papua, Indonesia
2. To determine the potential of currently available antimalarial drugs to reduce transmission of *P. vivax* in regions that are co-endemic for both falciparum and vivax malaria

Chapters 2 and 3 present focused literature reviews of the burden and pathogenesis of vivax-associated anaemia and the relative advantages and disadvantages of artemisinin combination therapies for *P. vivax* infections respectively. Primary data used in this thesis were gathered from Timika, Southern Papua, Indonesia and from the northwestern border of Thailand. The methods used to address the aforementioned aims are described in Chapter 4 and extra methodological details are given in the individual research chapters where necessary. Chapters 5 through 10 describe primary research, the first three of which address general aim number 1 and the second three, aim number 2. Chapters 5 and 6 examine the

haematological burden of *P. vivax* compared with other *Plasmodium* species in the health care setting and the community respectively. Chapter 7 aims to establish the incidence and nature of *P. vivax*-attributable mortality in the region. Chapters 8 and 9 use data from both Timika and the northwestern border of Thailand to explore the dynamics of *P. vivax* gametocytaemia and the effect of various drugs on gametocyte carriage and to investigate risk factors for, and the effect of antimalarial kinetics on, *P. vivax* recurrence following falciparum malaria respectively. Chapter 10 reports the results of a pragmatic assessment of the effectiveness of WHO-recommended regimens of primaquine for the prevention of *P. vivax* relapses in Timika. The thesis concludes with a general discussion and conclusions.

Both literature reviews and all of the research chapters in this thesis have been adapted from published articles or manuscripts that are in submission or soon to be submitted to peer-reviewed journals. Co-authors of these articles are gratefully acknowledged at the end of each chapter.

2. The anaemia of *Plasmodium vivax* malaria

2.1 Introduction

Plasmodium vivax threatens approximately 2.8 billion people globally and, because of its particular biological characteristics, will be more difficult to eradicate than *P. falciparum* [6,54,55,83,107-111]. Over recent years, case series [97,100,101,112], surveillance studies [49,50,96,99] and reviews [83,84,104-106,113-115] have linked vivax malaria with a number of severe manifestations similar to those found in *P. falciparum* infection; observations that challenge the notion that vivax malaria is a benign disease. The causative role of *P. vivax* in some of these severe manifestations remains to be proven and many are sufficiently rare that they are unlikely to represent significant public health problems. Anaemia, on the other hand, is a common and frequently severe consequence of vivax infection [49,50,96,116-118]. This review explores the epidemiology, pathophysiological mechanisms, relationship to transmission dynamics and consequences of anaemia caused by vivax malaria (highlighting similarities and differences as compared with *P. falciparum*) as well as the impact of antimalarial treatment on haematological recovery.

2.2 Literature search strategy and selection criteria

I searched PubMed, Medline and EMBASE using the terms “vivax” and (“hemato*” or “haemato*” or “haemoglobin” or “haemoglobin” or “anemi*” or “anaemi*”). I also referred to

the reference lists of relevant articles and asked experts in the field for information on any other relevant literature. In cases where articles were written in a language other than English or I was unable to obtain full-text versions, I relied on information from the abstracts. The most recent search was done in March 2011.

2.3 Epidemiology

Endemic vivax malaria is transmitted throughout the tropics in much the same geographical pattern as falciparum malaria [55,110]. The major exception is West Africa where *P. vivax* is largely absent, presumably due to selection over many millennia of individuals lacking the Duffy red blood cell surface antigen used for *P. vivax* red cell invasion [6,119].

Where *P. falciparum* and *P. vivax* co-exist, both the incidence of infection and severity of haematological morbidity attributable to *P. vivax* tends to peak at a younger age than for falciparum malaria and in many regions, before the end of the second year of life [46,49,118,120,121]. This pattern probably reflects greater parasite exposure through frequent relapse and consequently more rapid acquisition of immunity to vivax malaria. In tropical regions, relapses may occur as often as every three weeks [122,123]. In Papua, Indonesia, an area of comparatively high-level *P. vivax* transmission, approximately a quarter of infants hospitalised with vivax malaria have severe anaemia (haemoglobin less than 5g/dL), whereas in individuals 45 years or older this proportion decreases to less than 5% [49].

Females are at greater risk of hospitalisation with *P. vivax* malaria [49] and in one large analysis were more likely to present with anaemia (unpublished data). Pregnant women with *P. vivax* infection have a ~2-fold higher risk of moderate anaemia than uninfected pregnant women [33,34,124]. Whether the haematological impact of vivax malaria in pregnancy is greater than in non-pregnant women of child-bearing age is unknown.

Several red blood cell and haemoglobin variants have been associated with reduced susceptibility to anaemia caused by falciparum malaria, the best known being sickle cell anaemia. Hypothesised mechanisms for this protection include reduced red blood cell invasion, relative inhibition of intracellular parasite replication, more efficient removal of infected red blood cells by the spleen and more efficient presentation of parasite antigens to the immune system [125-127]. The high erythrocyte counts and relative microcytosis seen in conditions such as alpha thalassaemia result in a lower proportional reduction in haemoglobin with malaria infection [128]. Both alpha and beta thalassaemia have been associated with an increased risk of *P. vivax* parasitaemia in cross sectional studies [129-131] whereas the effects of Gerbich blood group and Melanesian ovalocytosis are less clear [132,133]. Glucose-6-phosphate dehydrogenase deficiency is associated with protection against clinical disease and reduced parasite density in *P. vivax* infections [134,135] but the effect on vivax-associated anaemia is unknown. The rarity of such polymorphisms in migrant Highland Papuan populations has been hypothesised to contribute to the higher risk of severe anaemia from *P. vivax* in Southern Papua compared with elsewhere in New Guinea [106].

Several helminth infections may cause anaemia through chronic blood loss, but the interaction with malarial anaemia is complex. In Africa, hookworm and *P. falciparum* malaria coinfection has been shown to cause an additive reduction in haemoglobin in children and pregnant mothers when compared with monoinfection with either parasite alone [136]. Helminthiasis may also be a risk factor for *P. falciparum* parasitaemia [137,138] but evidence is conflicting. Few studies have addressed the effect of intestinal helminthiasis on the risk of *P. vivax* infection and anaemia. Boel and colleagues showed a positive association between *A. lumbricoides* infection during pregnancy and risk of vivax malaria [88]. Another small study found that the reduction in haemoglobin associated with *P. vivax*

infection was attenuated by coinfection with hookworm, *Ascaris lumbricoides* and *Trichuris trichuria* [139].

The haematological effects of chronic blood loss caused by intestinal helminthiasis are exacerbated by nutritional iron deficiency which in turn may interact with the haematological effects of *P. vivax* malaria. Iron deficiency is protective against *P. falciparum* infection whereas iron supplementation increases the risk of falciparum malaria and high parasitaemia infections [140,141]. The evidence for a link between iron supplementation and morbidity associated with vivax malaria is conflicting. One study from Thailand showed that pregnant women given supplemental iron were at increased risk of morbidity associated with vivax malaria [142]. A randomised controlled trial from Peru showed that iron plus zinc reduced vivax-associated morbidity in children under 5 years but iron supplementation increased morbidity in those over 5 years [143]. A further study from Papua New Guinea showed no effect of iron supplementation on morbidity associated with vivax malaria [144].

2.4 Pathophysiology and mechanisms

The primary target of human *Plasmodium* species is the red blood cell. *Plasmodium vivax* has a very strong predilection for young red blood cells, in particular reticulocytes, whereas *P. falciparum* has a weaker predilection for young red blood cells and significant ability to infect older cells [145-147]. The natural history of erythrocytes infected by either species is to host the replicating parasite for approximately 48 hours before bursting and releasing daughter merozoites. The range of peripheral parasitaemias in *P. vivax* infections is lower than in symptomatic *P. falciparum* malaria and parasitaemia >2% is rare [111]. Despite this, mathematical models suggest that premature death of infected reticulocytes due to *P. vivax* infection should be sufficient to lead to extreme anaemia over a period of several months by choking the supply of mature red blood cells [26,27,148]. Direct evidence from two

malariatherapy patients studied in detail shows that severe anaemia may develop much more rapidly than this and that the proportion of infected reticulocytes after two to three weeks of vivax malaria can be less than 10% [145]. These observations suggest that other mechanisms of anaemia are likely to be important. In *P. falciparum* malaria, these include increased removal of infected, and to a greater extent, uninfected red blood cells from circulation, compounded, in subacute and chronic forms, by impaired erythropoiesis [149-153]. The same general processes appear to be important in vivax malaria but many of the cellular mechanisms differ (Table 2.1).

2.4.1. Removal of red blood cells from circulation

Although parasitaemias are typically lower in vivax compared with falciparum infections, the absolute number of red blood cells removed from circulation, and hence the degree of anaemia resulting from infection by the two species, is often similar. This is because in *P. vivax* malaria, approximately 34 non-infected cells are cleared for every one infected cell [154] whereas in *P. falciparum* malaria, this ratio is closer to 8 to 1 [150,153]. These figures are derived from observations in non-immune adults treated for neurosyphilis [150,154] and Thai patients over 5kg in weight [153]. There are no data on the proportional removal of uninfected red blood cells in infants, the age group bearing the greatest burden of anaemia due to vivax malaria. Although removal of uninfected red blood cells is an important component of vivax-associated anaemia, the mechanisms of removal are not fully understood.

As in falciparum malaria, vivax-infected erythrocytes adhere to uninfected red blood cells (rosetting) [155,156] but unlike falciparum-infected cells, they have limited propensity to adhere to endothelial cells and therefore sequestration in the deep microvasculature is not a major factor in the pathogenesis of vivax malaria [157,158]. Erythrocytes parasitised by *P. falciparum* become less deformable than uninfected red cells and have a reduced capacity to

pass through narrow inter-endothelial slits in the wall of splenic sinuses (mean dimensions $1.89 \times 0.65\mu\text{m}$) [159-162]. Vivax-infected cells become more deformable as the parasite matures and are thought to retain the ability to squeeze through splenic slits [162-164]. In falciparum malaria, sequestration reduces the proportion of parasitised red blood cells that traverse the spleen. In vivax malaria, increased deformability of parasitised red blood cells may limit the proportion of red cells that are removed during passage through the splenic microcirculation. Thus, it appears that *P. falciparum* and *P. vivax* have evolved two different means of escaping splenic filtration. In both vivax and falciparum malaria, parasitised, and possibly non-parasitised, red cells are hypothesised to be more fragile than red cells in non-infected individuals and more prone to damage from shear stresses [162,163,165,166]. This process is potentially a more important cause of red cell loss in falciparum malaria since major sequestration in the microvasculature impedes the passage of circulating erythrocytes and erythrocyte rosettes [167].

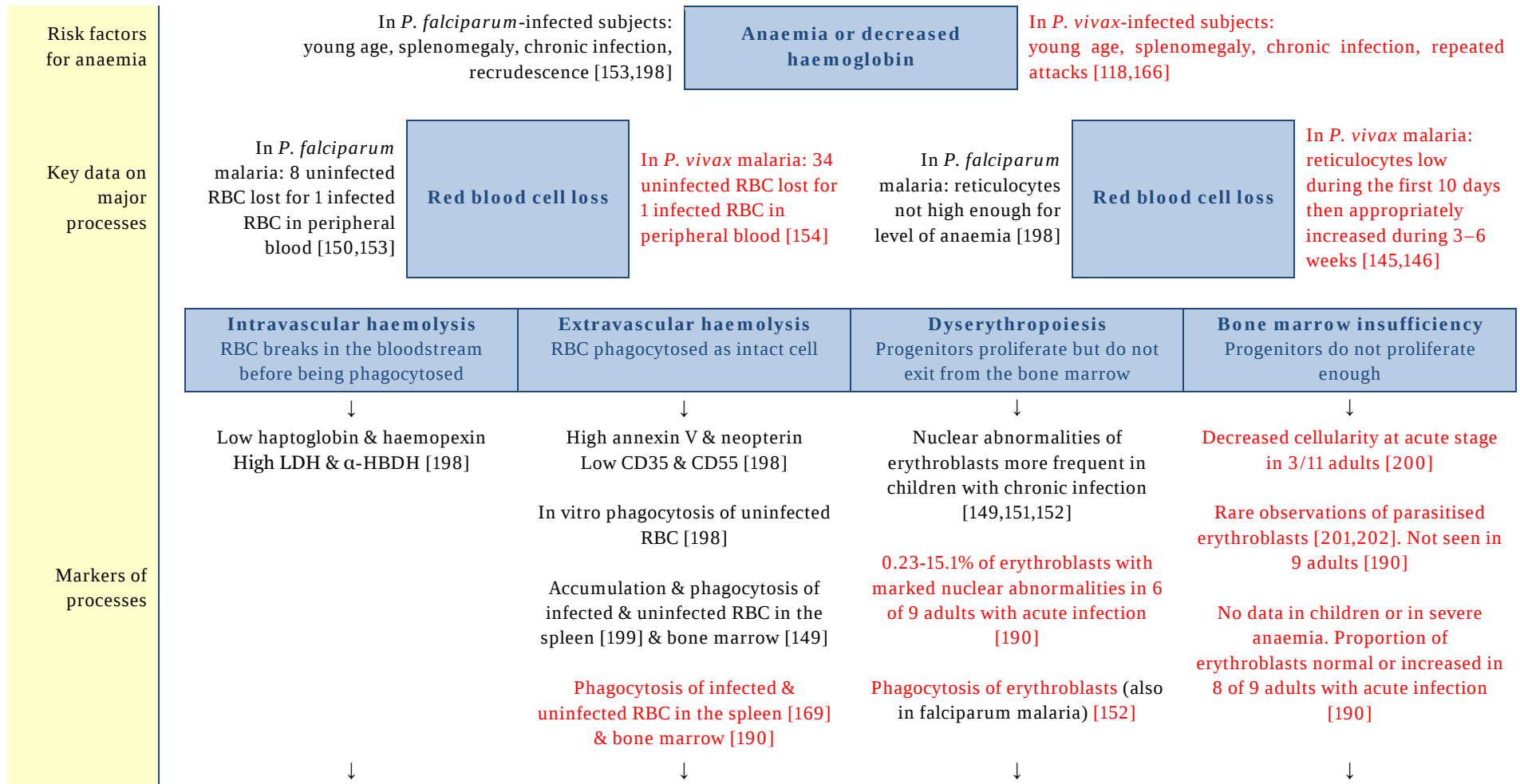
In addition to these mechanical processes, activation of the innate, cell-mediated and humoral immune systems in response to the presence of *P. vivax* antigens enhances the detection and removal of infected and abnormal uninfected red blood cells [152,168,169]. The non-specific immune response for a given parasitaemia is greater for *P. vivax* than *P. falciparum* and may partially explain the greater proportional removal of non-parasitised cells and lower fever threshold in vivax malaria [170-172]. Macrophage hyperplasia and increased phagocytic activity in both falciparum and vivax malaria results in a highly oxidative environment and may contribute to the shortened lifespan of non-infected erythrocytes [169,173-177]. To compound the problem, reduced glutathione, which is necessary for protecting red cells against damaging oxygen species, is reported to be depleted in vivax malaria [178,179]. Infection with *P. falciparum* causes altered expression of complement components and deposition of parasite proteins on infected and uninfected red

blood cells [180,181] (the latter sometimes associated with presence of specific immunoglobulins); facilitating opsonisation and complement-mediated phagocytosis [182-184]. It is unknown whether these processes also occur in vivax-associated anaemia.

Whatever the mechanisms leading to red blood cell alteration, the spleen is the most important site for the filtration, retention and phagocytosis of non-sequestered erythrocytes parasitised or altered by *P. falciparum* [41,160,185-187]. Splenic activity limits parasite density thereby reducing the risk of severe malaria. However, the more stringent the splenic clearance, the greater the likelihood of severe anaemia [185,186,188]. This may explain why concomitant severe malarial anaemia with spleen enlargement and cerebral malaria is relatively unusual with cerebral manifestations being more common in acute, fulminant infections and anaemia being more likely in chronic infections [118,188-190]. The role of the spleen in vivax malaria is poorly understood though splenic enlargement in this infection appears to be similar to falciparum malaria [191,192]. Indeed vivax malaria carries a very low but well-known risk of splenic rupture; considered greater than for falciparum malaria [193,194]. In 1974, Littman described a single patient with hereditary spherocytosis who developed severe anaemia secondary to vivax malaria. A relapse five months later after removal of the spleen did not cause anaemia suggesting that the spleen was the primary site of red blood cell removal (though the effect of strain specific immunity could not be excluded) [195]. A study from Papua showed that plasma haemoglobin concentrations in adults with uncomplicated vivax malaria were not increased compared to controls and were significantly lower than in falciparum malaria (unpublished data). This suggests that in adults with vivax malaria, the degree of intravascular haemolysis may be less than in falciparum malaria and that a greater proportion of uninfected red blood cells undergo extravascular removal.

Increased removal and destruction of both infected and uninfected red cells in vivax malaria is most prominent during the early stages of infection however enhanced removal of uninfected cells persists for 5 weeks or more after effective treatment of blood-stage infection [196,197]. In chronic, asymptomatic vivax parasitaemia, common in vivax-endemic areas, removal of both infected and uninfected red cells is likely to persist for the duration of infection.

Table 2.1 Comparative pathogenic mechanisms of anaemia associated with *P. vivax* and *P. falciparum* malaria (mechanisms in red font relate to vivax malaria and mechanisms in black relate to falciparum malaria)



Identified or suspected cellular mechanisms	Rupture of sequestered schizonts [203]	Mechanical retention of uninfected RBC in the spleen due to decreased deformability [167,204]	Macrophage activation by cytokines or parasite products enhancing phagocytosis of progenitors (including erythroblasts) [152] although cytokine levels are generally lower in severe malarial anaemia than in cerebral or uncomplicated attacks [209,210]	Inappropriate bone marrow response to appropriate EPO levels in children [213,214] though possibly not in adults [215,216]
	Rupture of circulating schizonts including parasite-harboured reticulocytes [26,148]. Intravascular haemolysis due to rupture of schizonts lower in <i>P. vivax</i> than in <i>P. falciparum</i> as parasitaemia is lower in <i>P. vivax</i> [111]	Mechanical retention of rings in the spleen due to decreased deformability [159-161,205]		
	Rupture of uninfected RBC (increased fragility) [163]	Phagocytosis & opsonisation of uninfected RBC decorated with RSP-2/RAP-2 [181], complement [182,183,206] or immunoglobulins [180]	Toxic effect of parasite products (eg haemozoin) on progenitors (including erythroblasts) (also in falciparum malaria) [152]	
		Oxidative stress on uninfected RBC & infected RBC [178] (also in falciparum malaria) [207,208]	RAP-2 on the surface of erythroblasts & phagocytosis in vitro [211]	
		Increased osmotic fragility & Heinz body formation of uninfected RBC [166]	Impaired iron utilisation (also in falciparum malaria) [212]	

Abbreviations: RBC; red blood cell, CD35; complement receptor 1, CD55; decay accelerating factor, LDH; lactate dehydrogenase, α -HBDH; alpha-hydroxybutyrate dehydrogenase, RSP-2; ring surface protein 2, RAP-2; rhoptry-associated protein-2, EPO; erythropoietin

2.4.2. Impaired production of red blood cells

Patients with vivax or falciparum malaria have bone marrow abnormalities reflecting impaired erythropoiesis. In the earliest stages of both infections, the typical marrow finding is of decreased cellularity [152,200]. In those with more chronic infections, marrow cellularity tends to be normal or increased but there is ineffective erythropoiesis [149,151,190], as indicated by impaired iron utilisation [152,212], presence of morphologically abnormal erythroblasts as a result of cellular injury [190] and phagocytosis of erythroblasts by marrow macrophages [152,190].

The exact mechanisms of impaired erythropoiesis in vivax malaria are unclear. Using electron microscopy, Ru and colleagues have shown parasitisation and subsequent degradation of erythroblasts in two patients with uncomplicated vivax malaria [202]. Yoeli demonstrated morphologically normal but non-pigmented intracellular *P. vivax* parasites in a sternal tap specimen but not in peripheral blood smears in a single patient with vivax malaria [201]. Wickramasinghe did not find any *P. vivax* parasites in the marrows of 9 Thai adults with uncomplicated *P. vivax* infections [190]. Because of the absence of any bone marrow data in children, or at any age with severe vivax-associated anaemia, the importance of *P. vivax* parasitisation of erythroblasts in severe vivax anaemia is not known. Hypoxia of the bone marrow resulting from obstruction of marrow sinusoids by parasitised red blood cells and inadequate erythropoietin production or response have been hypothesised to contribute to impaired erythropoiesis in *P. falciparum* infections [190,215-219]. In vivax malaria, hypoxia of the bone marrow is unlikely to be significant as there is minimal schizont sequestration. Erythropoietin metabolism is yet to be studied in this disease.

Wickramasinghe and colleagues proposed that *P. vivax* has a directly toxic effect on erythroblasts or their precursors [190]. Alternatively *P. vivax* may exert its effect on bone marrow macrophages leading to increased phagocytic activity and / or release of locally

cytotoxic molecules damaging surrounding haematopoietic cells [190]. Whatever the cause, some degree of impaired erythropoiesis has been shown to persist for at least two weeks after treatment of vivax malaria and therefore the effect is likely to be long-lasting [190].

2.5 The effects of transmission intensity, relapse patterns and strain diversity

Since a significant proportion of the anaemia of vivax malaria, at least in the acute phase, can be explained by removal of uninfected red cells in response to immune system activation, the magnitude of the immune response (of which intensity of symptoms can be taken as a proxy) is likely to be an important determinant of haematological impairment. Untreated primary sporozoite-induced infection in non-immune adult patients with neurosyphilis typically results in paroxysmal fever lasting 3-8 weeks followed by an extended period of increasing clinical 'tolerance' to persistent parasitaemia [220]. Anti-parasite immunity that suppresses parasitaemia to subpatent levels takes significantly longer to develop, in many cases more than 200 days [221]. Collins and colleagues have reviewed the natural history of haemoglobin changes associated with untreated *Plasmodium vivax* infection in adult neurosyphilis patients [154]. There was an exponential decay in mean haemoglobin concentrations during the first 4-5 weeks followed by a gradual climb in concentrations coinciding with development of parasite tolerance (see Figure 2.1). With persistent infection, mean haemoglobin levels had still not returned to normal by week 11 though the trajectory of the changes suggests that they might eventually have done so [154].

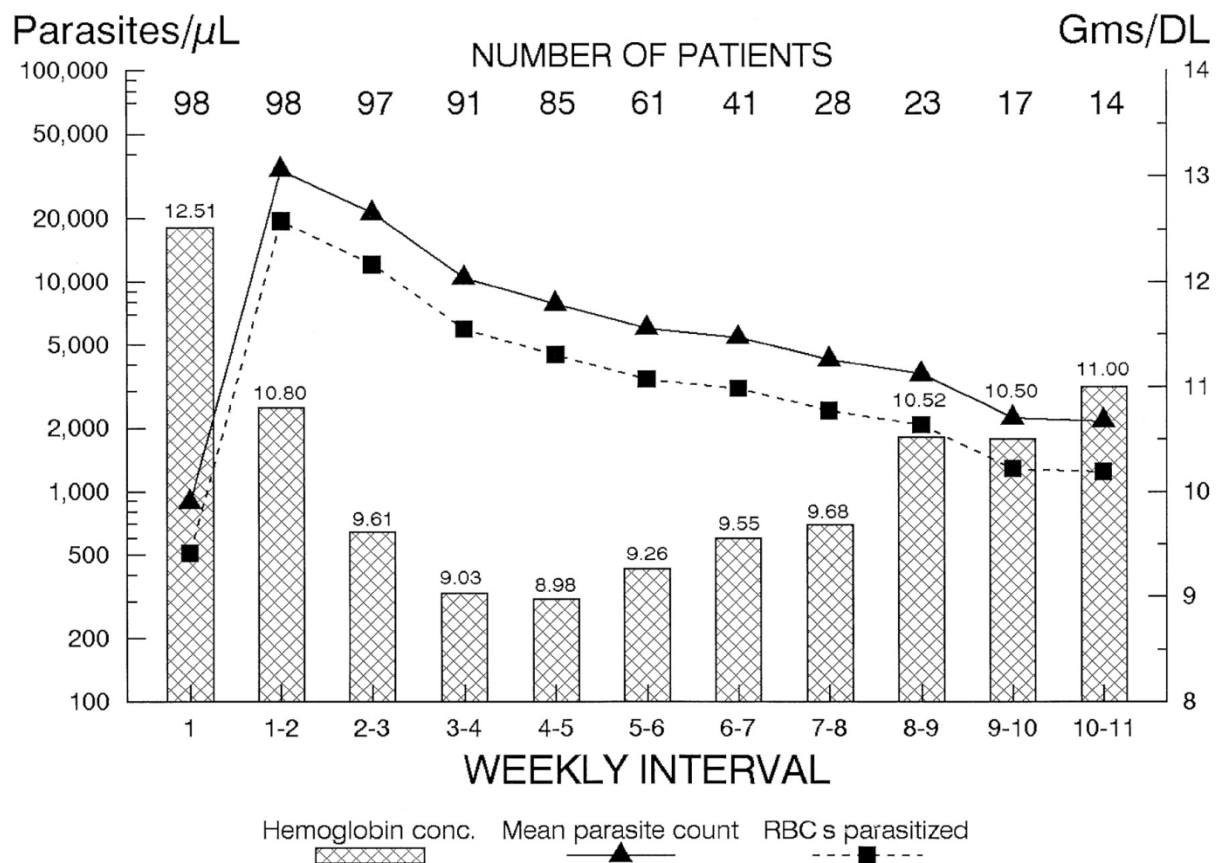


Figure 2.1 Mean haemoglobin concentration in relation to parasitaemia in patients with syphilis treated with induced *P. vivax* infections (98 with the St Elizabeth strain, 11 with the Chesson strain and 2 with the Korean strain). Reproduced with permission from Collins WE, et al. A retrospective examination of anemia during infection of humans with *Plasmodium vivax*. *Am J Trop Med Hyg* 2003; **68**: 410-2. [154]

Repeat infection with the homologous *P. vivax* strain, whether due to reinfection, recrudescence or relapse, during the period of parasite tolerance may result in a transient rise in parasitaemia but rarely recurrent or exacerbated symptoms [45,222,223]. Homologous re-exposure after an extended period of parasite exposure (approximately 6 months for the St Elizabeth strain [221]) from untreated or inadequately treated primary infection, relapse or reinfection results in neither patent parasitaemia nor clinical symptoms [45,222,224-227].

Since homologous re-exposure in tolerant or immune individuals causes little non-specific immune system activation, the haematological effects of this are likely to be minimal.

Heterologous immunity however, is weak and exposure to a new strain following primary infection will usually result in clinical malaria with non-specific immune system activation, recurrent symptoms and presumably a repeat haematological insult [223,228]. If this occurs before an individual's haemoglobin concentration has returned to normal following primary infection, the haematological effects are likely to be additive though direct evidence supporting this postulate is limited [122,166,229]. The likelihood of repeat heterologous infection, and the probability that it occurs before complete haematological recovery, is a function of, among other things, the relapse pattern of local *P. vivax* strains and the entomological inoculation rate, both of which are also likely to affect the rate of genetic recombination and hence parasite population diversity [230].

In endemic regions, a high proportion of *P. vivax* relapses are known to be caused by heterologous strains (with regard to the previous vivax infection) [231,232]. This is likely to be particularly common in areas with relatively intense *P. vivax* transmission and / or extensive parasite strain diversity. In such regions, there will also be a greater chance of simultaneous inoculation of two different strains of *P. vivax* which has been shown to cause deficient homologous immunity and therefore presumably greater susceptibility to the haematological effects of future homologous infection [233].

Different *P. vivax* strains have markedly different relapse patterns and in some instances may not even cause a primary infection [123,221,234]. In tropical regions, relapses tend to occur regularly every three to four weeks and rarely persist for more than 4 years from the time of initial inoculation [122]. In temperate climates, the period between relapses may be significantly longer and more variable with some strains showing a bimodal frequency

pattern [122]. Tropical strains with short relapse intervals will be more likely to cause repeat blood stage infection before complete haematological recovery has occurred and may therefore be expected to have the greatest haematological impact.

The virulence of an individual parasite strain may also modulate its haematological effects. There is some evidence that the Chesson strain causes fever at lower parasitaemia than other strains and therefore that it may be more immunogenic [235]. In 1947, Whorton and colleagues described the anaemia associated with this strain as “striking” stating that “after the second week of malaria [in malariatherapy patients], it was difficult to maintain patients’ erythrocyte count above 2 million per cubic mm [corresponding to a haemoglobin concentration of approximately 6g/dL] in spite of frequent erythrocyte transfusions” [235].

Given the aforementioned evidence, one might predict that the severest haematological impact of vivax malaria would be seen in tropical regions with high entomological inoculation rates and virulent circulating strains. These features are perhaps best exemplified by *P. vivax* on the island of New Guinea where the frequently relapsing Chesson strain is endemic and local populations experience particularly severe haematological impairment from vivax malaria [49,50,96,118]. In these regions, broad anti-disease immunity to all circulating parasite strains is also likely to develop more rapidly and therefore the haematological burden of vivax malaria will be most heavily skewed to very young children [46].

Most populations at risk of *P. vivax* infection are also exposed to *P. falciparum* and co-infection (not necessarily patent) with these two species is common [229,236-238]. In Thailand, mixed infection with *P. falciparum* and *P. vivax* has been shown to attenuate the risk of severe anaemia associated with falciparum malaria – possibly due to some degree of cross-species immunity [153,239-241]. Recent work has shown that in Papua New Guinea and Papua, Indonesia, mixed infection causes more severe haematological impairment than

infection with either species alone [49,96,118]. The explanation for these opposing findings is not obvious but may lie in the different transmission dynamics in these regions.

2.6 Effects of antimalarial treatment

Early treatment of malaria can truncate the impending reduction in haemoglobin and accelerate haematological recovery [2,153,242]. Despite the overall benefits of treatment, haemoglobin typically falls slightly following initiation of an antimalarial drug reaching a nadir between days 3 to 7 following treatment [153,243]. Data are sparse but there is some evidence that this initial fall may be less pronounced following treatment of vivax malaria with chloroquine (which gives faster clinical and parasitological responses against sensitive strains) compared with either sulfadoxine+pyrimethamine or chlorproguanil+dapsone [243]. The artemisinin derivatives cause an extremely rapid reduction in *P. vivax* parasite biomass. They also temporarily reduce red blood cell production [244-246]. In falciparum malaria, evidence suggests that the haematological benefit of the greater efficacy of the artemisinin drugs negates or outweighs the detrimental effects of this bone marrow suppression [247,248]. The only published comparative assessment of the acute haematological effects of treatment with an artemisinin derivative in patients with vivax malaria showed that artesunate+pyronaridine was associated with a greater mean reduction in haemoglobin at days 3 and 7 when compared with chloroquine alone, although no tests of statistical significance were given [245].

Complete removal of blood stage parasites following blood schizontocidal treatment allows faster haematological recovery (pre-infection haemoglobin concentrations are generally achieved in approximately 4-5 weeks following effective treatment [2,153,249,250]) but reduces total parasite exposure and hence limits the development of homologous immunity [225]. Treated individuals are therefore more likely to develop clinical malaria with

significant haematological impairment following repeat homologous infection [225,227,251]. Highly efficacious blood schizontocidal regimens containing slowly eliminated drugs (such as chloroquine, piperaquine or mefloquine) minimise the risk of recrudescence and also provide extended post-treatment prophylaxis against recurrent infection, allowing more time for full haematological recovery [2,3,108,229,252]. The haematological benefit of the long elimination half-life is likely to be greatest in equatorial regions where *P. vivax* strains relapse as often as every three weeks.

Plasmodium vivax has developed high-grade resistance to chloroquine in parts of Oceania, Asia, Africa and Latin America [83,108] and sulfadoxine+pyrimethamine in parts of Southeast Asia [253]. Clinically, drug resistance is manifest by delayed parasite clearance times, an increased likelihood of incomplete parasite clearance and subsequent recrudescence as well as a shorter period of post-treatment prophylaxis against early recurrence [108]. These factors are likely to result in a greater haematological insult associated with the initial infection (as demonstrated in *P. falciparum* malaria [153,242,254]) but may facilitate earlier development of anti-disease immunity.

Primaquine is a hypnozoitocidal drug that, if administered correctly, can prevent *P. vivax* relapses and thus reduce the total haematological impact of a given infection. Unfortunately this medication has the potential to cause lysis of old red blood cells in all patients, but particularly those with glucose-6-phosphate dehydrogenase deficiency [94,255]. G6PD deficiency is the most common heritable enzymopathy in the world, with a prevalence ranging from 7.5% in Africa as a whole to 2.9% in the Pacific [256]. Although G6PD deficiency increases the susceptibility of erythrocytes to oxidative damage this alone is not sufficient to account for primaquine-induced haemolysis [257,258]. Generally, the more severe the enzyme deficiency, the greater the severity of haemolysis [94,255]. Individuals

who have less than 10% of normal enzyme activity are at risk of life-threatening haemolysis after as little as one dose of primaquine [259] whereas those with milder variants may have negligible effects [255]. Weekly, as opposed to daily, dosing schedules mitigate primaquine-induced haemolysis [260] whilst retaining efficacy [261] though adherence to such regimens is likely to be poor unless therapy is supervised. In mildly deficient individuals, continuous daily primaquine dosing causes acute but self-limited haemolysis for approximately 10 days followed by reactive reticulocytosis and haematological recovery as the population of old, susceptible, red blood cells is replaced by young erythrocytes [260]. Since this is not an immunological phenomenon, repeat challenge with primaquine after a period of time in such patients causes equally severe haemolysis [94]. In severely deficient patients, haemolysis is progressive and can have a fatal outcome unless primaquine therapy is stopped and blood transfusion given [262,263].

2.7 Consequences of vivax anaemia

The impact of *Plasmodium vivax* infection on haemoglobin concentration varies from negligible to dramatic [49-51,96,101,102]. The clinical consequences of the reduction in haemoglobin depend on the haemoglobin concentration prior to infection. For example, an absolute reduction of 2g/dL would be more likely to have dramatic consequences if the initial haemoglobin was 6g/dL than if it was 12g/dL.

In Papua New Guinea, 1.6% of children under 5 years of age presenting to rural health clinics for treatment of vivax malaria were severely anaemic (haemoglobin <5g/dL) [96]. Across the border in Indonesian Papua, 22% of patients of all ages who were admitted to hospital with vivax malaria fulfilled criteria for severe anaemia [49]. In the D'Entrecasteaux Islands off Papua New Guinea, a cross-sectional survey of children between 0 and 6 years of age showed that the mean haemoglobin in those with *P. vivax* parasitaemia was 8.7g/dL, 0.3g/dL lower

than the equivalent value for those infected with *P. falciparum* [264]. In contrast, on the Thai-Myanmar border, less than 0.2% of patients presenting for treatment of vivax malaria were severely anaemic [51].

Although the spectrum of anaemia seen with vivax infection is reasonably well documented, the clinical, developmental and socioeconomic consequences are largely unknown. Severe anaemia in isolation is associated with a ~2-fold increased risk of death in African children with falciparum malaria and has an even higher mortality when combined with other manifestations of severe disease such as cerebral malaria or respiratory distress [189]. Severe anaemia of any cause in hospitalised children under 5 years has been associated with a case fatality of between 2% and 29.3% [265]. In pregnant women, presence of severe anaemia at delivery has been associated with maternal case fatality rates ranging from <1% to 56% in hospital-based studies [266].

Population-based estimates of mortality in severely anaemic individuals with vivax malaria have not been established but recent studies from Latin America, New Guinea and the Indian subcontinent have identified deaths in patients with severe vivax anaemia [49,50,99,101]. The extent to which the anaemia was responsible for those deaths is unclear.

Anaemia caused by vivax malaria is associated with requirement for blood transfusion [51,102]. Screening of blood products for pathogens is well known to be incomplete in many low and middle income countries and therefore has a significant attendant risk of pathogen transmission [267]. For example, in Sub-Saharan Africa, estimates for the risk of transfusion-associated infection with HIV, hepatitis B and hepatitis C are 1, 4.3 and 2.5 infections per 1,000 units of blood respectively [268].

Pregnant women with haemoglobin concentrations under 8g/dL in Papua New Guinea have been shown to have a 2.4-fold higher risk of delivering a low birth weight baby than non-anaemic mothers [269]. In this study, primigravidae with anaemia and parasitaemia at the time of delivery had the greatest risk of low birth weight [269]. Although vivax malaria is endemic in Papua New Guinea, attribution of these effects specifically to this species is not possible [269]. In Papua, Indonesia, *P. vivax* parasitaemia at delivery has been associated independently with an increased risk of moderate anaemia and a mean reduction in birth weight of 108g [33].

Although evidence is lacking it seems plausible that severe vivax anaemia may reduce resilience to other infectious and non-infectious diseases and therefore may be associated with indirect mortality. In 1938, Swellengrebel and de Buck reported that 62 (7.7%) of a series of 807 patients with syphilis who were treated with induced *P. vivax* infections subsequently died; those with other comorbidities were at particularly great risk [270].

Chronic or repeated episodes of malarial anaemia due to any *Plasmodium* species have been associated with adverse effects on physical and cognitive development as well as school attendance; all of which may be exacerbated by concomitant malnutrition [38,40,271-274]. Again, whether these outcomes are generalisable to vivax malaria, and more specifically the haematological effects of this species, is unknown.

2.8 Conclusions

Haematological morbidity associated with *P. vivax* infection is greatest in young children, especially in tropical countries such as Papua New Guinea and Eastern Indonesia where transmission is intense and local parasite strains relapse very frequently. In these regions, vivax malaria is commonly associated with severe anaemia both in the health care and community setting. The haematological effects of vivax malaria are likely to have complex

interactions with gastrointestinal helminth infection, haemoglobin and red blood cell abnormalities and malnutrition.

Removal of uninfected red blood cells is a particularly important mechanism of anaemia in acute vivax malaria. *Plasmodium vivax*-infected red blood cells are minimally adherent and are more deformable than *P. falciparum*-infected erythrocytes resulting in relatively little red blood cell sequestration in the microvasculature and marrow sinuses and passage of a greater proportion of red cells through the spleen and other reticuloendothelial organs. The role of the spleen in the pathogenesis of vivax anaemia, particularly the removal of uninfected red blood cells, is an important area for future research.

As the global control and elimination of malaria progresses, *P. vivax* is set to become the dominant *Plasmodium* species [83], yet the health, developmental and socioeconomic consequences of vivax malaria and vivax-associated anaemia have received very little attention. Severe vivax anaemia may cause significant morbidity and indirect mortality via association with impaired resilience to infectious and non-infectious comorbidities, obstetric complications and requirement for blood transfusion (with attendant risk of blood-borne pathogen transmission). Early treatment with an efficacious blood schizontocide can reduce the initial fall in haemoglobin associated with vivax infection and thus help to prevent adverse outcomes associated with severe anaemia. Reliable prevention of recurrent haematological insults caused by relapses will require hypnozoitocidal therapy. Primaquine is the only licensed hypnozoitocidal agent available and can exacerbate haemolysis in individuals with G6PD deficiency. Policymakers need to weigh the potential benefits of this drug against the risks based on the local prevalence of this enzymopathy as well as the availability of G6PD testing. Vivax-associated anaemia is an important public health concern that underscores the importance of reducing global transmission of *Plasmodium vivax*.

2.9 Acknowledgements

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3. Artemisinin combination therapy for vivax malaria

3.1 Introduction

Calls for the global elimination of malaria and availability of new funding sources have reinvigorated malaria control programmes. A central theme for these programmes is the development of infrastructure and treatment policy that ensures that all patients with malaria are diagnosed rapidly and have access to highly effective antimalarial drugs. Artemisinin combination therapies effect rapid and sustained parasitological cure in patients with *Plasmodium falciparum* malaria [275] and have been shown to reduce transmission of this species in areas with moderate and low endemicity [89,276-279]. If ACTs can also fulfil their promise of delaying the emergence of further antimalarial resistance [89,278], these effects are likely to be sustained at least in the medium term. Consequently, by 2009, 81 malarious countries had adopted ACTs for first-line treatment of uncomplicated falciparum infection [280].

Outside of Africa, *P. falciparum* almost invariably co-exists with other human *Plasmodium* species. Of these, *Plasmodium vivax* is the most important and is currently transmitted in 95 countries [55]. While vivax malaria is less frequently severe than falciparum malaria, it has

been associated with death [49,50,99,113] and causes substantial morbidity and socioeconomic disruption in endemic regions [33,34,49,83,117,281].

The use of ACTs for first-line treatment of vivax malaria has received comparatively little attention, probably because this is seen as “an expensive and inefficient approach to treating a disease which can be readily treated in most cases with chloroquine” [282]. By 2009, only the Solomon Islands, Vanuatu, Papua New Guinea (PNG) and Papua, Indonesia had adopted a unified ACT-based treatment policy for malaria of any cause [280]. Although laudably targeted, the resultant ‘separate’ treatment scenario for falciparum and vivax malaria in the other co-endemic nations has disadvantages that could potentially hamper global malaria elimination efforts. This review explores the effectiveness of ACTs for vivax malaria and canvasses the relative benefits and disadvantages of the existing ‘separate’ treatment approach versus a ‘unified’ ACT-based strategy for treating both *P. falciparum* and *P. vivax* infections in co-endemic countries.

3.2 Literature search strategy and selection criteria

I searched PubMed, Medline, EMBASE, Global Health and the Cochrane libraries of systematic reviews and randomised controlled trials using the keywords: “vivax” and “artemisinin” or “artemether” or “arteether” or “dihydroartemisinin” or “artesunate” (expanded to all relevant MeSH headings when available) in order to determine the effectiveness of ACTs for vivax malaria and: “vivax” and “chloroquine” and “resistance” to determine the extent of chloroquine resistance. I also searched the Australian and New Zealand, American, United Kingdom and WHO clinical trial registries, the reference lists of relevant articles and asked experts in the field for information on any other relevant published or unpublished research. In cases where articles were written in a language other

than English or I was unable to obtain full text versions, I relied on information from the abstracts. The most recent searches were done in April 2010.

3.3 Considerations on the potential for antimalarial drugs to reduce transmission of *Plasmodium vivax*

Plasmodium vivax has a number of characteristics that make it comparatively refractory to the transmission-blocking effects of blood-stage antimalarials. The first and most important of these is its ability to relapse from dormant liver forms. These relapses help to ensure transmission of the parasite, even in seasonal environments that are hostile to mosquito vectors for much of the year. It remains unclear whether the total number of relapses is predetermined or adaptive – an important distinction that will partially determine the utility of slowly eliminated schizontocidal antimalarials that have the potential to suppress the first, but not subsequent relapses.

As outlined in Chapter 2, primaquine can prevent *P. vivax* relapses if given in sufficient doses but has several shortcomings including the propensity to cause gastrointestinal upset in all individuals and significant haemolysis in those with glucose-6-phosphate dehydrogenase deficiency. Moreover, it is contraindicated in the patients who are at greatest risk of morbidity associated with *P. vivax* infection: namely pregnant women and young children [78]. Adherence to the standard 14-day course is thought to be poor though supportive evidence for this is relatively sparse [283,284].

Mature, infective *Plasmodium vivax* gametocytes appear much earlier in the course of primary or recrudescent infections than *P. falciparum* gametocytes [109,111] with approximately 50-80% [2,285] versus 10-40% [286] of patients having patent

gametocytaemia on presentation respectively. It follows that *P. vivax* is much more likely to be transmitted before treatment can be commenced.

Plasmodium vivax gametocytes are also more efficiently transmitted to mosquitoes than *P. falciparum* [287,288] and once ingested, develop into sporozoites faster than any of the other human *Plasmodium* species [289]. Ostensibly, this would suggest that insecticide-treated bed nets (ITNs) are highly appropriate means of targeting *P. vivax* transmission and indeed this has been shown to be the case in some countries [290]. However in other areas, particularly those with unstable *P. vivax* transmission, studies have shown ITNs to be a relatively poor control mechanism for this species [290,291], possibly because of the greater propensity for vectors of *P. vivax* to bite during daytime hours [291].

In most co-endemic areas, morbidity associated with vivax malaria peaks at a younger age than for falciparum malaria [46,51,118,292,293], a phenomenon that Maitland and colleagues postulate is due to greater ease of transmission and more rapid acquisition of immunity [239]. In these settings, older children and adults with vivax malaria are more likely to be asymptomatic than their falciparum-infected counterparts [1,46]. This inherently limits the comparative transmission-blocking potential of interventions aimed at effective treatment of symptomatic disease.

A small number of studies in regions where *P. vivax* and *P. falciparum* cohabit have shown high rates of *P. vivax* parasitaemia following treatment for falciparum malaria [2,236,252,294]. Indeed in many sites, the force of these recurrences may rival that of *P. falciparum* infection in hyperendemic regions of Africa. Whatever the cause for this phenomenon, these ‘heterologous’ relapses (which are the subject of Chapter 9) intensify local *P. vivax* transmission and may have important clinical implications.

3.4 Artemisinin combination therapies

Artemisinin was first isolated from *Artemisia annua* in 1972 [295]. Its use has now been superseded by other derivatives (notably the water-soluble hemisuccinate artesunate, the lipophilic ester artemether and dihydroartemisinin, their common metabolite). The artemisinin derivatives induce the greatest reduction in parasitaemia per asexual cycle of any of the widely available antimalarials [296]. However, because they are rapidly eliminated, their use as monotherapy is associated with high rates of recrudescence unless 7 or more days of therapy is administered to cover 3-4 asexual cycles [297-299]. Combining the artemisinins with partner drugs that have longer half-lives and different mechanisms of action provides protection against subsequent recrudescence and limits the development of drug resistance [74,75,300]. Over the last decade the role of artemisinin combination therapy has been extensively debated and subsequently endorsed by the WHO as a central component of antimalarial treatment policy. By 2009, 81 countries had changed policy to ACT for uncomplicated falciparum malaria. The most common combinations selected were: artemether+lumefantrine (AM+LUM, n=50), artesunate+amodiaquine (AS+AQ, n=23), artesunate+sulfadoxine+pyrimethamine (AS+SP, n=12) and artesunate+mefloquine (AS+MQ, n=8) [280] (note: total exceeds 81 since some countries use more than one ACT). Four countries had adopted ACTs for the treatment of vivax malaria. The Solomon Islands, Vanuatu and PNG have opted for AM+LUM nationwide and Indonesia has adopted dihydroartemisinin+piperaquine (DHA+PIP) in Papua only [301].

The pharmacokinetic and pharmacodynamic properties of the partner drugs have important implications for the effectiveness and post-treatment prophylaxis provided by the ACTs. Chloroquine and piperaquine have the longest terminal elimination half-lives (1-2 months [302] and 23-28 days respectively [303,304]), followed by amodiaquine (1-3 weeks [302]), mefloquine (~12 days [305]), sulfadoxine (6.7 days [306]), pyrimethamine (3.2 days [306])

and lumefantrine (3.2 days [307]). Of these partner drugs, chloroquine has the greatest intrinsic activity against *P. vivax* and sulfadoxine the lowest [80,296].

3.5 Artemisinin combination therapies for treating *P. vivax* malaria

In areas where *P. vivax* is known to be chloroquine-sensitive, the WHO recommends three days of chloroquine plus two weeks of primaquine (provided the affected individual is not severely G6PD-deficient). Where ACT has been adopted for treatment of falciparum malaria and / or in areas where *P. vivax* is known to be resistant to chloroquine, ACT plus primaquine is seen as an appropriate alternative, with the exception of artesunate+sulfadoxine+pyrimethamine which is regarded as ineffective against *P. vivax* in most areas [78].

3.5.1. Parasitological response

All of the artemisinins and most of the commonly used partner drugs are known to be active against asexual stages of *P. vivax* [308]. Comparing the overall efficacy of these drugs *in vivo*, however, is challenging since it is currently impossible to determine whether recurrent parasitaemia is due to recrudescence, reinfection or relapse [231,232]. The rapidity of parasite and fever clearance is indicative of the intrinsic activity of the artemisinins against *P. vivax* but does not necessarily correlate with the subsequent risk of recrudescence. Since hypnozoites are resistant to all but the 8-aminoquinoline antimalarials, the occurrence of early relapses is predominantly dependent on the elimination half-life of the partner drug rather than the level of schizontocidal activity. The cumulative risk of recurrent parasitaemia within 28-63 days of initial treatment therefore indicates the degree of post-treatment prophylaxis provided. All of these indices of treatment efficacy are dependent on pre-existing levels of parasite resistance and acquired immunity.

My literature search revealed 11 published studies of varying design that specifically report on the efficacy or effectiveness of one or more combinations of an artemisinin derivative plus a blood schizontocide for the treatment of *P. vivax* malaria (Table 3.1) [2,3,252,285,303,309-315]. Ten out of 11 of these studies were from Asia and 5 were from the island of New Guinea. The most commonly investigated combinations were DHA+PIP (6 studies), AM+LUM (4 studies) and AS+SP (3 studies). Since the literature review for this chapter was conducted there have been two further randomised trials of ACT in *P. vivax* malaria published (included in red in Table 3.1 below but not included in the subsequent discussion). The first of these was a multi-country study demonstrating non-inferiority of artesunate+pyronaridine compared with chloroquine for uncomplicated vivax malaria (cumulative risks of *P. vivax* recurrence by day 42 were 4.5% and 7.9% respectively in the per-protocol populations, $p>0.05$) [245]. The second study from Northwestern Thailand compared DHA+PIP with chloroquine for uncomplicated vivax malaria. At day 63 following treatment, the cumulative risk of *P. vivax* recurrence in the DHA+PIP group was 54.9% versus 79.1% in the chloroquine group ($p<0.001$) [316]. The results of these two recent studies do not change the conclusions of this review.

Table 3.1 Studies of the effectiveness of an artemisinin derivative combined with a blood schizontocide for the treatment of *Plasmodium vivax* malaria

First Author	Year	Location	Study design	Drug (days)	n	PCT	FCT	Prop. free of recurrence				
								Day 14	Day 28	Day 42	Day 56	Day 63
Li et al. [309]*	1999	China	Efficacy study, not otherwise specified	AM (3) + LUM (3) (high dose)	36	33.5 h	22.3 h					
				AM (3) + LUM (3) (low dose)	41	30.5 h	23.2 h					
				CQ + PIP†	55	44.9 h	25.0 h					

LeYuan et al. [310]*	2001	Eritrea	Efficacy study, not otherwise specified	DHA† + PY†	?	24.0 h			
				PY†	?	32.0 h			
Tjitra et al. [311]	2002	Papua, Indonesia	Non-randomised, pilot efficacy study	AS (3) + SP (1)	22	1.1 d	1.4 d	100%	89.5%
				CQ (3) + SP (1)	6			67%	
				CQ (3)	9			11%	
Hung et al. [303] & Karunajeewa et al. [312]	2004	Cambodia	Non-randomised, population PK and safety evaluation	DHA (2) + PIP (2)	10	12 h		100%	
Hasugian et al. [3]	2007	Papua, Indonesia	Open-label, randomised controlled trial	DHA (3) + PIP (3) + PQ (14)	74			84%	
				AS (3) + AQ (3) + PQ (14)	75			52%	
Kolaczinski et al. [313]	2007	Afghanistan	Open-label, randomised controlled non-inferiority trial	AS (3) + SP (1)	94			99%	76%
				CQ (3)	96			96%	54%
Krudsood et al. [314]	2007	Bangkok, Thailand	Open-label, randomised controlled trial	AM (3) + LUM (3) + PQ (14)	47	41.6 h	21.8 h	97.4%	
				CQ (3) + PQ (14)	51	55.8 h	25.3 h	100%	
Ratcliff et al. [2]	2007	Papua, Indonesia	Open-label, randomised controlled trial	DHA (3) + PIP (3) + PQ (14)‡	147			86%	
				AM (3) + LUM (3) + PQ (14)‡	141			43%	
Karunajeewa et al. [315]	2008	Papua New Guinea	Open-label, randomised population PK and efficacy trial	DHA (3) + PIP (3)	3			66.7%	
				CQ (3) + SP (3)	1¶				
Karunajeewa et al. [252]	2008	Papua New Guinea	Open-label, randomised controlled trial	AM (3) + LUM (3)	39	1.4 d	2.1 d	48.5%	30.3%
				DHA (3) + PIP (3)	44	1.2 d	1.9 d	84.2%	69.4%

			AS (3) + SP (1)	51	1.1 d	2.1 d	51.3%	33.3%
			CQ (3) + SP (1)	61	3.1 d	2.3 d	51.0%	13.0%
Awab et al. [285]	2010	Afghanistan	Open-label, randomised controlled trial	CQ (3)	268		100%	91.1%
				DHA (3) + PIP (3)	268		100%	97.2%
Poravuth et al [245]	2011	Cambodia, Thailand, India, Indonesia	Double-blind, randomised, non-inferiority trial	AS (3) + PY (3)	228	23.0 h 15.9 h	99.5% [§] 97.1% [§]	95.5% [§]
				CQ (3)	228	32.0 h 23.8 h	100% [§] 98.0% [§]	92.1% [§]
Phyo et al [4]	2011	Thailand	Open-label, randomised controlled trial	DHA(3) +PIP (3)	250		95.9%	45.1%
				CQ (3)	250		90.2%	20.9%

Abbreviations: PK; pharmacokinetics, DHA; dihydroartemisinin, PY; pyronaridine, AS; artesunate, SP; sulfadoxine+pyrimethamine, CQ; chloroquine, PIP; piperazine, PQ; primaquine, AQ; amodiaquine, AM; artemether, LUM; lumefantrine, n; number, PCT; parasite clearance time, FCT; fever clearance time. *, assessment based on abstract alone, †; unknown duration, ‡; primaquine delayed until day 28, ¶; lost to follow-up, §; according to per-protocol analyses. Excludes studies of artemisinin derivatives plus primaquine since the latter has no activity against asexual *P. falciparum* parasites and is therefore not an option as the sole partner drug for widespread use against both species. Studies by Ratcliff, Hasugian and Karunajeewa (2008) included patients with *P. vivax* and mixed *P. vivax* / *P. falciparum* infections in their analyses of *P. vivax* recurrence. Table reproduced with permission from Douglas NM. Artemisinin combination therapy for vivax malaria. *Lancet Infect Dis* 2010; **10**: 405-16.

The studies in Table 3.1 show a shorter time to parasite clearance in patients receiving ACTs (median parasite clearance time (PCT) = 28.8h, range 12–41.6h) compared to chloroquine-based monotherapy or non-ACT combination therapies (median PCT = 50.4h, range 32–74.4h). Only three studies reported fever clearance times for non-ACT regimens. In all cases these were longer than the corresponding times for the ACT regimens.

In support of these findings, clinical studies have shown that vivax malaria patients treated with an artemisinin derivative plus primaquine [317-321] or an artemisinin derivative alone [308,322-324] have faster parasite clearance times (median PCT in these studies = 37.2h, range 14.2–50h) than patients treated with chloroquine ± primaquine (median PCT = 53.5h, range 24.0–65h). Artesunate and artemether also have significantly higher *P. vivax* parasite reduction ratios than chloroquine (844, 508 and 36 respectively) [308].

Where local parasite strains are completely sensitive, chloroquine provides good post-treatment prophylaxis against the first and possibly even second liver-stage relapse; a feature attributable to its very long terminal elimination half-life. Nevertheless the studies in Table 3.1 show that beyond two weeks, the proportion of individuals who remained free of *P. vivax* parasitaemia after ACT treatment was at least as high, if not higher than for the individuals treated with chloroquine. This probably either reflects a degree of chloroquine resistance in the study areas or comparison with one of the longer-acting ACTs. Of the ACTs, DHA+PIP has the longest half-life and correspondingly was shown to be particularly effective at preventing *P. vivax* relapse up to as many as 56 days following initial treatment [2,252,285]. In separate studies, artesunate+mefloquine has also provided good protection against *P. vivax* parasitaemia up to 63 days following mixed [325] or *P. falciparum* [326] infections [327]. The shorter half-life combinations such as artemether+lumefantrine, although equally effective at rapidly reducing the parasite biomass, provide comparatively little cover against early relapses.

3.5.2. Effects on the emergence and spread of parasite resistance

Whereas chloroquine-resistant (CQR) *P. falciparum* was first documented over 50 years ago, resistant strains of *P. vivax* have taken much longer to emerge. Several factors are likely to have contributed to this disparity. Firstly, *P. vivax* gametocytes appear earlier in the course of disease and are more susceptible to blood schizontocides than the gametocytes of *P.*

falciparum [328]. This results in a greater likelihood of transmission prior to drug exposure. Secondly, adults with *P. vivax* infections are more likely to be asymptomatic than those with *falciparum* malaria leading to less antimalarial drug usage and therefore less selective pressure for resistance-conferring mutations. Thirdly, *P. vivax* reaches lower blood densities than *P. falciparum* and therefore there is a statistically smaller chance of *de novo* resistance-conferring mutations arising and being propagated [74,75].

The first cases of CQR *P. vivax* were documented in Australian soldiers repatriated from Papua New Guinea in 1989 [329]. Since then, reports of chloroquine resistance have been published from throughout the *vivax*-endemic world (Figure 3.1). Although some of this apparent spread is likely to be attributable to increased recognition and therefore greater reporting of the problem, this cannot explain the increasing degree of resistance in many places. In Papua, Eastern Indonesia, the proportion of chloroquine-resistant parasites is between 64 and 84% [330-334]. Failure rates at day 28 exceeding 10% have also been reported from other parts of Indonesia [335], Papua New Guinea [252], India [336], Myanmar [337], Turkey [338] and Madagascar [339]. Elsewhere, resistance has been described but generally falls below 5% [311,313,322,340-355]. With continued use of chloroquine in these regions, the situation is likely to deteriorate.

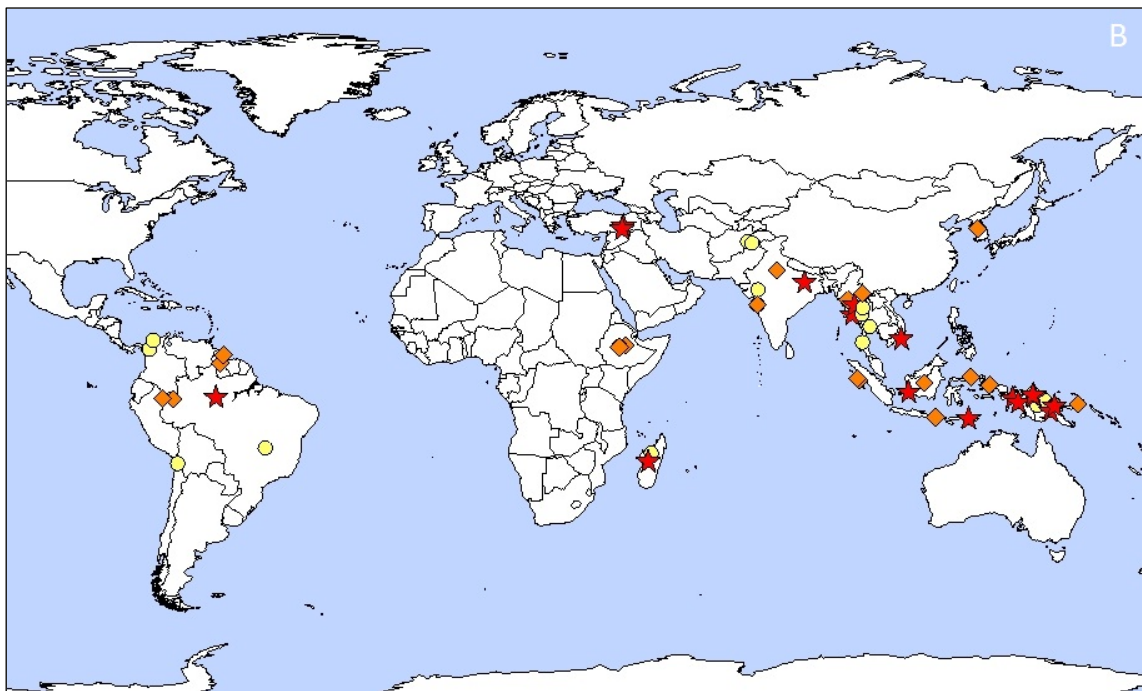
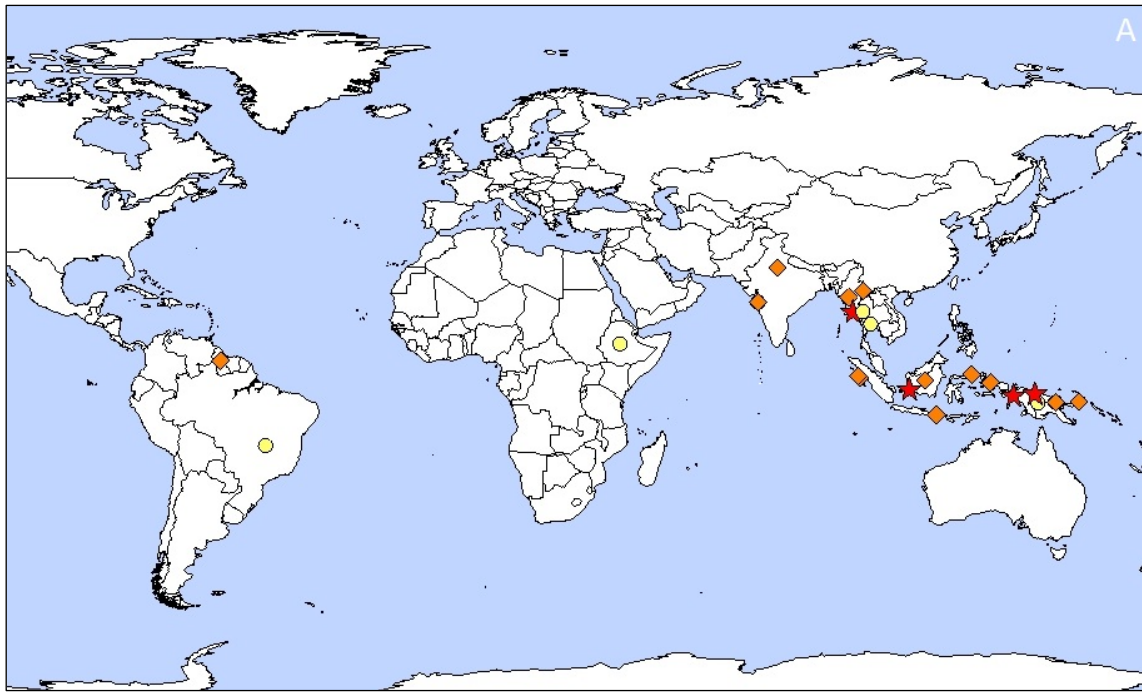


Figure 3.1 Reports of chloroquine-resistant *Plasmodium vivax* by 1999 (a) and 2009 (b). Red stars = >10% recurrence (and greater than 5 absolute failures) by day 28 with or without chloroquine levels; orange diamonds = <10% recurrence (or fewer than 5 absolute failures) by day 28, with chloroquine levels; yellow circles = <10% recurrence (or fewer than 5 absolute failures) by day 28, without

chloroquine levels. Modified with permission from Douglas NM, et al. Artemisinin combination therapy for vivax malaria. *Lancet Infect Dis* 2010; **10**: 405-16.

Various ACTs have been shown to be effective against highly chloroquine-resistant strains of *P. vivax* [2,3,252]. In line with current rationale for ACTs in falciparum malaria, the protection afforded by combining drugs with different mechanisms of action and the very rapid reduction in parasite biomass induced by the artemisinins suggests that the ongoing efficacy of the artemisinin component is likely to be more assured than the ongoing efficacy of chloroquine. However empirical evidence supporting this is lacking. Conversely, slowly eliminated partner drugs, such as piperaquine, may be comparatively prone to the development of *P. vivax* resistance since they are more likely to be present at low levels in the bloodstream at the time of the first, and possibly even second, relapse long after any therapeutic trace of the artemisinin derivative has been eliminated. Since asexual relapses are frequently associated with concurrent gametocytaemia [2], partially resistant parasites that break through low concentrations of the partner drug will have a selective transmission advantage.

The ongoing efficacy of the artemisinins against *P. vivax* would require their exclusive use in combination with effective partner drugs. There would also need to be sufficient monitoring in place to enable early detection of resistance and thus a timely change of partner drug before there was any threat to the artemisinin. These major operational concerns apply for the entire malarious world, not just countries with co-endemicity.

3.5.3. Transmission-blocking potential

Malaria is transmitted between humans by the female *Anopheles* mosquito which must first ingest *Plasmodium* gametocytes from an infected host. Factors determining the likelihood of this event include the duration an individual has viable gametocytes in the peripheral

circulation, the level of gametocytaemia and the infectiousness of the gametocytes to the local anopheline vectors. The ACTs prevent or decrease the risk of infectious *P. falciparum* gametocytaemia by rapidly reducing the biomass of precursor asexual forms, killing immature gametocytes and minimising the risk of recrudescence [279,356].

Even in regions where chloroquine retains high efficacy, treatment of *P. vivax* with an artemisinin-containing regimen results in faster reduction of gametocyte biomass. In Bangkok, the median duration of gametocytaemia in hospitalised patients treated with artesunate was significantly shorter than patients treated with chloroquine (24 hours, range 0-96 hours versus 24 hours, range 0-264 hours respectively, $p=0.005$) [357]. However, such rapid clearance is of relatively minor transmission-blocking benefit given that gametocytes are likely to have appeared and been transmitted prior to symptom onset. Since most ACTs are eliminated faster than chloroquine, there is a theoretical potential for the shorter duration of post-treatment prophylaxis to lead to greater recurrence and associated gametocytaemia [2]. However, in Afghanistan, where *P. vivax* retains susceptibility to chloroquine, the slowly eliminated combination DHA+PIP was associated with fewer asexual recurrences by day 63 than chloroquine, even though both regimens were associated with 100% cure at 28 days [285].

As chloroquine resistance emerges, the duration of post-treatment protection against relapse or reinfection will decline (as demonstrated in Table 3.1) and recrudescences will become more frequent [279,356]. Introduction of ACTs for the treatment of vivax malaria in these circumstances should lead to the full range of potential transmission-blocking benefits including more rapid gametocyte clearance, fewer recrudescences and greater post-treatment prophylaxis; the latter probably only being significant for combinations with slowly eliminated partner drugs. In Southern Papua, an area with relatively high *P. vivax*

transmission intensity, gametocyte carriage by day 42 was almost 7 fold lower in those treated with DHA+PIP compared to the more rapidly eliminated combination artemether+lumefantrine [2].

It should be noted however, that it is still not known whether suppressing the first relapse will reduce the total number of relapses from a particular parasite strain or will simply delay their onset. The effect that a long period of post-treatment prophylaxis has on limiting transmission remains uncertain.

3.6 Separate versus unified treatment approach

The artemisinin derivatives are clearly highly active against *P. vivax* and, if coupled with certain other blood schizontocides, may have advantages over chloroquine for this species. But should a unified ACT-based protocol replace the “separate” treatment approach used in most co-endemic nations? Policy-makers must weigh-up wide-ranging malariometric, operational and economic factors.

3.6.1. Malariometric considerations

Perhaps the greatest potential compromise associated with instituting a unified ACT-based treatment strategy is the use of a combination that is unequally effective against the different *Plasmodium* species. Artemisinin combination therapies are assumed to be effective against infections by *P. malariae* and the blood stages of *P. ovale*, though confirmatory data are sparse [358,359] and the relative advantages and disadvantages of the different combinations are unknown. The slowly eliminated combination dihydroartemisinin+piperaquine has been shown to be particularly effective for vivax infections, inducing rapid reduction in parasitaemia and high rates of parasitological cure at 42 days [2,3,252]. Given that mefloquine and pyronaridine have long elimination half-lives and good activity against

chloroquine-resistant *Plasmodium* species [360,361], ACTs containing these antimalarials are likely to have similar pharmacodynamic advantages.

Globally, artemether+lumefantrine is the most widely used artemisinin combination for malaria and has been heavily subsidised by various international funding agencies. Although AM+LUM is a good option for falciparum malaria, it provides comparatively little post-treatment prophylaxis against *P. vivax* relapse and is thus unlikely to be the drug of choice for this species (recurrence rates for AM+LUM at day 42 in studies from Papua and PNG were 57% and 70% versus 14% and 31% for DHA+PIP) [2,252]. However, if antirelapse treatment can be combined with ACTs in a reliable, safe and effective way, then the superior efficacy against *P. vivax* afforded by the longer-acting combinations would be limited to a reduction in the rate of post-treatment reinfection which, in most vivax endemic regions, is relatively low. Of course, any unified ACT-based strategy would be contingent on the continued effectiveness of these combinations for falciparum malaria – a prerequisite that now seems less assured than previously thought [362,363].

The activity of primaquine against *P. vivax* hypnozoites is potentiated by co-administration of blood schizontocides [364]. A small study of *P. cynomolgi* in Rhesus monkeys suggested that chloroquine may be better than quinine in this regard [365]. In humans, however, chloroquine and quinine appear to be equally and highly efficacious at preventing relapse when given concurrently with primaquine for the treatment of fully drug-sensitive parasites [364]. The activity of the ACTs in combination with primaquine is unknown and therefore there is a potential that their introduction for treatment of vivax malaria in conjunction with primaquine antirelapse therapy could lead to a relative reduction in relapse prevention. However, the only 8-aminoquinoline-blood schizontocide combination administered concurrently that has not shown good efficacy at preventing relapse is pentaquine plus

chlorguanide, an unsurprising observation given the relatively poor activity of antifolates against *P. vivax* [365]. In view of the excellent blood schizontocidal activity of the artemisinins and partner drugs such as piperaquine and lumefantrine, lack of synergy with primaquine seems unlikely, but confirmatory studies are warranted.

Inflammation plays an important role in the pathogenesis of *P. vivax* infection and may be responsible for some of the manifestations of severe disease such as acute lung injury [106,158,366]. Since chloroquine has anti-inflammatory activity, it has been hypothesised that its use might ameliorate the development of these manifestations – an effect that could be lost if chloroquine was replaced by an ACT [158].

Continued use of chloroquine rather than ACTs for the treatment of vivax malaria also has hypothetical disadvantages. Perhaps the greatest of these relates to the emergence and spread of chloroquine resistance. Diagnosis of declining drug efficacy in *P. vivax* malaria is difficult and therefore low-grade resistance often goes unnoticed. Sufficient studies have been done, however, to show that chloroquine resistance is both more widespread and severe than previously recognised (see Figure 3.1). If chloroquine remains the mainstay of treatment for vivax malaria, not only will it continue to be deployed in areas where its efficacy is declining, it is likely to gradually propagate the emergence and spread of further chloroquine resistance.

The ‘separate’ treatment approach leads to inadvertent use of chloroquine for *P. falciparum* infections. Field microscopy results in substantial species mis-identification and under-diagnosis of mixed parasitaemia [49,367,368]. On the Thai-Myanmar border, 11% of *P. vivax* monoinfections diagnosed by field microscopy were actually found to be *P. falciparum* or mixed species infections on cross-checking [368]. Furthermore, even if microscopic diagnosis of *P. vivax* is correct, subpatent coinfection with *P. falciparum* is relatively common [237,369]. New generation rapid diagnostic tests can distinguish *P. falciparum* from *P. vivax*

but the sensitivity and specificity of these tests is often poor [73]. Therefore, in routine practice in co-endemic regions, a significant proportion of patients with *P. falciparum* infections are likely to be treated with chloroquine alone. Since this drug is partially or completely ineffective against falciparum malaria in most parts of the world, its inadvertent use will result in increased transmission and morbidity from this species, as well as a greater risk of progression to severe disease or death.

Continued use of separate treatment strategies may exert unwanted selective pressure on *P. vivax* parasites, especially for drugs with long half-lives. In Thailand, use of mefloquine for falciparum malaria (either alone or in combination with artesunate) has led to an increased prevalence of *P. vivax* isolates with *pvm-dr1* amplification - a molecular marker associated with increased resistance to mefloquine [370,371]. Selection for the *pvdhfr* and *pvdhps* resistance-conferring mutations has also been observed following antifolate exposure in Thailand [371], Papua, Indonesia [311] and Madagascar [372]. These observations highlight that use of antimalarial drugs specifically for *P. falciparum* infection may limit their future utility against *P. vivax*.

One of the major rationales for artemisinin combination therapies is their potential to delay the emergence of *de novo* parasite resistance [74]. Once resistance has emerged, however, combinations of pharmacokinetically mismatched drugs will still be vulnerable to selective transmission of resistant parasites [373]. Mathematical models have shown that simultaneously deploying multiple first-line antimalarials may retard the emergence and fixation of drug resistant *P. falciparum* by decreasing total parasite exposure to a single agent [374]. However, these models assume concurrent use of highly effective drugs and therefore would not necessarily apply to inadvertent exposure to chloroquine in areas where

chloroquine resistance is already present. Similar multi-treatment strategies have yet to be investigated for *P. vivax*.

3.6.2. Operational considerations

One of the greatest challenges for the malarious world is getting the right drugs to all of the people that need them at the right time. In most endemic areas, a high proportion of patients will seek treatment in the private or informal sector in the first instance [1,375,376]. Since diagnosis of malaria in such settings is usually based on clinical symptoms alone, it is critically important that the drugs prescribed at these facilities are effective against all local species of *Plasmodium*. Continued use of chloroquine in public health care systems could hypothetically sustain the use of chloroquine in the private sector through the legitimisation of its use and potentially also through shared supply channels.

Overall, a unified treatment strategy would be easier for health care providers to implement, would not be dependent on correct parasite species identification and might have a greater chance of being adopted in the private sector. Drug resistance monitoring and antimalarial supply chains could be simplified and patients might develop a greater expectation of receiving the most effective drug. However, there is also a potential that a unified treatment strategy would decrease the impetus for health care providers to set up and implement parasitological testing. This might result in a greater proportion of aparasitaemic patients receiving antimalarial medications with associated implications for the development of ACT resistance, misdiagnosis of other febrile illnesses and reduced cost-effectiveness. Furthermore, since species identification is necessary for targeting primaquine therapy, it could reduce the likelihood that patients with vivax malaria receive this critically important drug.

3.6.3. Economic considerations

Chloroquine is a cheap and widely available drug whereas ACTs are considerably more expensive, even with subsidy, and are limited by supply issues. Table 3.2 shows current estimates for the purchase price of full co-packaged adult courses of various ACTs compared with chloroquine [377]. The additional global cost associated with using DHA+PIP or AS+AQ as opposed to chloroquine for the treatment of vivax malaria can be estimated to be between 60 and 364 million US dollars per year (Table 3.2). It must be noted that these figures do not account for any potential cost-savings associated with the use of ACTs, such as reductions in the number of recurrent *P. vivax* infections requiring retreatment, decreases in the overall incidence of vivax malaria and a reduction in the number of recrudescence, severe and fatal cases of falciparum malaria arising due to inappropriate use of chloroquine. With worsening chloroquine resistance throughout the world, these potential savings are likely to become more significant with time.

Table 3.2 Costs of artemisinin combination therapies compared with chloroquine, 2008

Drug	Total dose for full adult course (60kg)	Minimum cost per full adult course (US\$)	Additional purchase cost per course (US\$)	Additional global purchase cost per year* (US\$)
Artemether+lumefantrine	120mg/720mg	1.474	1.405	98-549 million
Artesunate+amodiaquine	600mg/1836mg	0.918	0.849	60-332 million
Artesunate+mefloquine	600mg/1500mg	3.85	3.781	0.26-1.5 billion
Artesunate+sulfadoxine+pyrimethamine	600mg/2000mg/100mg	1.38	1.311	92-513 million
Dihydroartemisinin+piperazine†	135mg/1080mg	1.00	0.931	65-364 million
Chloroquine	1500mg	0.069	-	-

* Assumes: a) there are 70-435 million *P. vivax* infections per year, b) all of these infections are treated, c) adherence to World Health Organization dose recommendations and d) the average total dose administered is 2/3rds of a full adult dose.

† As DHA+PIP is not yet manufactured according to International Good Manufacturing Practice standards, the cost in this table is conservatively set at one US dollar per treatment course based on a predicted public sector price of “less than one US dollar in adults and less than 0.5 US dollars in children” (Duparc, Medicines for Malaria Venture, personal communication). Table reproduced with permission from Douglas NM, et al. Artemisinin combination therapy for vivax malaria. *Lancet Infect Dis* 2010; **10**: 405-16.

In addition to savings associated with a reduction in the burden of malaria, a unified treatment strategy would streamline antimalarial procurement and distribution systems and provide greater impetus for drug companies to reduce ACT manufacturing costs. These potential savings are unavoidably speculative since to date there have been no comprehensive cost comparisons or cost-effectiveness analyses of the use of ACTs versus chloroquine for vivax malaria.

3.7 Conclusions

Several artemisinin combination therapies have shown excellent efficacy against asexual and sexual stages of both chloroquine sensitive and resistant *P. vivax*. Where chloroquine resistance has emerged, slowly eliminated ACTs such as dihydroartemisinin+piperaquine and artesunate+mefloquine will provide greater post-treatment prophylaxis against early recurrence of infection. This advantage will become more pronounced as chloroquine resistance increases.

In areas of established high-grade *P. vivax* chloroquine resistance, such as across the island of New Guinea, policymakers are already implementing unified ACT-based treatment policy.

In regions of low-grade resistance and where *P. vivax* retains susceptibility to chloroquine, the best treatment strategy is less obvious and the relative malariometric, operational and economic costs and benefits of ACTs versus chloroquine need to be compared. ‘Separate’ treatment protocols for the two species in such areas may be justifiable if diagnostic tests reliably distinguish *P. vivax* from chloroquine-resistant *P. falciparum*. However, with the relatively high frequency of misdiagnosis in routine practice and the rise of chloroquine-resistant *P. vivax*, there may be a compelling rationale for a unified ACT-based strategy for both species in all co-endemic settings. To date, consideration of the use of ACTs for vivax malaria has been stifled by the supposedly prohibitive additional expense this would imply. This view is based on assumption rather than scientific evidence and overlooks the potential malariometric advantages of ACTs, their falling cost and the operational efficiencies of a pragmatic, unified ACT-based treatment protocol. The global burden of *P. vivax* and its unique biological characteristics remain a major hurdle to the goal of malaria elimination. Studies of the cost-effectiveness of unified ACT-based strategies for malaria treatment should be prioritised to assess the role of ACTs in vivax malaria control and elimination efforts.

3.8 Acknowledgements

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4. Background and methods

Most of the research described in this thesis was conducted in the municipality of Timika in South-central Papua, Indonesia. This chapter provides a description of the geography and social characteristics of the region followed by an introduction to the local epidemiology of malaria. It then outlines past and ongoing malaria research activities relevant to this thesis. Proceeding chapters will refer back to this introduction and add extra methodological detail where required. Two chapters also include data from the Shoklo Malaria Research Unit in Northwestern Thailand. This region will be described in the relevant research chapters only.

4.1 Site description

4.1.1. Geography

Papua (formerly Irian Jaya) is the eastern-most province of Indonesia, lying directly to the west of Papua New Guinea and to the east of West Papua Province. Mimika District is in South-central Papua and covers a land area of 21,522km². It encompasses extensive lowland, tropical rainforest and estuarine coastal regions (Figure 4.1). The northern aspect of the district is formed by the Sudirman mountain range which runs east to west, reaching an altitude of 4,884m at the summit of Puncak Jaya (Carstensz Pyramid).

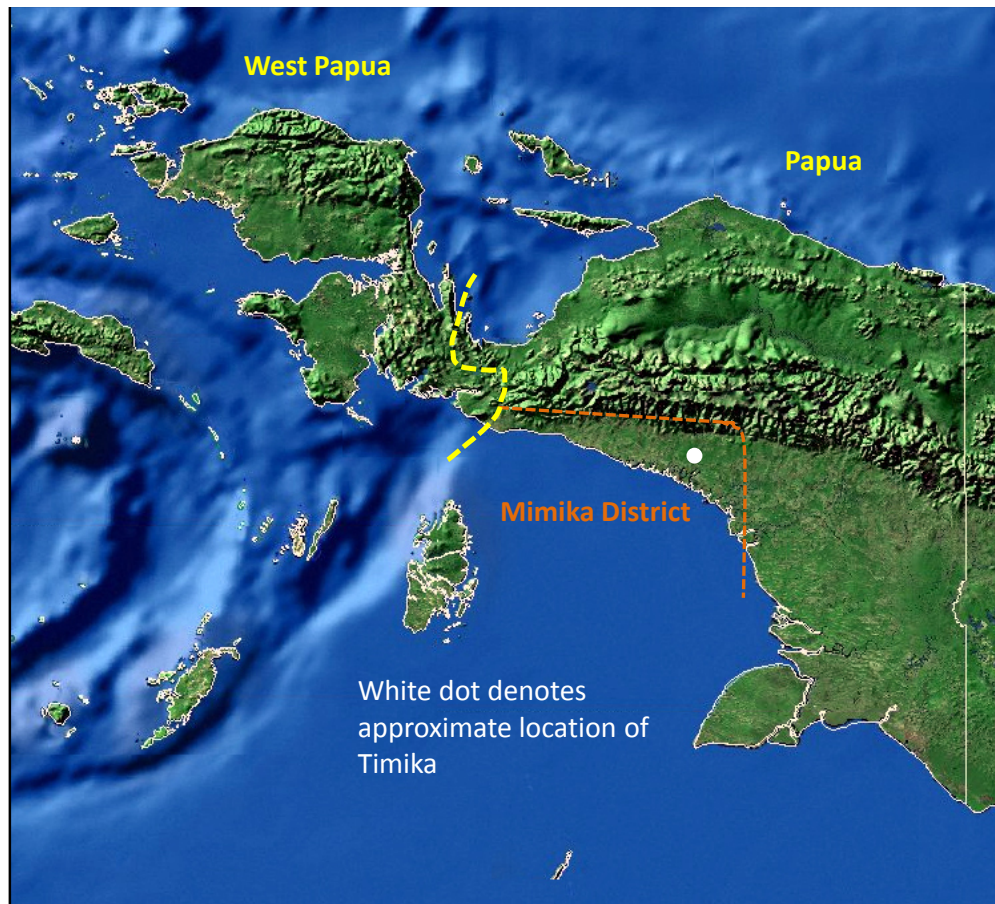


Figure 4.1 Satellite image of Papua showing the approximate boundary of Mimika District and location of Timika.

Timika is by far the most populous municipality in Mimika District and is situated close to the banks of the Mimika River amongst dense, lowland rainforest. Other much smaller settlements are scattered throughout the district, most of which are close to the southern coast or in the highlands near the Grasberg mine operated by PT Freeport Indonesia. Timika itself is composed of a central administrative and business region with surrounding, geographically discrete, communities (Figure 4.2). Many of these communities were formed to accommodate Indigenous Papuans displaced by the formation of the mine approximately 50km to the north or government sponsored transmigrants from elsewhere in Indonesia.

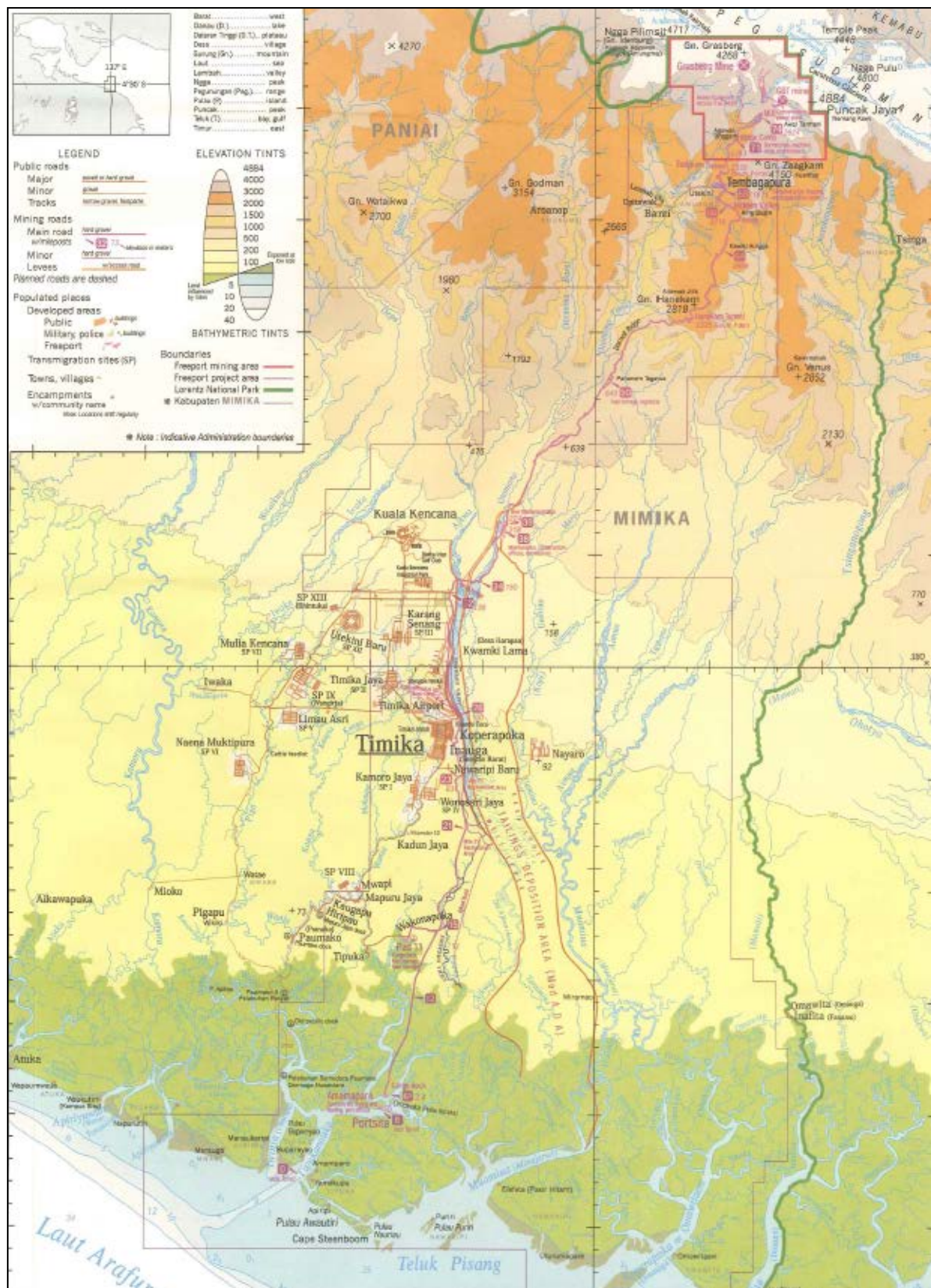


Figure 4.2 Map showing municipality of Timika (courtesy of Dr Michael Bangs, PT Freeport Indonesia)

4.1.2. Climate

The climate in Mimika District varies widely depending on proximity to the ocean and altitude but has little seasonal variability (Figure 4.3). Rainfall in the lowlands is approximately 5,000mm/year and tends to occur in the afternoons and evenings following evaporation of water from sodden, hygroscopic rainforest earlier in the day. Mean lowland maximum and minimum temperatures in January, the hottest month, are approximately 32°C and 23°C respectively and in July, the coldest month, are 29°C and 22°C respectively. Mean lowland humidity is approximately 89%, again with little seasonal variation.

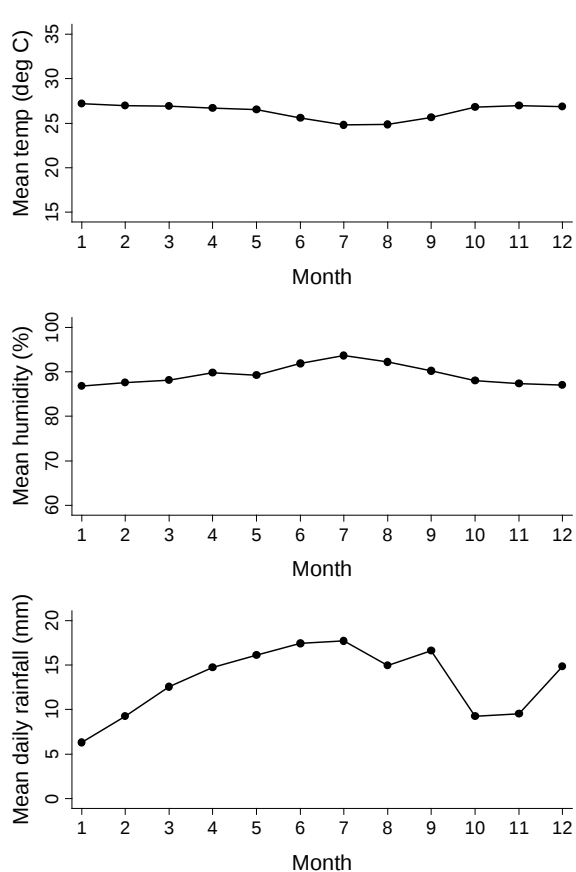


Figure 4.3 Mean temperature, humidity and daily rainfall by month in Timika

4.1.3. Recent political history

In 1848, the Dutch annexed Papua¹ to their already significant Indies empire [378]. Despite having administrative rule of the region, they had little presence there. In 1942, during the Second World War, the Dutch lost control of Papua and the rest of Indonesia-to-be to the invading Japanese who used Papua as a strategic point from which to launch attacks on Australia. Dutch control of the Indonesian archipelago was temporarily regained following Japan's surrender but only with significant assistance from the British military. By this time, there was a strong independence movement spearheaded by General Sukarno's nationalists. Indonesian independence was declared in 1949 but the Dutch negotiated a separate transfer of sovereignty for Papua [379]. After an interim United Nations administration, Papua was handed to Indonesian rule in 1963.

Indonesian colonisation of Papua began in earnest during the 1960s and continued apace throughout the rest of the century. In 1967, Indonesia signed a contract with Freeport McMoRan to exploit a copper belt known as Ertzberg in the Carstensz Massif. Mining activity officially began in 1973 and increased dramatically in scale in 1988 with the discovery of the enormous Grasberg copper and gold deposit just 3km from the Ertzberg site. Commencement of mining activities provided a major economic stimulus attracting Indonesian transmigrants as well as expatriate mine workers and their families – the latter residing in geographically isolated, purpose-built settlements in the mine's operational territory. In 2003, Papua was divided into two provinces, Papua and West Papua, each with “Special Autonomy” allowing extended powers of self-government.

¹ In this section, the regions now encompassed by Papua and West Papua Provinces are referred to collectively as Papua. Since 1848, this area has had many name changes.

The legitimacy, motives and fairness of many of the aforementioned political machinations have been, and continue to be, contested [378,380]. As a result, Papua remains a volatile region with sporadic outbursts of politically-motivated violence and a strong Papuan freedom movement [381].

4.1.4. People

The original settlers of New Guinea arrived from Asia at least 40,000 years ago at a time when New Guinea was joined to Australia. These people subsequently diversified and disbanded to become the Aboriginal people of Australia and the tribespeople of New Guinea [380]. Within New Guinea, culture, language, diet and lifestyle diversified depending on geographical location. Highland tribes, such as the Amungme and Dani of Papua, tended to be shifting cultivators of sweet potato and pandanus with a heavy reliance on pig farming whereas lowland tribes, such as the Kamoro, tended to be riverine dwellers with subsistence systems based on fishing and sago cultivation. Historically, different Papuan tribes had relatively little interaction with each other, except at times of war.

The demographics of Mimika District, and in particular Timika, have been irrevocably changed by mining activities and the influx of Indonesian transmigrants. Since the 1970s, highland and lowland tribespeople that have either been displaced by the mine activities or attracted to urban and economic opportunities have come to be living in close proximity to each other.

The population of Timika has swelled from 3,000 in 1967 to 130,000 in 2004 to an estimated 170,000 in 2007, the highest rate of growth of any urban area in Indonesia [382]. In 2005, 28% of the population was estimated to be Highland Papuan, 26% Lowland Papuan and 45% of non-Papuan origin, the vast majority of whom were Indonesians from other provinces [1].

4.1.5. Health care facilities

Health care in Mimika District is variously provided by the Freeport mine (or its contractors), the Indonesian Government and myriad private practitioners. The mine contracts the International SOS to provide primary health care services to its employees in various mining settlements as well as to maintain a small hospital in Tembagapura (translating to 'Copper Town') accessible to mine employees and their families only. In addition, Freeport mine has its own Public Health Malaria Control programme which is charged with the responsibility for primary health care in various communities throughout Timika and for malaria control efforts in those regions. The mine funds Rumah Sakit Mitra Masyarakat (RSMM) which, until 2009, was the only referral hospital in the district and remains the primary base for our research activities.

The Indonesian Ministry of Health (Dinas Kesehatan) funds and administers primary care clinics in most of the remaining communities in Timika and is responsible for malaria control activities in these regions. From November 2009, it has also been responsible for Rumah Sakit Umum Daerah, the second referral hospital in Timika. Patients in Timika may also seek diagnostic and treatment services at various private institutions ranging from regulated specialist doctors' practices and pharmacies to unregulated shop owners and spirit healers. A survey in 2005 showed that 60% of patients with malaria sought treatment in the private sector in the first instance and that 32% of deaths in the community occurred at RSMM [1].

Reliable malaria microscopy in the region is usually available at all International SOS and Public Health Malaria Control clinics, both hospitals and at the larger of the Dinas Kesehatan clinics. Rapid diagnostic tests are now used as the primary means of malaria diagnosis at the smaller Dinas Kesehatan clinics and occasionally as a supportive diagnostic tool at the two hospitals and some of the larger clinics.

Health care at Rumah Sakit Mitra Masyarakat is available to all, free of charge for Papuans belonging to the 7 tribes most severely affected by mining operations and at a small cost for others. The hospital has 110 inpatient beds, a high dependency unit with facilities for monitoring and intravenous infusions but not mechanical ventilation, a 24-hour emergency department and an active “polyclinic” that sees over 300 patients per day, 6 days per week. Radiological services at the hospital include X-ray and limited access to ultrasound. Laboratory facilities are also available for haematological and biochemical analysis, malaria microscopy and basic serology. Microbiological culture and testing of blood and other biological fluids is not available. The hospital is staffed by consultant specialist doctors, junior house doctors and nurses. A limited range of medicines is available as is typical in resource poor settings.

4.1.6. General health indicators

The disease burden in Papua is typical of a region in stage one of the epidemiologic transition with high rates of infectious disease and malnutrition. The maternal mortality rate is estimated to be 1,145 deaths per 100,000 live births and the infant mortality rate is 68 deaths per 1,000 live births [383]. The reported seroprevalence of HIV in individuals between the ages of 15 and 49 years was 2.4% [384] in 2006 and in 2008-9, 13% of patients newly diagnosed with tuberculosis in Timika had HIV coinfection [385].

4.1.7. Malaria epidemiology

Four of the five *Plasmodium* species that infect humans are known to be endemic in Papua; *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* [1,386]. Transmission is most intense in the transitional zone between approximately 400m and 800m in altitude corresponding to the region between the southern coastal mangrove swamps and the northern mountainous escarpments. Local mosquito vectors include *Anopheles punctulatus*, *An. koliensis*, *An.*

farauti, *An. longirostris*, *An. karwari* and *An. bancrofti* [387]. *Anopheles koliensis* is the most efficient of these vectors and is particularly prevalent in the 400-800m zone (Dr Michael Bangs, Public Health Malaria Control, Freeport Indonesia, personal communication). Based on nightly mosquito catching, individuals in Timika can expect to receive 136 bites by anopheline mosquitoes each year. The entomological inoculation rates for *P. falciparum*, *P. vivax* and mixed *P. falciparum* / *P. vivax* infections have been estimated at 0.50, 0.28 and 0.06 infectious bites per year respectively (unpublished data).

In 1992, a survey of various communities in Timika revealed an overall prevalence of splenomegaly of 44.0% [387]. The prevalence of parasitaemia by microscopy ranged from 17.1% in those greater than 15 years to 60.0% in those between one and two years of age [387]. At this time, pure or mixed *P. falciparum* infection accounted for 77.1% of detected parasitaemia and *P. vivax* for 29.7% [387].

A survey of 800 households in 2005 revealed an overall point prevalence of asexual parasitaemia according to light microscopy of 16.3%; 46% due to *P. falciparum*, 39% due to *P. vivax*, 4% due to *P. malariae* and 11% due to mixed infections (mostly *P. falciparum* and *P. vivax*). *Plasmodium ovale* infection was not found. Asexual parasitaemia was present in 19.0% of Papuans and 12.7% of non-Papuans. The median age of those with *P. falciparum* parasitaemia was 19 years with a peak prevalence of 10% in those between 15 and 24 years. The median age of those with vivax parasitaemia was 13.5 years with a peak prevalence of 9.5% in those between one and 4 years [1]. Overall, 66% of survey participants with falciparum parasitaemia were afebrile, versus 71% with vivax parasitaemia and 65% with mixed infection. The overall incidence of parasitaemia (symptomatic or asymptomatic) in 2005 was estimated to be 876 episodes per 1,000 people per year with a species breakdown

of 512 per 1,000 for *P. falciparum*, 322 per 1,000 for *P. vivax*, 15.7 per 1,000 for *P. malariae* and 26.3 per 1,000 for mixed infections.

Papuan strains of *P. vivax* tend to relapse at intervals of approximately three weeks, as is typical in tropical regions [2,3]. The Chesson strain, first discovered in an American soldier returning from “New Guinea” in 1944 [388] is thought to be prevalent in Papua [389-391]. This strain is characterised by frequent relapses with no prolonged latent periods [122,235]. It causes fever at lower parasitaemias than many *P. vivax* strains and there is evidence to suggest that it is relatively ‘tolerant’ to primaquine, requiring 30mg/day for 14 days for reliable cure [235,389,390,392-394]. Extensive *P. vivax* strain diversity has been found in Papua New Guinea and the same is likely to be true in Papua, Indonesia [395,396]. Multiple concurrent *P. vivax* infections are also likely to be relatively prevalent [395,397].

Both *P. falciparum* and *P. vivax* are known to cause severe disease in Papua and severe malarial illness due to either species may be seen throughout life. A prospective surveillance study between January 2004 and December 2007 showed that of all presentations to RSMM with slide confirmed malaria, 20.2% resulted in admission to hospital, 19.8% for patients with *P. falciparum*, 18.2% for *P. vivax* and 37.4% for mixed infection [49]. Impaired consciousness, respiratory distress and / or severe anaemia (defined according to modified World Health Organization criteria [41,49]) were present in 22% of malaria inpatients, 20% for *P. falciparum*, 23% for *P. vivax* and 31% for mixed infection. Severe anaemia was easily the most common manifestation of severe disease in patients with *P. falciparum* (73%), *P. vivax* (87%) and mixed infections (81%). Respiratory distress was present in 11.3% of *P. vivax* patients with severe malaria and impaired consciousness in 6.1%. The overall case fatality of patients admitted with malaria was estimated to be 2.0%; 2.2% for patients with *P. falciparum*, 1.6% for *P. vivax* and 2.3% for mixed infection [49].

The worldwide distribution of chloroquine-resistance in *P. vivax* strains has been addressed in Chapter 3 and in published reviews [83,108,394]. *Plasmodium vivax* chloroquine-resistance in Papua was first documented in the Arso region in 1991 where 16 of 24 study participants developed *P. vivax* infection during 8 weeks of surveillance despite weekly doses of chloroquine [398]. Subsequent reports from elsewhere in the province suggested that this problem was widespread [311,330-333,360,399]. In 2004, 6 of 40 patients (15%) with *P. vivax* infections treated with chloroquine monotherapy in Timika had early treatment failure and 65% had had a recurrence of *P. vivax* by day 28 [332]. Although sulfadoxine+pyrimethamine resistance has been demonstrated in *P. vivax* isolates from Papua, it is unlikely to be as prevalent as in Thailand [253,311,400].

Plasmodium falciparum in Papua is also extensively drug resistant [386]. Chloroquine resistant strains were first documented in 1975 [401] and resistance to sulfadoxine+pyrimethamine was first established in 1979 [402]. In 2004, 48% of falciparum malaria patients treated with a combination of chloroquine and sulfadoxine+pyrimethamine in Timika had had a recrudescence by day 42 [332]. In vitro assessments suggest that there may also be low-grade resistance to quinine in local isolates [386].

4.1.8. Malaria treatment and control activities

Antimalarial treatment guidelines in the formal health care sector in Mimika District have changed substantially over the last 7 years. Between 2004 and February 2006, patients presenting to clinics or hospitals with uncomplicated malaria due to any of the locally prevalent *Plasmodium* species were treated with a three day course of oral chloroquine or, more commonly, a 7-day unsupervised course of oral quinine. Pregnant women and infants less than 5kg in weight were treated with a 7-day course of oral quinine plus clindamycin or chloroquine. In March 2006, following a series of local drug trials [2,3,332], the protocol for

blood schizontocidal treatment of uncomplicated malaria due to any *Plasmodium* species changed to a three-day unsupervised course of dihydroartemisinin+piperaquine. Women in the first trimester of pregnancy and infants less than 5kg in weight continued to be treated with a combination of quinine and clindamycin. Supplies of DHA+PIP ran out at Rumah Sakit Mitra Masyarakat and surrounding clinics for a brief period in 2007 during which first-line antimalarial therapy was switched to artesunate+amodiaquine. Prior to May 2005, first-line treatment of severe malaria in Mimika District was intravenous quinine and after this date it was intravenous artesunate.

Throughout the entire period, a single gametocytocidal dose of primaquine has been recommended, but inconsistently given, for patients with *P. falciparum* malaria. A 14-day unsupervised course of primaquine has also been recommended for all patients with vivax or mixed species malaria although glucose-6-phosphate dehydrogenase deficiency testing is not available. At least at RSMM, there has been a gradual adoption of the higher 0.5mg/kg daily dose of primaquine in preference to the lower 0.25mg/kg daily dose.

Various antimalarial treatments are available in the informal health care sector. In 2005, these included intravenous and oral quinine, chloroquine, sulfadoxine+pyrimethamine, amodiaquine, primaquine, intravenous and oral artesunate, doxycycline and clindamycin (unpublished data). The quality of these drugs is unknown.

Aside from passive detection and treatment of symptomatic malaria, both the Public Health Malaria Control Program and Dinas Kesehatan conduct a range of malaria control activities. These activities have remained relatively constant over the period 2004-2011 and include distribution of insecticide-treated bed nets to communities within the respective departments' remit, indoor residual spraying and a limited amount of larvaciding. A small number of clinics in Mimika District also conduct occasional active case-finding and

treatment surveys in their local communities. The proportion of individuals that regularly sleep under insecticide-treated bed nets is unknown.

4.2 Research activities

In 2003, Menzies School of Health Research renewed a memorandum of understanding with the National Institute of Health Research and Development (NIHRD) in Indonesia that allowed the expansion of a collaborative research programme based at Rumah Sakit Mitra Masyarakat focused on the control of malaria in Papua. In May 2010 the 'Papuan Health and Community Development Foundation', a local Non-Government Organization headed by local researchers, was established and is now leading a new three-way collaboration with Menzies School of Health Research and the University of Gadjah Mada in Yogyakarta. The following is an outline of the research activities of the NIHRD-Menzies collaboration that are relevant to this thesis.

4.2.1. Malaria surveillance

During the latter part of 2003 and early 2004, the research collaboration garnered support from local government and private institutions to form an extensive malariometric surveillance network in Timika. At the time of formation, this network encompassed 14 community health care clinics, variously run by International SOS, Public Health Malaria Control and Dinas Kesehatan, as well as Mitra Masyarakat Hospital. In November 2009, the network extended to cover the newly-opened Umum Daerah government hospital. In many cases, malariometric data collection was already occurring at these clinics in accordance with institution-specific requirements. The surveillance network formalised this data collection, adding new mechanisms where necessary. In 2005, it was estimated that 40% of patients with symptomatic *Plasmodium* infections in Timika sought treatment at one of these network institutions [1].

4.2.2. Community surveillance

At community clinics, staff were asked to fill out a data entry form showing the total number of blood slides examined and the number of slides positive for malaria (stratified by species and age) at the end of each week. After the introduction of dihydroartemisinin+piperaquine for treatment of uncomplicated malaria in March 2006, staff were also asked to record the number of courses of dihydroartemisinin+piperaquine prescribed and, for the first year following introduction, any major observed side effects of treatment. From the outset, local meteorological and entomological data collected by staff of Public Health Malaria Control have been submitted to the surveillance network. Meteorological data include rainfall, temperature and humidity at various sites in Timika. Entomology data include the number and species of mosquitoes caught during more than 30,000 man-hours of catching per year as well as sporozoite positivity by ELISA (stratified by *Plasmodium* species).

4.2.3. Hospital surveillance

Two surveillance systems have been established at Rumah Sakit Mitra Masyarakat. The first of these is a passive system administered by hospital staff. Each patient presenting to the hospital (regardless of department) is assigned a unique, individual identification number. This unique number, along with basic demographic information (age, sex and ethnicity) and the diagnoses given by the attending doctor (classified according to the International Classification of Diseases) are then entered into a computer database using Q-Pro® software (Jakarta, Indonesia). Although not intended as part of the malariometric surveillance system, two further passive data collection systems at the hospital have subsequently proved very useful. Haematology results for each patient (including haemoglobin concentration, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, platelet count and non-differentiated white blood cell count) from the hospital's Coulter Counter® are automatically recorded in a separate database and linked to the unique identifier.

Pharmaceutical prescription data (including date, drug name, formulation of drug and dose prescribed) are recorded in a further separate database and linked indirectly with the unique patient identifier.

A second, active malaria surveillance system is administered by staff of the NIHRD-Menzies collaboration. With the aid of an automated alert system based on the results of malaria microscopy at the lab, a research nurse searches the wards for any patient with parasitemia at least once per day, 6 days per week. This nurse records basic demographic and clinical details including age, sex, ethnicity, pregnancy status, date of admission, date of discharge, *Plasmodium* species, haemoglobin concentration and respiratory rate. A research physician then reviews patients to determine the presence of severe disease according to a modified version of the World Health Organization criteria (WHO) (see Chapter 7 for details) [41,49].

Protocols dictate that all patients presenting to the RSMM outpatients department with a fever or symptoms potentially consistent with malaria and all inpatients, regardless of diagnosis, should have a malaria blood film. Diagnosis of malaria is usually based on a thick film examination and parasite density is given semiquantitatively as 1+ to 4+. Confirmatory thin films and histidine rich protein-2 (HRP2)-based rapid diagnostic tests for *P. falciparum* (Paracheck Pf®, Orchid Biochemical Systems, Goa, India) are also performed in a minority of cases where clinical suspicion of malaria is high but thick film microscopy fails to reveal parasites. A further minority of patients who are enrolled in clinical and *in vitro* studies have their blood films re-read by a research microscopist with over 10 years of experience. Research microscopists only declare slides negative after examination of at least 100 high-power fields and although this is the protocol for hospital microscopists, in reality, such stringent examination does not always occur. In 2004, a random selection of 1,158 positive slides from the hospital was re-read by one of the research microscopists. Concordance was 89.1% (1,032/1,058). Of the 235 slides initially read as *P. vivax* infections, 192 (81.7%) were

confirmed to be *P. vivax* monoinfections, 28 (11.9%) were shown to be mixed *P. falciparum* / *P. vivax* infections, 9 (3.8%) were reclassified as *P. falciparum* monoinfections and 6 (2.6%) were reclassified as being negative for parasitaemia. Of the 730 slides initially read as *P. falciparum* infections, 696 (95.3%) were confirmed to be falciparum monoinfections, 17 (2.3%) were shown to be mixed infections, 6 (0.8%) were reclassified as *P. vivax* monoinfections and 11 (1.5%) were reclassified as being negative for parasitaemia. Clinical diagnosis of malaria at the hospital is actively discouraged and uncommon. Complete blood counts are ordered according to clinical indication and are performed using a Coulter Counter®.

Data from the passive hospital surveillance systems are integral to Chapters 5, 7 and 10 and are further described therein. Data from the active surveillance system are used in Chapter 7.

4.2.4. Clinical trials

Detection of extensive resistance to standard antimalarials in Papuan *P. vivax* and *P. falciparum* strains during the 1990s and 2000s clearly mandated a search for more effective alternatives in Timika. Between July 2004 and December 2005, patients presenting to primary health care clinics in SP9 and SP12 communities with uncomplicated, slide-confirmed malaria due to *P. vivax* and / or *P. falciparum* were enrolled in two large randomised controlled trials of artemisinin combination therapy [2,3]. The first of these two studies compared dihydroartemisinin+piperaquine with artemether+lumefantrine and included 754 patients, 175 with *P. vivax* monoinfections and 113 with mixed *P. vivax* / *P. falciparum* infections [2]. The second study compared dihydroartemisinin+piperaquine with artesunate+amodiaquine and included 334 patients, 80 of whom had *P. vivax* monoinfections and 69 of whom had mixed infections [3]. Follow-up in both studies was for 42 days.

Dihydroartemisinin+piperaquine was found to be superior to artemether+lumefantrine and artesunate+amodiaquine for both *P. vivax* and *P. falciparum* infections. Subsequent to these studies, the protocol for first-line treatment of uncomplicated malaria due to any *Plasmodium* species in Timika was changed to dihydroartemisinin+piperaquine, as described earlier in this chapter. Important methodological details of these two randomised controlled trials are given in Chapter 8.

4.2.5. House-to-house survey

Prior to the introduction of dihydroartemisinin+piperaquine for treating uncomplicated malaria, a large house-to-house survey was conducted to establish the prevalence of parasitaemia in the region as well as the treatment seeking behaviour of those with fever. A second survey was planned for two years after introduction to determine if there had been any changes in these indices but for a number of reasons, in particular the end of the NIHRD-Menzies collaboration, this will not be conducted until the second half of 2011.

The first survey was carried out between July and December 2005 and included 825 households housing 5,255 individuals in the 4 largest subdistricts of Mimika District. Overall, 3,890 (74%) individuals were home at the time of the survey and consented to providing a finger prick sample of blood for parasite microscopy and haemoglobin measurement. Demographic, clinical and laboratory data from these 3,890 individuals are used in Chapter 6 and the methodological details of the survey are given therein.

4.2.6. Ethical approval

The Papuan studies described in this thesis were approved by the ethics committees of the National Institute of Health Research and Development, Indonesian Ministry of Health (Jakarta, Indonesia) or University of Gadjah Mada as well as the ethics committee of the Menzies School of Health Research (Darwin, Australia). The studies from Shoklo Malaria

Research Unit that contribute data to this thesis were approved by the ethics committees of the Faculty of Tropical Medicine, Mahidol University (Bangkok, Thailand) and Oxford University (OXTREC, Oxford, United Kingdom). Written informed consent was obtained from patients or their parents or guardians prior to enrolment in all studies other than the routine surveillance systems in Papua.

4.2.7. Statistical analysis software

Unless stated otherwise, all data cleaning, merging and analysis in this thesis was done using STATA® version 10.1 (StataCorp, College Station, Texas, USA).

5. The burden of anaemia associated with vivax malaria in the health care setting

5.1 Introduction

The following three chapters of this thesis focus on the haematological morbidity and mortality associated with *Plasmodium vivax* infection in Southern Papua.

Anaemia is a common manifestation of *Plasmodium* infection and is responsible for substantial morbidity [403-406] as well as direct [189,407,408] and indirect mortality [265,409-411]. In falciparum malaria, haemoglobin loss results from increased removal of infected, and to a greater extent, uninfected red blood cells from circulation, compounded, in subacute and chronic forms, by impaired erythropoiesis [149-152]. Removal of uninfected red blood cells is thought to be an especially important mechanism of anaemia in *P. vivax* infection but in general the pathogenesis of haematological impairment caused by *P. vivax* malaria is comparatively poorly described (see Chapter 2) [154].

Where there is high *P. falciparum* endemicity, intense parasite exposure from an early age induces robust immunity to clinical disease resulting in a low risk of severe anaemia beyond childhood [412]. Outside of Africa, low or moderate malaria transmission and co-endemicity

of two or more *Plasmodium* species, each with a different age distribution, is the norm [55,81,109]. In these regions, less intense parasite exposure during early life delays the development of immunity and gives rise to the potential for symptomatic and complicated infections at all ages. This, in combination with interactions between the different *Plasmodium* species, complicates analyses of the pattern and public health impact of malarial anaemia.

Recent studies suggest that *P. vivax* monoinfections are a significant, and underestimated, cause of severe anaemia on the island of New Guinea [49,50,96]. There is also evidence that in this region, mixed *P. vivax* / *P. falciparum* infections cause greater, as opposed to less, haematological impairment than monoinfection with *P. falciparum* [49,96].

The aim of this study was to describe the comparative haematological impact of the different *Plasmodium* species at Mitra Masyarakat Hospital in Southern Papua at all ages from infancy through to adulthood.

5.2 Methods

5.2.1. Laboratory and data collection procedures

This study used prospective data collected as part of the routine surveillance system operated by staff of Mitra Masyarakat Hospital. The details of this system were introduced in Chapter 4. In brief, hospital administrators record basic demographic information along with the diagnoses given by the attending doctor for all patients presenting to hospital, regardless of department. These data are entered into an electronic database and are linked to a unique hospital identification number. All blood results from the hospital's Coulter counter are recorded in a separate database and are also identified using this unique hospital number.

Protocols dictate that all patients presenting to the outpatients department with fever or symptoms potentially consistent with malaria and all inpatients, regardless of diagnosis, should have a malaria blood film. Diagnosis of malaria is usually based on a thick film examination, though confirmatory thin films and HRP2-based rapid diagnostic tests for *P. falciparum* (Paracheck Pf®) are also performed in some cases. Clinical diagnosis of malaria at the hospital is actively discouraged and uncommon. Complete blood counts are ordered according to clinical indication.

5.2.2. Data merging and statistical analysis

Clinical data were merged with haematology data by creating all possible pairwise combinations for each unique hospital number. Pairs in which the laboratory record fell between the date of presentation and discharge (the same day for outpatient visitations) were kept and if more than one haemoglobin measurement was available for a single event, the lowest was taken (Figure 5.1). For the purposes of these analyses, mixed infection was defined as concomitant infection with any combination of *Plasmodium* species. The vast majority of cases in this group were individuals infected with *P. falciparum* and *P. vivax*.

The primary outcomes in this study were the absolute reduction in haemoglobin, the odds of severe anaemia (haemoglobin <5g/dL) and the population attributable fraction of severe anaemia associated with infection by the different *Plasmodium* species at all ages from infancy through to adulthood. Continuous haemoglobin data were analysed using linear regression and binary anaemia data were analysed using logistic regression. Since some patients appeared in the database multiple times, I corrected the variance-covariance matrices of these models for intra-patient correlation (giving robust standard errors).

Univariable analyses were performed for each of the following variables: *Plasmodium* species (negative, *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* or mixed species), sex, self-reported

ethnicity (non-Papuan, Highland Papuan, Lowland Papuan), age group (<1 year, 1 to <5 years, 5 to <15 years, ≥15 years) and year of presentation (2004 through to 2009). All of these factors as well as the interaction between age and *Plasmodium* species were associated with clinically important differences in haematological status and were therefore included in multivariable models. Fractional polynomials were used to allow for the non-linear relationship between age and mean haemoglobin and log odds of severe anaemia because they make no assumption regarding the shape of this association [413]. To maintain stability of the fractional polynomial models it was necessary to exclude infants under one week of age (n=409, 0.4%) and adults over 40 years of age (n=11,173, 9.6%), the former predominantly representing neonates who would not have had *ex utero* exposure to malaria.

Adjusted population-based fractions of severe anaemia attributable to parasitaemia were calculated from multivariable, unconditional logistic regression models using the aflogit module for STATA® [414]. This was done for multiple age groups from infancy through to adulthood. The attributable fractions cannot be summed as the model assumes a mutually exclusive scenario where each risk factor is deemed to be the first to be eliminated [415]. Outputs can therefore be interpreted as the proportion of severe anaemia that could be prevented by addressing the particular factor of interest in isolation. I did not make allowances for intra-subject correlation in these analyses since the outcome of interest was total hospital workload rather than the impact on individuals. Due to very small numbers, patients with *P. ovale* infections (n=21, 0.02%) were excluded from all multivariable models.

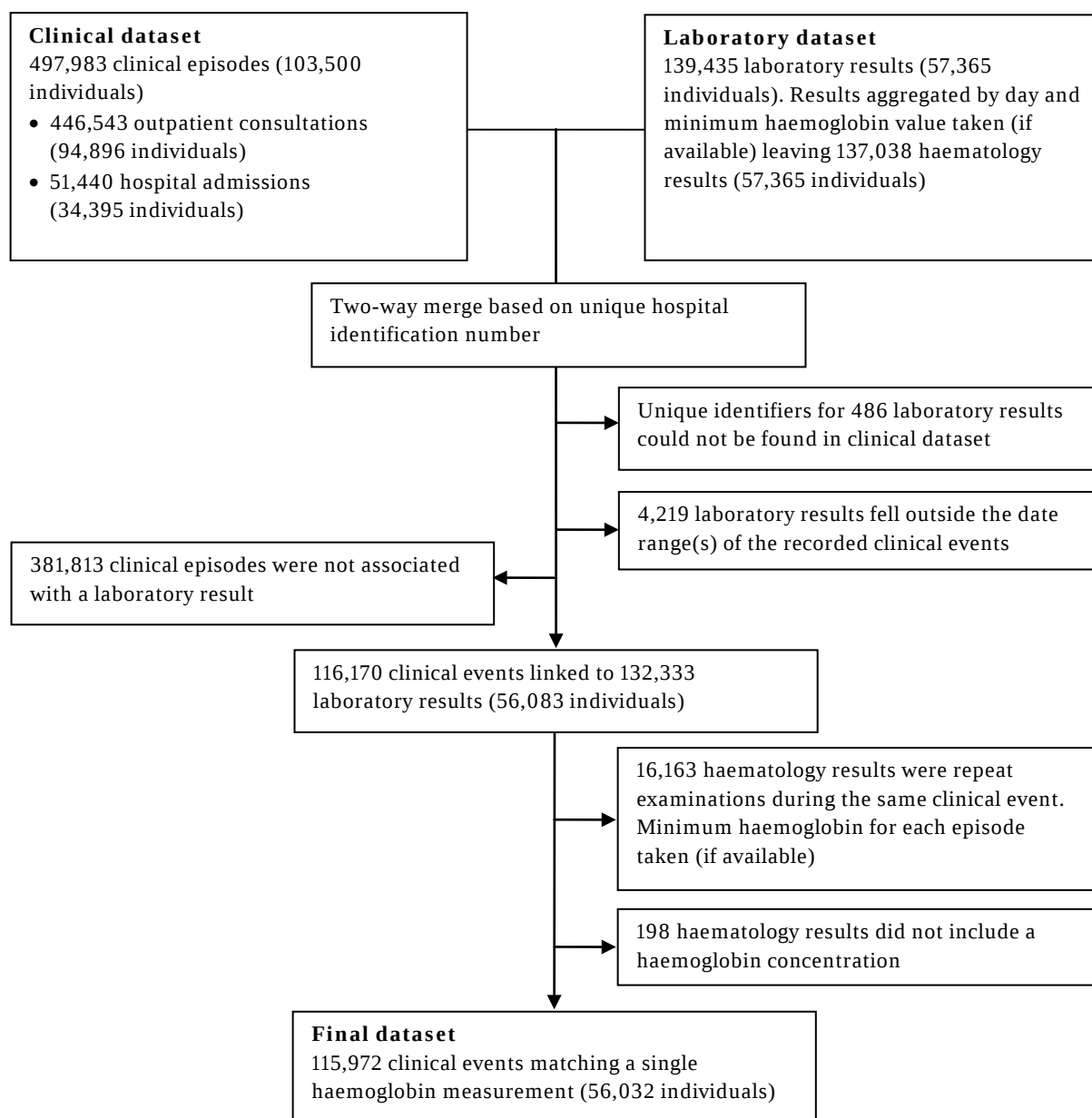


Figure 5.1 Flow diagram demonstrating the dataset merge process and hospital workload. Note, individuals may have had both outpatient consultations and inpatient admissions.

5.3 Results

Between April 2004 and May 2009, there were 497,983 presentations to Mitra Masyarakat Hospital constituting 446,543 (89.7%) outpatient visitations and 51,440 (10.3%) inpatient admissions (Table 5.1). Overall, 115,972 (23.3%) of these clinical events were matched to a single haemoglobin measurement. Excluding *P. ovale* (which was rare), 29.6% (range 27.6-31.9%) of malaria outpatient visitations were linked with a haemoglobin measurement compared to 14.1% of non-malaria presentations. The corresponding figures for patients admitted to the wards were 91.5% (range 91.2-92.4%) and 76.5% respectively. Patients who had a haemoglobin measurement were slightly younger than those who did not (median age in those without malaria, 23.1 years versus 24.2 years, $p<0.001$ and for those with malaria; 16.7 years versus 18.0 years respectively, $p<0.001$).

The age distribution of all clinical events combined showed a peak in infancy and a second peak during the late 20s (Figure 5.2). The absolute number of malaria presentations over this period was highest during the second year of life but as a proportion of admissions was highest during the early teens. Vivax malaria was the dominant cause of malaria in patients under three years of age in both the outpatient and inpatient setting. Thereafter, *P. falciparum* was the most common malaria parasite.

Table 5.1 Distribution of clinical and laboratory data plus haematological status by clinical and demographic group

	Distribution of clinical and laboratory data					Haematological status				
	Total clinical events	OP events	OP events with Hb (%)	IP events	IP events with Hb (%)	Mean Hb	Robust SE	<i>p</i>	Hb<5g/dL n (%)	<i>p</i>
Malaria status										
Negative	409,238	375,550	52,946 (14.10)	33,688	25,777 (76.52)	10.30	0.015	Ref	2,553 (3.24)	Ref
<i>P. falciparum</i>	54,001	41,805	12,566 (30.06)	12,196	11,150 (91.42)	9.04	0.022	<0.001	2,006 (8.46)	<0.001
<i>P. vivax</i>	26,395	22,775	6,491 (28.50)	3,620	3,300 (91.16)	8.93	0.032	<0.001	851 (8.69)	<0.001
Mixed infection	6,013	4,340	1,386 (31.94)	1,673	1,545 (92.35)	8.33	0.056	<0.001	414 (14.12)	<0.001
<i>P. malariae</i>	2,260	2,001	552 (27.59)	259	238 (91.89)	8.52	0.092	<0.001	72 (9.11)	<0.001
<i>P. ovale</i>	76	72	17 (23.61)	4	4 (100)	10.37	0.65	0.9	0 (0)	-
Sex										
Male	229,358	206,606	35,116 (17.00)	22,752	18,290 (80.39)	10.31	0.022	Ref	2,658 (4.98)	Ref
Female	268,619	239,934	38,841 (16.19)	28,685	23,723 (82.70)	9.48	0.016	<0.001	3,238 (5.18)	0.2
Ethnicity										
Non-Papuan	87,923	80,254	14,100 (17.57)	7,669	6,057 (78.98)	11.92	0.025	Ref	281 (1.39)	Ref
Highland Papuan	335,831	301,369	48,859 (16.21)	34,462	29,146 (84.57)	9.34	0.016	<0.001	5,010 (6.42)	<0.001
Lowland Papuan	71,801	63,562	10,817 (17.02)	8,239	6,431 (78.06)	9.83	0.030	<0.001	580 (3.36)	<0.001
Age										
<1 year	39,674	31,213	6,102 (19.55)	8,461	4,341 (51.31)	9.17	0.031	<0.001	543 (5.20)	<0.001
1 to <5 years	74,565	65,523	13,046 (19.91)	9,042	7,234 (80.00)	8.79	0.025	<0.001	1,574 (7.76)	<0.001
5 to <15 years	53,605	49,067	8,669 (17.67)	4,538	4,100 (90.35)	9.12	0.031	<0.001	905 (7.09)	<0.001

≥15 years	330,138	300,740	46,141 (15.34)	29,398	26,339 (89.59)	10.40	0.017	Ref	2,874 (3.97)	Ref
Year										
2004	63,067	56,190	8,595 (15.30)	6,877	4,605 (66.96)	9.08	0.028	Ref	930 (7.05)	Ref
2005	88,667	78,357	17,336 (22.12)	10,310	8,710 (84.48)	9.64	0.022	<0.001	1,277 (4.90)	<0.001
2006	96,284	86,251	13,067 (15.15)	10,033	8,411 (83.83)	9.89	0.024	<0.001	1,032 (4.80)	<0.001
2007	106,036	95,115	14,338 (15.07)	10,921	9,247 (84.67)	9.75	0.025	<0.001	1,480 (6.28)	0.01
2008	98,036	88,886	12,040 (13.55)	9,150	7,604 (83.10)	10.45	0.027	<0.001	831 (4.23)	<0.001
2009	45,893	41,744	8,582 (20.56)	4,149	3,437 (82.84)	10.44	0.030	<0.001	346 (2.88)	<0.001
Total	497,983	446,543	73,958 (16.56)	51,440	42,014 (81.68)	9.86	0.013	-	5,896 (5.08)	-

Abbreviations: OP; outpatient, IP; inpatient, Hb; haemoglobin, n; number, std dev; standard deviation, Ref; reference category

* *p* values based on univariable linear regression with correction of the variance-covariance matrix for within-patient correlation

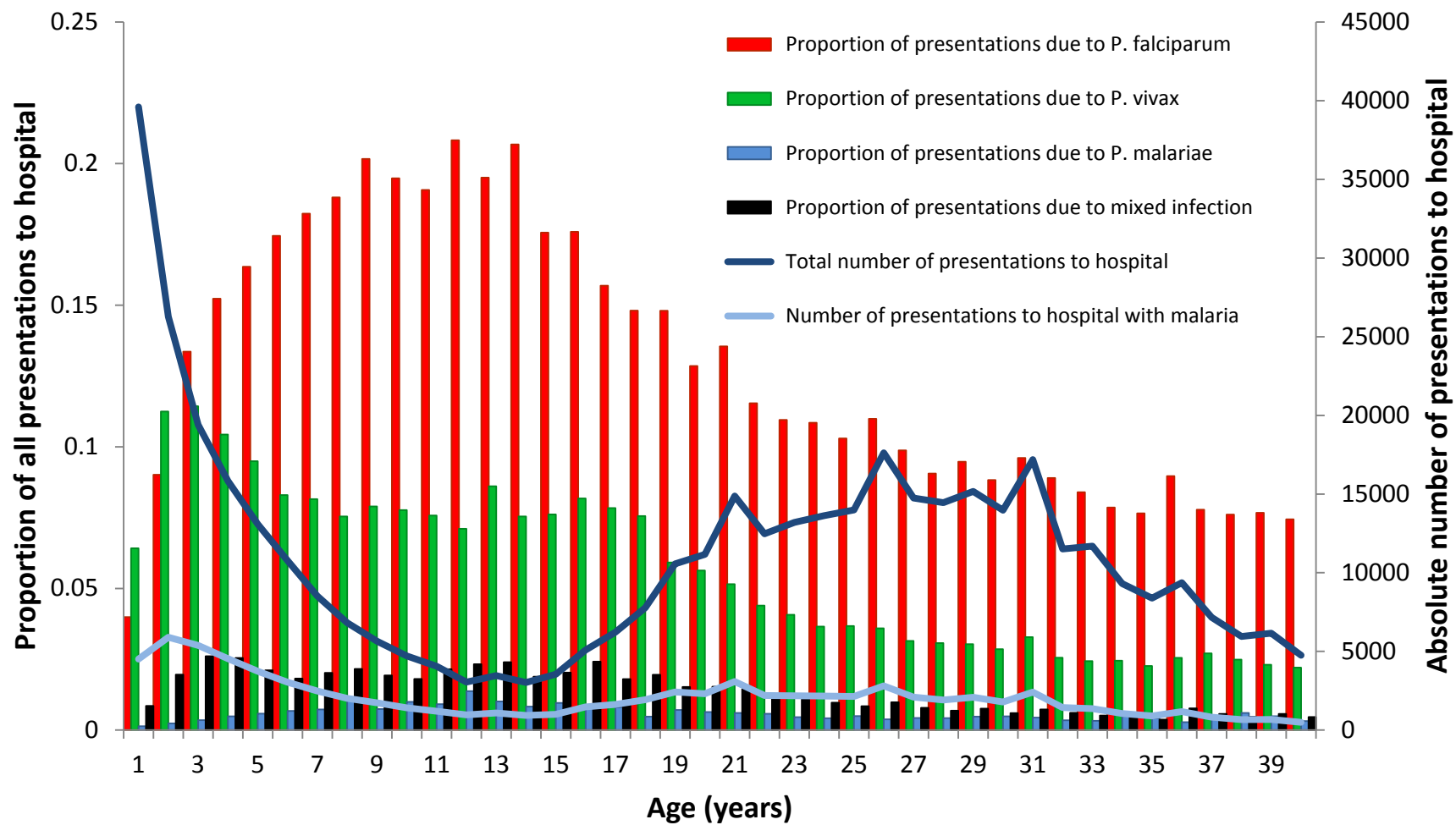


Figure 5.2 Presentations to hospital due to malaria as a proportion of all presentations

In univariable analyses, absolute haemoglobin concentration and prevalence of severe anaemia varied significantly by *Plasmodium* species (Table 5.1). Mixed infection and *P. malariae* infection were associated with particularly poor haematological status (mean haemoglobin 8.33g/dL and 8.52g/dL respectively, prevalence of severe anaemia 14.1% and 9.1% respectively) while the haematological effects of *P. vivax* and *P. falciparum* monoinfection were less severe and broadly similar (Table 5.1). Highland Papuans had a mean haemoglobin of 9.34g/dL compared to a mean of 9.83g/dL in Lowland Papuans and 11.92g/dL in non-Papuans; *p* for comparisons with non-Papuans <0.001.

After correcting for confounding factors, patients presenting to hospital with malaria had lower mean haemoglobin concentrations and higher odds of severe anaemia than those without malaria at all ages but most noticeably during childhood (Figure 5.3). Overall, *P. malariae* was associated with the greatest mean reduction in haemoglobin compared with uninfected individuals (-1.52g/dL [95% CI -1.70- -1.35g/dL]) followed by mixed infection (-1.47g/dL [95% CI -1.58- -1.37g/dL]), *P. falciparum* (-0.91g/dL [95% CI -0.95- -0.87g/dL]) and *P. vivax* (-0.87g/dL [95% CI -0.93- -0.81g/dL]), *p* for all comparisons <0.001. Mixed infection was associated with the greatest odds of severe anaemia overall (Adjusted Odds Ratio [AOR] = 4.11 [95%CI 3.64-4.64]) followed by *P. malariae* (AOR = 2.74 [95% CI 2.12-3.53]), *P. falciparum* (AOR = 2.43 [95% CI 2.27-2.61]) and *P. vivax* (2.43 [95% CI 2.23-2.66]), *p* for all comparisons <0.001). The comparative haematological impact of *P. malariae* was greatest in adulthood whereas for *P. falciparum*, *P. vivax* and mixed infections, it was worst during infancy. At six months of age the probability of severe anaemia was 14% (95% CI 12-16%) in patients with *P. falciparum*, 12% (95% CI 11-14%) with *P. vivax*, 4% (95% CI 3-7%) with *P. malariae* and 17% (95% CI 14-20%) with mixed infection. Beyond the first year of life, the haematological impact of *P. vivax* infection decreased rapidly whereas for *P.*

falciparum infection, and in particular mixed infection, the improvement in mean haemoglobin with increasing age was slower.

In patients presenting to hospital the overall adjusted fraction of severe anaemia attributable to *P. falciparum* was 11.7% (95% CI 10.6-12.7%), compared to 6.0% (95% CI 5.2-6.7%) for *P. vivax*, 0.6% (95% CI 0.4-0.8%) for *P. malariae* and 2.8% (95% CI 2.5-3.2%) for mixed species infections. Overall, the fraction of severe anaemia attributable to *P. vivax*, *P. malariae* or mixed species infections was 9.7% (8.8-10.6%). The impact of *P. vivax* peaked in infancy when it was responsible for 23.4% (95% CI 19.6-27.1%) of severe anaemia compared to 15.4% (95% CI 12.3-18.3%) for *P. falciparum*. The peak impact of falciparum malaria occurred in the third year of life with a second peak, apparent for all species, between the ages of approximately 15 and 25 years (Figure 5.4).

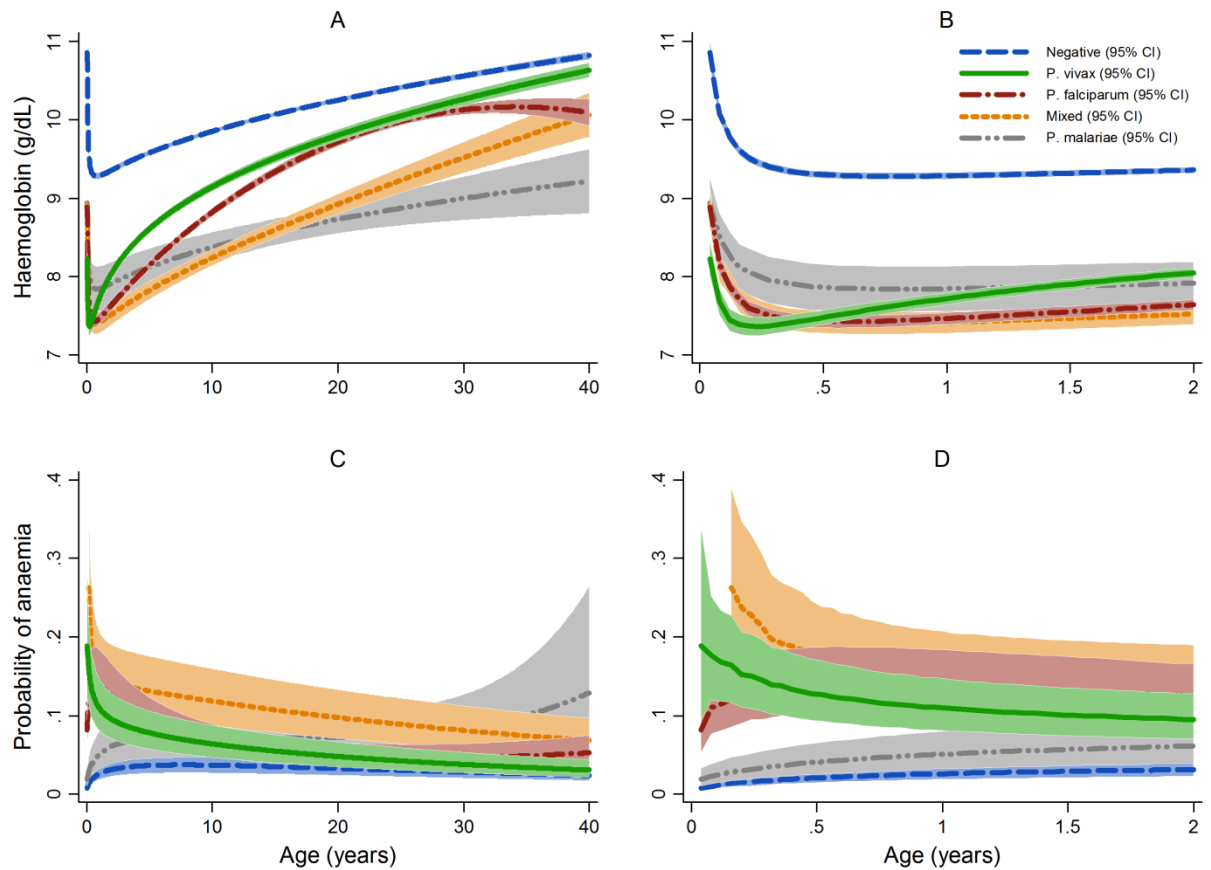


Figure 5.3 Multiple fractional polynomial regression analysis showing absolute haemoglobin by *Plasmodium* species from infancy to adulthood (A) and during the first two years of life (B) and the probability of severe anaemia (haemoglobin <5g/dL) by *Plasmodium* species from infancy to adulthood (C) and during the first two years of life (D). Model covariables include: *Plasmodium* species, age, *Plasmodium* species by age, sex, age by sex, ethnic group (Highland Papuan, Lowland Papuan, non-Papuan) and year.

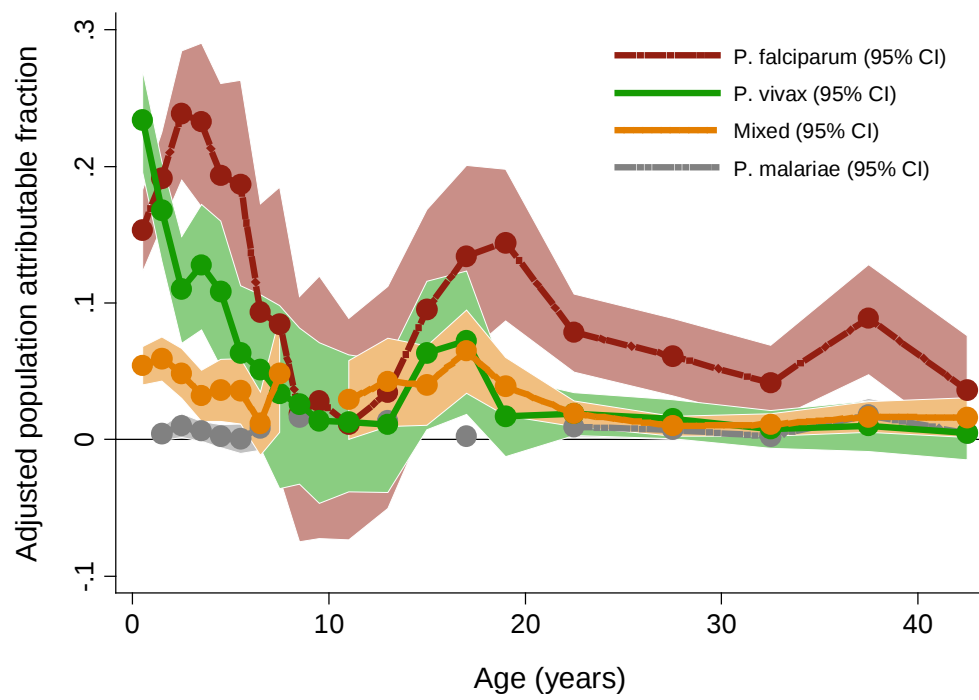


Figure 5.4 Adjusted population attributable fractions of anaemia (haemoglobin <5g/dL) by *Plasmodium* species and age. Model covariables for each age stratum include: *Plasmodium* species, sex, ethnic group (Highland Papuan, Lowland Papuan, non-Papuan) and year.

5.4 Discussion

Anaemia is highly prevalent throughout the tropics and has major consequences for human health and socioeconomic prosperity [416]. Our very large hospital-based study in Southern Papua, Indonesia provides a comparative assessment of the pattern of haematological changes associated with the non-falciparum malarias as well as an estimate of their public health importance. It shows that *P. vivax* is associated with a high burden of severe anaemia in infancy (almost one quarter of all cases at the hospital), that mixed *P. vivax* / *P. falciparum* infection causes significantly more haematological impairment than monoinfection with either species alone and that in adulthood, *P. malariae* infection is associated with the greatest overall reduction in haemoglobin. Overall, the fraction of severe anaemia attributable to *P. vivax*, *P. malariae* and mixed species infections was only marginally lower than the fraction attributable to *P. falciparum* (9.7% compared to 11.7%).

Infancy is a time of rapid physical and cognitive development as well as increased vulnerability to infectious diseases. Our study shows that during early life, both the incidence of vivax malaria and severity of vivax-associated anaemia are greater than for other *Plasmodium* species. This probably reflects the greater non-specific immune response for a given parasite density in *P. vivax* infections [171,172] and higher subsequent parasite exposure from relapsing disease. Together these factors will result in more frequent haematological insults and faster acquisition of immunity when compared with other *Plasmodium* species. *Plasmodium vivax* has a strong predilection for reticulocytes which are most abundant at approximately three months of age [417]. It is therefore possible that young infants are also more susceptible to high density *P. vivax* infections than other age groups.

Young African children with severe malarial anaemia have an increased risk of mortality [189,265,416] and blood transfusion (with attendant risk of blood-borne disease

transmission) [189] and may have reduced resilience to other infectious and non-infectious diseases. It therefore seems likely that infantile *P. vivax* infection may be associated with indirect morbidity, and potentially significant mortality in co-endemic regions. The long-term effects of malarial anaemia are also likely to include deleterious developmental and socioeconomic sequelae although defining these is challenging since anaemia in malaria-endemic areas is often multifactorial [40,265,406,416]. Malaria has been linked with impaired cognitive development but it is unclear if this is due to the haematological effects of infection [40].

Previous work from Thailand has found an association between concomitant *P. vivax* infection and reduced severity of *P. falciparum* infection [153,241]. Beyond three years of age, mixed infection in Southern Papua was associated with both a lower mean haemoglobin concentration and greater odds of severe anaemia than monoinfection with either species. This could reflect greater antigenic diversity of local parasite strains and thus less cross-reactive immunity, though evidence for this is lacking. Alternatively, it may relate to greater transmission intensity. In Southern Papua, the majority of severe malarial anaemia beyond early childhood is likely to be the result of repetitive infections in individuals who have already developed partial immunity. In this situation, the haematological effects of the two species are likely to be additive and any immunomodulatory effect of *P. vivax* on *P. falciparum* is likely to be negligible. In Thailand, where transmission is less intense, severe anaemia is likely to result from a single fulminant infection in a non-immune individual and therefore any immunomodulatory effect of *P. vivax* is likely to be proportionately more important.

Unexpectedly, *P. malariae* was found to be associated with the greatest overall reduction in haemoglobin of all locally prevalent *Plasmodium* species as well as a high frequency of severe

anaemia. Its haematological effects were most apparent in adulthood whereas in childhood it was associated with minimal impairment. *Plasmodium malariae* is a slow-growing parasite that only ever achieves low parasite density [418]. It is believed to have a predilection for old red blood cells [418] and hence should have a minimal effect on the average lifespan of circulating red blood cells [148]. A high proportion of *P. malariae* infections is asymptomatic and therefore unlikely to prompt treatment seeking [1]. The marked reduction in haemoglobin associated with *P. malariae* infection seen in our study may therefore relate to prolonged red cell destruction and bone marrow suppression caused by chronic, asymptomatic or minimally symptomatic parasitaemia. *Plasmodium malariae* is prevalent in much of sub-Saharan Africa [419] and according to the results of this study may be associated with an underappreciated burden of anaemia in adults.

Our study has several strengths. The very large number of patients enabled precise depiction of the evolution of haemoglobin changes associated with malaria infection from infancy right through to adulthood. Cases of malaria were microscopically confirmed by quality-assured microscopists and data entry was carried out in a consistent fashion. Due to strict hospital policy, the vast majority of symptomatic malaria infections were likely to have been ascertained. There are also a number of limitations of this study. Haemoglobin measurement was done on an as-needed basis and therefore a degree of selection bias has almost certainly occurred. Those without malaria were less likely to have a complete blood count, both in the outpatient and inpatient setting. Since those who did not receive a blood test were unlikely to have been severely anaemic, this will have resulted in underestimation of the malaria-attributable fractions of severe anaemia. Overall, the majority of patients with malaria did not have a haemoglobin measurement. However the proportion of patients who did have a measurement was the same for all species (*P. ovale* excluded) and hence, comparisons of the haematological effects of these species are likely to be valid.

The aflogit module for STATA® calculates adjusted population attributable fractions using odds ratios as approximations of relative risk. Since severe anaemia was not a particularly rare outcome, this may have resulted in slight overestimation of the population attributable fractions, particularly in young children in whom severe anaemia was the most prevalent.

The analyses in this study may have been subject to residual confounding. Haemoglobin and red cell polymorphisms are known to influence the likelihood of severe malarial anaemia [128,420,421] and in some cases may also modulate the risk of uncomplicated parasitaemia [129,422-426]. Adjusting the multivariable models for self-reported ethnicity (which, in Papua, is strongly indicative of the geographic location and altitude of tribal lands) should have accounted for at least some of the potential variation in the prevalence of these disorders by malaria status.

Other potential confounders include nutritional status, helminth infestation, bacteraemia and chronic disease. Malaria and malnutrition may cluster in the same geographic areas but the mechanisms and direction of any biological link have not been elucidated conclusively. Chronic vivax malaria has been implicated in a state of malnourishment akin to kwashiorkor [37], whereas iron deficiency may provide some protection against malaria infection [427]. Helminth infection causes gastrointestinal blood loss which may exacerbate malarial anaemia [136]. Two oft-cited studies by Nacher and Spiegel respectively indicate that intestinal helminthiasis increases the risk of falciparum malaria by a factor of 1.5 and 2.2 [137,138]. If this is indeed the case, the severity of anaemia associated with *Plasmodium* infection in this study may have been slightly overestimated. This is unlikely to have been important for children under 3 years old since helminth density typically peaks in adulthood and previous work has shown minimal impact on haemoglobin prior to 30 months of age [428].

The results from this hospital-based study cannot be generalised to individuals with malaria in the community, many of whom will have asymptomatic disease. The burden of anaemia attributable to *Plasmodium* species outside of the health care setting in Timika is addressed in Chapter 6.

In conclusion, our study demonstrates that the haematological impact of malaria in Southern Papua is dependent on host age, parasite species and presence of *Plasmodium* coinfection. The non-falciparum malarias make a major contribution to the burden of anaemia in this region, with almost 10% of all severe anaemia at the hospital attributable to *P. vivax*, *P. malariae* or mixed species infections. *Plasmodium vivax* was associated with the greatest attributable fraction of severe anaemia in infancy and is likely to be an important cause of indirect morbidity and possibly mortality in this age group. Conversely, the haematological impact of *P. malariae* was most apparent in adults. In contrast to earlier reports from regions of low mixed-species endemicity, mixed *P. falciparum* / *P. vivax* infections in Southern Papua are associated with a significantly greater absolute reduction in haemoglobin and odds of severe anaemia than monoinfection with either species alone. These findings highlight the public health importance of integrated genus-wide rather than species-specific malaria control strategies in areas of *Plasmodium* co-endemicity.

5.5 Acknowledgements

A modified version of this chapter is soon to be submitted to a peer-reviewed journal. The co-authors are Daniel A Lampah,¹ Enny Kenangalem,^{1,2} Julie A Simpson,³ Jeanne R Poespoprodjo,^{1,2} Paulus Sugiarto,⁴ Yati Soenarto,⁵ Nicholas M Anstey^{6,7} and Ric N Price.^{6,7,8}

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6. The burden of anaemia associated with vivax malaria in the community

6.1 Introduction

The haematological impact of malaria is most apparent and most easily measured in the health care setting. Outside of health care facilities, the burden of malarial anaemia is less well understood and its contribution to ‘indirect’ malaria morbidity and mortality is largely unknown [404]. There are two major explanations for this uncertainty. Firstly, the adverse effects of mild or moderate anaemia *per se* are not clearly understood. Haemoglobin concentrations below 7g/dL probably confer an increased risk of poor pregnancy outcomes such as haemorrhagic shock [266] and low birth weight [269,429] but other, less tangible, effects such as decreased resilience to infectious diseases and poor cognitive performance remain unproven. Secondly, in hyper- and holoendemic regions, the majority of individuals will be parasitised at any point in time making it difficult to disentangle the impact of malaria from other concomitant causes of anaemia. To circumvent this problem, researchers in highly endemic settings have typically limited their assessments to individuals with clinical malaria based on the presence of fever and a probabilistic cut-off for parasite density [430,431]. This approach inevitably misses the haematological effects of low-density symptomatic infections and asymptomatic parasitaemia. Evidence for the likely importance of the latter comes from

several sources including intervention trials that have demonstrated much greater improvements in haemoglobin and mortality than could have been predicted by preventing symptomatic infections alone [409-411] as well as observational studies showing large variations in haemoglobin concentrations coinciding with seasonal fluctuations in parasite prevalence [432]. These and other findings led Molineaux to suggest that ‘total’ falciparum malaria mortality in Africa is likely to be twice as high as ‘direct’ malaria mortality [433]. Although there is relatively less information for *P. vivax*, one could assume that the ratio for this species would be even greater since direct deaths are rare (but probably underestimated [49,50,99]) and infections are associated with significant morbidity [33,34,83,96,117,124].

Papua has a lower *Plasmodium* entomological inoculation rate than most malarious tropical African nations but a disproportionately high direct malaria attributable mortality rate [49]. Such a setting provides a unique opportunity to establish the combined haematological impact of both symptomatic and asymptomatic parasitaemia since at any point in time between 80 and 90% of the population can be expected to be aparasitaemic [1]. Moreover, the similar incidence of *P. falciparum* and *P. vivax* infections enables valid comparisons between the two species. This chapter uses data from a cross-sectional prevalence survey of 5,255 individuals in Southern Papua conducted between July and December 2005 to determine and compare the haematological impact of *P. falciparum*, *P. vivax* and mixed parasitaemia in the community.

6.2 Methods

6.2.1. Cross-sectional survey methods

Households for this survey were chosen by cluster random sampling. First, the 4 largest of the 12 subdistricts in Mimika District were chosen purposively. Second, the number of clusters required in each subdistrict was apportioned according to the relative populations of the subdistricts. In most cases, clusters constituted discrete villages however in Mimika Baru, the very large population size dictated that villages within this subdistrict be subdivided into

census blocks. Once mapped, clusters, and 25 houses within each cluster, were chosen randomly according to WHO recommendations [434]. Household members were defined as people who lived under one roof, ate from one kitchen and who had resided in the study area for at least 6 months. For each household member, socio-demographic information and history of fever were recorded using a standardised questionnaire. Those present at the time of the survey had their weight and temperature recorded and a finger prick sample of blood taken for blood film examination and haemoglobin measurement. Patients with microscopically confirmed malaria were treated according to the Indonesian Ministry of Health Guidelines. Those with anaemia were given iron supplementation according to local protocols.

6.2.2. Laboratory methods

Blood films were read locally by experienced hospital microscopists. Parasitaemias were calculated assuming a white cell count of 7,300 cells/ μ L. All positive films and 10% of the negative slides were cross-checked at the National Institute of Health Research and Development reference laboratory in Jakarta. Results that differed were reviewed by the two lead microscopists for final assessment. Haemoglobin concentrations were determined using a calibrated portable HemoCue® photometer (Hb201+, Angelholm, Sweden).

6.2.3. Statistical analysis

The primary outcomes in this study were the absolute reduction in haemoglobin, the odds of moderate-to-severe anaemia and the population attributable fraction of moderate-to-severe anaemia associated with infection by the different *Plasmodium* species. For the purposes of this study, a haemoglobin concentration of 7g/dL was chosen *a priori* as an appropriate distinction between mild and moderate or severe anaemia (as recommended by Snow and colleagues [404]).

Univariable analyses were stratified by *Plasmodium* species (*P. falciparum*, *P. vivax*, *P. malariae* and mixed *P. falciparum* / *P. vivax* species infections), age group (<1 year, 1-<5

years, 5-<15 years, ≥ 15 years), self-reported ethnicity (non-Papuan, Highland Papuan, Lowland Papuan), pregnancy status, weight for age/sex/ethnicity ($\geq$ mean, $<$ mean) and household income per person ($>75^{\text{th}}$ centile, $25^{\text{th}}\text{--}75^{\text{th}}$ centile, $<25^{\text{th}}$ centile). Age strata were chosen with reference to the local hospital data presented in Chapter 5 and evidence from other mixed endemicity settings which suggests marked differences in the rate of acquisition of immunity to *P. falciparum* and *P. vivax* during early childhood [46,51,292,435]. Weight for age/sex/ethnicity was established by creating a nomogram based on the sample data. Normally distributed continuous data were compared using two-tailed independent sample t-tests or analysis of variance and proportions were compared using chi-squared tests with Yates' continuity correction or the chi-squared test for trend. Pearson's correlation coefficients were used to examine the relationship between log-transformed parasite density and haemoglobin concentration.

Multivariable analyses were done for the *a priori* age strata as well as for the study population as a whole. Linear regression was used for continuous haemoglobin data and logistic regression for binary anaemia data. To account for the study design, I included the categorical variable 'subdistrict' in all multivariable models and adjusted the variance-covariance matrices for within-household correlation (giving robust standard errors). The association between haemoglobin concentration and age group differed for males and females, therefore the interaction term 'age by sex' was included in all analyses of the whole study population.

Adjusted population fractions of moderate-to-severe anaemia attributable to parasitaemia were calculated using the aflogit module for STATA® introduced in the previous chapter [414]. Patients with *P. malariae* infections were excluded from all multivariable analyses due to small numbers.

6.3 Results

6.3.1. Parasitaemia

In total, 5,255 individuals resided in the 825 households surveyed of whom 3,890 (74%) were present and consented to providing a finger prick sample of blood (Figure 6.1). Those who either refused to provide a sample or were not present at the time of the survey were an average of 4 years older (24.7 years versus 20.6 years) and more likely to be male (71.2% versus 48.2%) than their counterparts who provided blood samples. Parasitaemia was demonstrated in 17.0% of the participants with *P. falciparum* present in 8.1% (n=315), *P. vivax* in 6.4% (n=250) and mixed infections in 1.9% (n=72) (Table 6.1). More infants (<1 year) and children between the ages of one and less than 5 years were infected with *P. vivax* compared to *P. falciparum* (12 versus 5 children and 62 versus 50 children respectively) whereas the opposite was true for all other age groups. After infancy, there was a statistically significant trend to decreasing prevalence of *P. vivax* parasitaemia with increasing age (χ^2 test for trend, $p=0.003$) but no such trend for *P. falciparum* (χ^2 test for trend, $p=0.13$). Unlike in highly endemic regions, there was no age-associated decrease in the likelihood of having concomitant fever with parasitaemia for either species (χ^2 test for trend, $p=0.55$ for *P. vivax* and $p=0.92$ for *P. falciparum*). Overall, a higher proportion of Highland and Lowland Papuans were parasitised (21.2% and 17.3% respectively) than non-Papuans (13.1%, χ^2 test, $p<0.001$ and $p=0.01$ respectively).

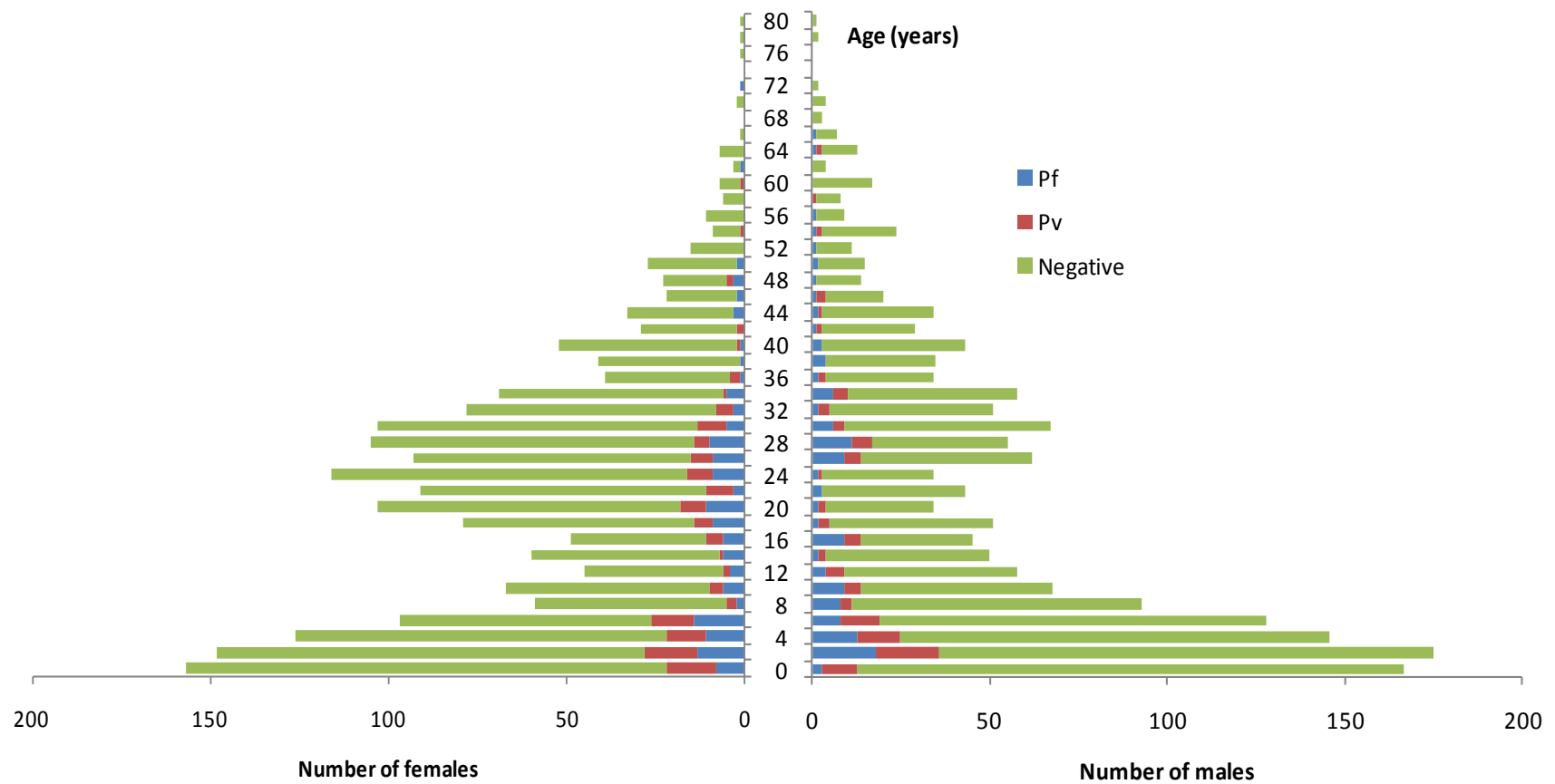


Figure 6.1 Population structure of those who provided a blood sample by age, sex and *Plasmodium* parasitaemia

Table 6.1 Demographic features of participants in the household survey for whom blood samples were available

n (%)		Sex		Pregnancy status		Age-group				Ethnicity			All
		Female	Male	Pregnant	Not pregnant	<1 year	1-<5 years	5-<15 years	≥15 years	Non-Papuan	Highland Papuan	Lowland Papuan	
Malaria negative	slide	1,699 (84.3)	1,530 (81.6)	74 (85.1)	1,625 (84.2)	159 (89.8)	507 (79.0)	668 (81.4)	1,895 (84.2)	1,582 (86.8)	813 (77.9)	834 (81.5)	3,229 (83.0)
<i>P. falciparum</i>		149 (7.4)	166 (8.9)	7 (8.0)	142 (7.4)	5 (2.8)	50 (7.8)	70 (8.5)	190 (8.4)	117 (6.4)	106 (10.2)	92 (9.0)	315 (8.1)
<i>P. vivax</i>		128 (6.3)	122 (6.5)	6 (6.9)	122 (6.3)	12 (6.8)	62 (9.7)	52 (6.3)	124 (5.5)	103 (5.7)	93 (8.9)	54 (5.3)	250 (6.4)
<i>P. malariae</i>		11 (0.5)	13 (0.7)	0 (0)	11 (0.6)	0 (0)	3 (0.5)	11 (1.3)	10 (0.4)	3 (0.2)	9 (0.9)	12 (1.2)	24 (0.6)
Mixed species		29 (1.4)	43 (2.3)	0 (0)	29 (1.5)	1 (0.6)	20 (3.1)	20 (2.4)	31 (1.4)	18 (1.0)	23 (2.2)	31 (3.0)	72 (1.9)
Total		2,016 (51.8)	1,874 (48.2)	87 (4.3)	1,929 (95.7)	177 (4.6)	642 (16.5)	821 (21.1)	2,250 (57.8)	1,823 (46.9)	1,044 (26.8)	1,023 (26.3)	3,890 (100)

Abbreviations: n; number

6.3.2. Anaemia

The mean haemoglobin in the sample as a whole was 11.0g/dL (95% reference range 6.1-15.9g/dL) (Table 6.2) with 5.7% of individuals having a concentration less than 7g/dL (222/3,890). Presence of parasitaemia shifted the haemoglobin distribution curve markedly to the left (Figure 6.2). Indigenous Papuans and females had significantly lower haemoglobin concentrations than their counterparts in univariable analyses while mean haemoglobin increased with increasing age up to 15 years. No correlation existed between haemoglobin and log parasite density for *P. falciparum* (Pearson's correlation coefficient (r) = -0.08, $n=290$ (95% CI -0.19-0.04), $p=0.2$) whereas for *P. vivax* there was a weak but statistically significant inverse correlation (r = -0.24, $n=248$ (95% CI -0.35- -0.12), $p<0.001$). Those with parasitaemia and fever at the time of the survey or within the last 24 hours ($n=205$) had the same haemoglobin concentrations as parasitaemic individuals without fever ($n=456$) (mean haemoglobin = 9.85 versus 9.93g/dL, $p=0.4$).

After adjusting for age, sex, ethnicity, pregnancy and malnourishment, presence of *P. falciparum* parasitaemia was associated with an absolute reduction in haemoglobin of 1.2g/dL (95% CI 0.9-1.4), $p<0.001$) (Table 6.3). The corresponding values for *P. vivax* and mixed infections were 0.7g/dL (95% CI 0.3-1.0) and 1.3g/dL (95% CI 0.7-1.8) respectively ($p<0.001$ for both). Although numbers were small, *P. vivax* was associated with a large mean reduction in haemoglobin of 1.9g/dL (95% CI 1.0-2.9), $p<0.001$) in infants. There was a smaller and non-statistically significant reduction for infants with *P. falciparum* (mean reduction = 0.7g/dL (95% CI -0.9-2.2), $p=0.4$).

Table 6.2 Haemoglobin by clinical and demographic group

	n	Mean Hb	Std. dev.	<i>p</i>
<i>Plasmodium</i> species				
Negative	3,229	11.2	2.41	<0.0001
<i>P. falciparum</i>	315	9.8	2.43	
<i>P. vivax</i>	250	10.2	2.58	
<i>P. malariae</i>	24	8.9	2.42	
Mixed infection	72	9.4	2.65	
Sex				
Male	1,874	11.4	2.70	<0.0001
Female	2,016	10.6	2.17	
Ethnicity				
Non-Papuan	1,823	11.9	2.27	<0.0001
Highland Papuan	1,044	10.0	2.48	
Lowland Papuan	1,023	10.5	2.32	
Age				
< 1 year	177	9.4	1.64	<0.0001
1-5 years	642	9.8	1.98	
5-15 years	821	10.4	2.03	
> 15 years	2,250	11.7	2.56	
Pregnancy status				
Pregnant women	1,929	10.1	1.86	0.05
Non-pregnant women	87	10.6	2.18	
Weight for age/sex/ethnicity				
≥ mean	1,809	11.2	2.47	<0.0001
< mean	2,081	10.8	2.47	
Household income per person				
>75 th centile	823	11.3	2.35	<0.0001
25 th – 75 th centile	1,776	11.2	2.51	
<25 th centile	974	10.7	2.43	
Missing	317	9.7	2.30	
All	3,890	11.0	2.48	

Abbreviations: Hb; haemoglobin, Std. dev.; standard deviation, n; number

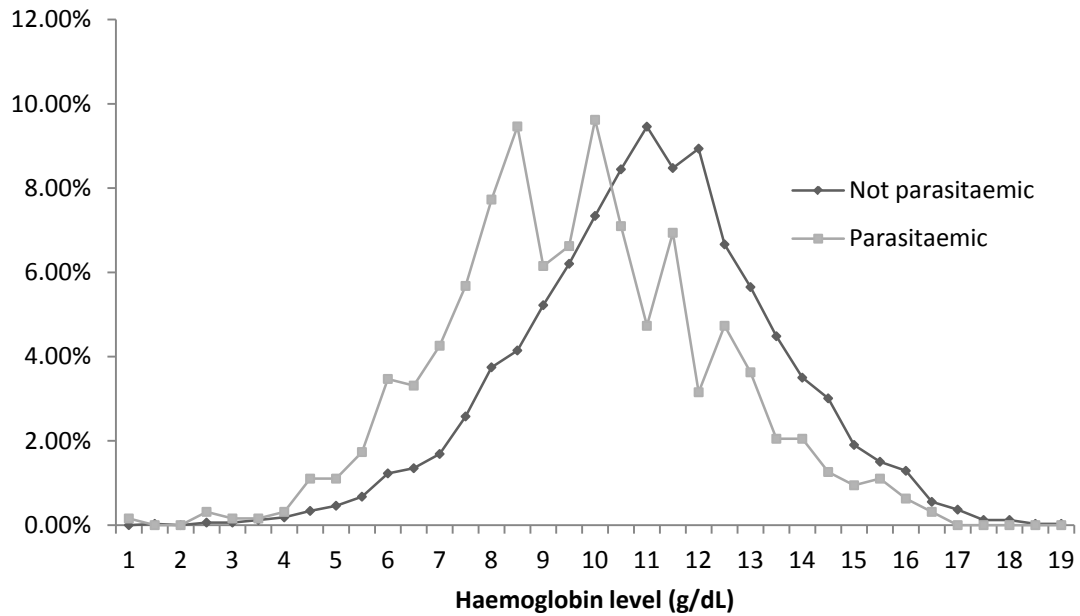


Figure 6.2 Frequency distribution of haemoglobin concentrations by presence or absence of *Plasmodium* parasitaemia

Table 6.4 shows adjusted odds ratios for having a haemoglobin concentration less than 7g/dL. Individuals with mixed infections were most likely to have moderate or severe anaemia (AOR = 3.2 [95% CI 1.7-6.1], $p < 0.001$) followed by patients with *P. falciparum* (AOR = 2.3 [95% CI 1.6-3.5], $p < 0.001$) and *P. vivax* respectively (AOR = 1.9 [95% CI 1.1-3.2], $p = 0.02$). For infants with *P. vivax* the adjusted odds ratio was 37 (95% CI 2.8-500), $p = 0.006$ and for infants with *P. falciparum* it was 6.7 (95% CI 0.6-76), $p = 0.1$.

Table 6.3 Multiple linear regression showing the effect of *Plasmodium* infection on mean haemoglobin concentration (g/dL)

	< 1 year ^a			1-<5 years ^a			5-<15 years ^a			≥15 years ^a			All ^b		
	Coef	95% CI	p	Coef	95% CI	p	Coef	95% CI	p	Coef	95% CI	p	Coef	95% CI	p
Malaria															
Negative	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
All species	-1.4	-2.2- -0.5	0.003	-1.3	-1.7- -0.9	<0.001	-1.1	-1.5- -0.7	<0.001	-1.1	-1.4- -0.8	<0.001	-1.0	-1.2- -0.8	<0.001
<i>P. falciparum</i>	-0.7	-2.2- 0.9	0.4	-1.6	-2.1- -1.1	<0.001	-1.4	-1.9- -0.9	<0.001	-1.3	-1.6- -0.9	<0.001	-1.2	-1.4- -0.9	<0.001
<i>P. vivax</i>	-1.9	-2.9- -1.0	<0.001	-0.8	-1.3- -0.3	0.001	-0.4	-0.9- 0.1	0.2	-0.8	-1.2- -0.3	<0.001	-0.7	-1.0- -0.3	<0.001
Mixed	-	-	-	-1.9	-2.9- -0.9	<0.001	-1.8	-2.4- -1.3	<0.001	-1.1	-2.0- -0.3	0.01	-1.3	-1.8- -0.7	<0.001

Abbreviations: Coef; coefficient, 95% CI; 95% confidence interval

^a Models also include sex, ethnicity (non-Papuan, Highland Papuan, Lowland Papuan), weight for age/sex/ethnicity (< mean, ≥ mean) and pregnancy status (where applicable)

^b Model also includes age (as a continuous variable) by sex, ethnicity (non-Papuan, Highland Papuan, Lowland Papuan), weight for age/sex/ethnicity (< mean, ≥ mean) and pregnancy status

Table 6.4 Adjusted odds ratios for having a haemoglobin concentration less than 7g/dL

	< 1 year ^a			1-<5 years ^a			5-<15 years ^a			≥15 years ^a			All ^b		
	AOR	95% CI	<i>p</i>	AOR	95% CI	<i>p</i>	AOR	95% CI	<i>p</i>	AOR	95% CI	<i>p</i>	AOR	95% CI	<i>p</i>
Parasite negative	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-
Any species	12	2.3-64	0.003	2.5	1.2-4.9	0.01	2.5	1.2-5.0	0.01	2.1	1.3-3.5	0.004	2.3	1.6-3.2	<0.001
<i>P. falciparum</i>	6.7	0.6-76	0.1	2.9	1.3-6.9	0.01	3.0	1.2-7.4	0.02	2.0	1.0-3.7	0.04	2.3	1.6-3.5	<0.001
<i>P. vivax</i>	37	2.8-500	0.006	1.5	0.5-3.9	0.5	1.8	0.6-5.5	0.3	2.1	0.9-4.7	0.08	1.9	1.1-3.2	0.02
Mixed species	-	-	-	4.8	1.6-14	0.005	2.1	0.5-9.6	0.3	3.1	0.7-14	0.1	3.2	1.7-6.1	<0.001

Abbreviations: AOR; adjusted odds ratio, 95% CI; 95% confidence interval

^a Models also include sex, ethnicity (non-Papuan, Highland Papuan, Lowland Papuan), weight for age/sex/ethnicity (< mean, ≥ mean) and pregnancy status (where applicable)

^b Model also includes age (as a continuous variable) by sex, ethnicity (non-Papuan, Highland Papuan, Lowland Papuan), weight for age/sex/ethnicity (< mean, ≥ mean) and pregnancy status

6.3.3. Population attributable fractions of anaemia due to malaria

Parasitaemia due to any *Plasmodium* species was responsible for 17% (95% CI 9-24%) of cases of moderate or severe anaemia in the community sample (Table 6.5). The corresponding values for *P. falciparum*, *P. vivax* and mixed infections were 9% (95% CI 3-13%), 5% (95% CI 0-9%) and 3% (95% CI 1-6%) respectively. The attributable fraction was greatest in infants (34%, [95% CI -3-58%]) and decreased with increasing age thenceforth. *Plasmodium vivax* was responsible for greater than three times the proportion of moderate or severe anaemia in infants compared with *P. falciparum* (28% versus 8%) though the precision of these estimates was poor (95% CI -3-49% and -6-20% respectively). Figure 6.3 indicates that in general, a greater proportion of moderate or severe anaemia (haemoglobin [Hb] <7g/dL) was attributable to malaria than mild anaemia (Hb <11g/dL).

Table 6.5 Adjusted population attributable fractions of moderate or severe anaemia (haemoglobin concentration less than 7g/dL) by presence or absence of *Plasmodium* parasitaemia

	< 1 year ^a		1-5 years ^a		5-15 years ^a		> 15 years ^a		All ^b	
	aPAF	95% CI	aPAF	95% CI	aPAF	95% CI	aPAF	95% CI	aPAF	95% CI
Any species	34%	-3-58%	23%	2-39%	19%	1-34%	12%	2-21%	17%	9-24%
<i>P. falciparum</i>	8%	-6-20%	11%	0-20%	13%	0-25%	6%	-1-12%	9%	3-13%
<i>P. vivax</i>	28%	-3-49%	5%	-7-13%	4%	-5-12%	4%	-2-10%	5%	0-9%
Mixed species	-	-	3%	1-15%	2%	-3-7%	2%	-2-5%	3%	1-6%

Abbreviations: aPAF; adjusted population attributable fraction, 95% CI; 95% confidence interval

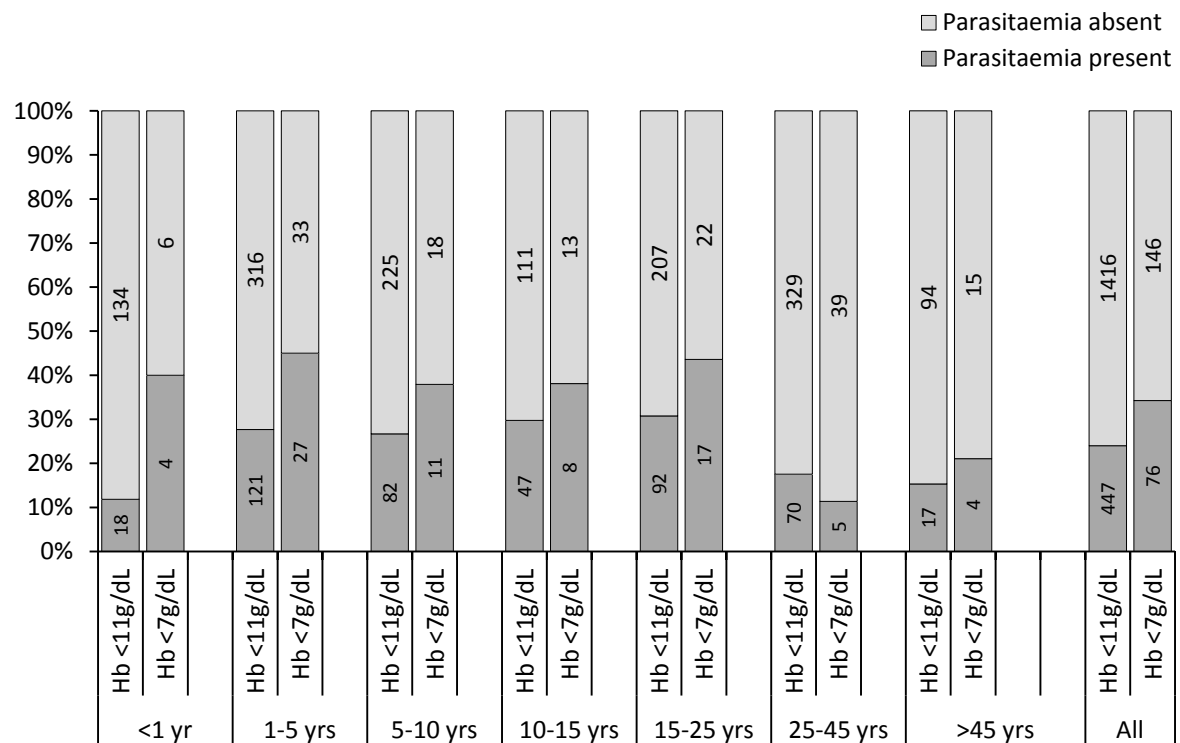


Figure 6.3 Proportion of participants with haemoglobin concentrations <11g/dL and <7g/dL who had parasitaemia (bar labels = absolute numbers)

6.4 Discussion

Despite comparatively low-level endemicity, malaria in Southern Papua is associated with 17% of all moderate to severe anaemia in the community. In infants and young children, the corresponding proportions rise to 34% and 23% respectively. Although *P. vivax* is less prevalent than *P. falciparum* overall, this analysis shows that in the community in Mimika District it is the commoner species in children under 5 years of age and that it is associated with a much higher population attributable fraction of anaemia in infants. Similar to the hospital setting, mixed species infections in the community are associated with a greater reduction in haemoglobin than *P. falciparum* or *P. vivax* infections alone.

In many parts of Africa, only a small minority of individuals will be aparasitaemic at any point in time making it difficult to determine the haematological impact of asymptomatic infection. In this community sample, 83% of individuals were aparasitaemic at the time of the survey enabling a valid assessment of the combined impact of symptomatic and asymptomatic parasitaemia. Despite this, our results are still likely to represent an underestimate of the true total effect of malaria on haemoglobin in the community. Full haematological recovery takes several weeks following acute malaria [153] suggesting that many aparasitaemic individuals in our sample may have been experiencing the haematological after-effects of recent malaria infection. Forty-two percent (1629/3890) of the survey participants had a history of fever in the last month and these individuals had both a significantly lower mean haemoglobin concentration and an increased unadjusted odds ratio for moderate or severe anaemia compared to those without a history of fever (mean haemoglobin concentration, 10.6 versus 11.3g/dL, $p < 0.001$, OR for moderate or severe anaemia, 1.7 (95% CI 1.3-2.5), $p < 0.001$. Since 35% of fevers in the community are estimated to be due to malaria, parasitaemia is likely to have been responsible for a sizeable, but unmeasured, proportion of all anaemia in the aparasitaemic group.

Our estimates for the adjusted population attributable fraction of moderate or severe anaemia in infants are based on very small numbers. Nevertheless, the finding that *P. vivax* accounts for a greater fraction of anaemia than *P. falciparum* is in agreement with results from Mitra Masyarakat Hospital presented in Chapter 5 [49,118] as well as a previous cross-sectional survey in Papua New Guinea [264]. Chapter 5 shows that *P. vivax* is the most common cause of malaria-associated presentation to hospital in the first year of life and in this age group causes an equal or greater reduction in haemoglobin than *P. falciparum* [49,118]. Both the greater prevalence of vivax malaria and the severity of the associated anaemia in infancy can probably be related to multiple relapses causing repetitive insults to the haematological system in turn inducing early development of immunity. Two pieces of evidence from this community study support this assertion. First, there was a statistically

significant reduction in the odds of *P. vivax* parasitaemia with age, whereas there was no such reduction in odds for *P. falciparum*. Second, infants with *P. vivax* parasitaemia had had significantly more episodes of fever in the last month than infants with *P. falciparum* (1.1 versus 0.4, $p=0.007$). The subsequent decline in the fraction of anaemia attributable to either species of *Plasmodium* with increasing age is likely to relate to three main factors: the acquisition of some degree of immunity, especially in the case of vivax malaria, the increasing importance of alternative causes of anaemia, such as intestinal helminthiasis and chronic infections, and lastly the increasing likelihood that absence of parasitaemia represents a state of remission or a period between primary infections rather than a state of complete malaria naivety.

The results of this analysis demonstrate that mixed *P. vivax* / *P. falciparum* infections are associated with a greater drop in haemoglobin and a higher risk of moderate or severe anaemia than monoinfections with either species in the community as well as in the hospital. This reiterates that the dynamics of mixed infections in Papua are significantly different to Thailand where *P. vivax* infection attenuates the severity of *P. falciparum* infections [153]. Maitland and colleagues have previously suggested that reducing transmission of *P. vivax* malaria may have unexpected and potentially adverse effects on morbidity associated with *P. falciparum* infection [239]. Our results provide reassurance that at least in Papua, reducing the burden of vivax malaria will result in a fall rather than an increase in total haematological morbidity associated with malaria.

This study has several limitations. Due to the cross-sectional design it was not possible to draw solid conclusions about the direction of the observed associations. Although anaemia is an established sequel of both falciparum and vivax malaria, there is evidence that iron deficiency anaemia reduces the risk of falciparum malaria [427] and conversely, that administering iron supplements to iron replete individuals may slightly increase the risk [141,436]. The comparative effect of this reverse causation is likely to be negligible since there

were no special community-wide iron supplementation programmes at the time of the survey.

Selection bias may have affected the population attributable fractions due to their heavy reliance on the prevalence of parasitaemia in the sample. Those who did not provide a blood sample (mostly due to absence at the time of the survey) tended to be older males. Overall, there was relatively little effect of increasing age or sex on the odds of parasitaemia, however those who did not provide a blood sample could conceivably have been at greater risk of malaria acquisition due to behavioural or lifestyle factors. If this was true, the fraction of anaemia attributable to malaria may have been underestimated.

As in Chapter 5, it was not possible to control for some potentially important confounders including intestinal helminth infection and haemoglobin and red blood cell abnormalities. I adjusted all multivariable models for the effects of ethnicity on haematological status which should have accounted for some of the potential variation in prevalence of haemoglobin and red cell disorders in the study population. Malnutrition is unlikely to have been a major confounder since the models also incorporated the effects of weight for age/sex/ethnicity. Given that iron deficiency is thought to be protective against *Plasmodium* infection [427], any residual confounding caused by malnutrition is likely to have biased the fractions of moderate-to-severe anaemia attributable to malaria towards the null.

Despite comparatively low-level *Plasmodium* endemicity, parasitaemia (whether symptomatic or not) is an important and preventable cause of anaemia in the community in Southern Papua. Young children bear the brunt of this burden but the haematological effects also extend into adulthood. *Plasmodium vivax* is an especially important cause of community anaemia in infants and as such is likely to be an important and underestimated contributor to indirect malaria mortality and developmental morbidity in regions where this species is prevalent.

6.5 Acknowledgements

A modified version of this chapter is soon to be submitted to a peer-reviewed journal. The co-authors are Muhammad Karyana,¹ Lenny Burdarm,² Shunmay Yeung,³ Enny Kenangalem,^{4,5} Julie A Simpson,⁶ Emiliana Tjitra,¹ Nicholas M Anstey^{7,8} and Ric N Price.^{7,8,9}

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7. Mortality attributable to *Plasmodium vivax* malaria

7.1 Introduction

Chapters 5 and 6 have demonstrated that *Plasmodium vivax* is associated with significant haematological morbidity both in the hospital setting and in the community in Southern Papua. However *Plasmodium vivax* is not generally regarded as being sufficiently virulent to cause death. Notwithstanding this benign reputation, over the last two to three decades, there have been multiple case reports of fatal vivax malaria variously attributed to acute respiratory distress syndrome, cerebral malaria, renal failure, metabolic acidosis, liver dysfunction and cardiac arrest [84,100,437-440]. More recently, hospital and outpatient surveillance systems in endemic regions have shown that mortality in those with *P. vivax* parasitaemia may be occurring with greater frequency than previously thought – in particular in those who are severely anaemic or in respiratory distress [49,50,97,99]. Whether vivax malaria was the primary cause of death in these patients was not established.

In Papua, Indonesia, the fatality of hospitalised patients with *P. vivax* parasitaemia has been estimated to approach that of patients with *P. falciparum* infections (1.6% versus 2.2% respectively) [49]. The aim of the present study was to determine the extent to which *P. vivax* contributed to those deaths and thence to estimate the case fatality of vivax infections in Southern Papua. To this end I conducted a review of all clinical records and investigations for

patients with pure *P. vivax* infections who died at Mitra Masyarakat Hospital between January 2004 and September 2009.

7.2 Methods

7.2.1. Prospective hospital surveillance procedures

This study uses data from the active malariometric surveillance system (separate to the hospital-run surveillance system) operated by staff of the Menzies School of Health Research-National Institute of Health Research and Development collaboration. The details of this system were introduced in Chapter 4. In brief, a research nurse searches the wards at Mitra Masyarakat Hospital for any patient with parasitaemia at least once per day. A research physician then reviews patients to determine the presence of severe malaria according to a modified version of the World Health Organization criteria [41]. Respiratory distress (oxygen saturation [off supplemental oxygen] of less than 94% or an age-adjusted elevation in respiratory rate [>32 breaths per minute in adults, >40 breaths per minute in children aged 5-14 years, >50 breaths per minute in children aged 2 months to 5 years and >60 in babies less than 2 months), coma (Glasgow Coma Score <11 or Blantyre Coma Score <3) and anaemia (haemoglobin $<5\text{g/dL}$) are routinely assessed whereas assessment of the other severity criteria (which tend to rely on more specialised laboratory tests) is only done if the clinical indication arises and is therefore less systematic.

Hospital guidelines dictate that all inpatients should have blood taken for malaria microscopy at the hospital laboratory. If parasitaemia is present the density is given semiquantitatively as 1+ to 4+. In a minority of cases films may also be read by a research microscopist and blood may be tested for HRP2 using the Paracheck Pf[®] rapid diagnostic test (RDT).

7.2.2. Death audit

All patients who, according to the prospective surveillance data, had had pure *P. vivax* infection at any stage during their final admission and who subsequently died at Mitra Masyarakat Hospital between January 2004 and September 2009 were eligible for inclusion in the death audit. Those diagnosed by RDT alone and those who only received a microscopic diagnosis of vivax malaria before referral to the hospital were excluded. Data from the clinical notes were extracted by three physicians experienced in internal medicine. Information was recorded on a standardised death audit form and then entered into an EpiData database (version 3.1, EpiData Association, Denmark). The audit form documented presenting symptoms, past medical history, examination findings, laboratory and radiological investigation results, fulfilment of 'severity' criteria, diagnoses given during admission, treatment received and progress. If *Plasmodium* species identification was available from both hospital and research cross-check microscopy, the latter was taken as the best available evidence. A positive HRP2-based rapid antigen test (Paracheck Pf®) was taken as confirmation of *P. falciparum* infection even if this was not detected on standard microscopy. If available, X-rays were interpreted by an independent certified infectious diseases specialist who was blinded to case history. Indicators of severity were only deemed to be present if the relevant criteria were fulfilled prior to the periterminal period (defined as within 6 hours of death).

After all relevant information had been extracted, three independent infectious diseases specialists who had not been involved in the data extraction process independently reviewed each case record and classified the degree to which *P. vivax* was responsible for the deaths. This was done according to *a priori* criteria based on current understanding of plausible pathological mechanisms in severe vivax malaria [104,106,194,441] and prior clinical experience (Figure 7.1). Any disagreements in classification of the cause(s) of death were

resolved by consensus. The sole exception to the criteria was for patients who did not fulfil the rules for inclusion in Category 1 (vivax malaria as primary cause of death) but who had no plausible alternative cause of death. In these instances, vivax malaria was classified as the prime cause of death. Respiratory distress was only considered to be due to vivax malaria if there was no evidence of concomitant bacterial sepsis, conservatively defined as infiltrates on chest X-ray or any deviation in white cell count from the age-adjusted normal range [442]).

7.2.3. Statistical analysis

Data were analysed in STATA® version 10.1 (StataCorp, College Station, Texas, USA) and EpiInfo® version 3.4.3 (Centers for Disease Control and Prevention, USA). Proportions were compared using the chi-square test with Yates' continuity correction or Fisher's exact test and nonparametric continuous data such as patient age were compared using the Mann-Whitney U test. For the purposes of incidence and mortality rate estimations, I assumed a constant population of 150,000 people (mid-way between the Indonesian government census estimates obtained in 2003 and 2007).

1	Pure vivax malaria the primary cause of death	Potential mechanisms include: coma, extreme anaemia (haemoglobin <3g/dL), respiratory distress not associated with evidence of sepsis, ^a acidosis if associated with severe anaemia or splenic rupture
2	Pure vivax malaria likely to have been a major contributor to death	Alternative cause(s) more likely to have led to death but vivax malaria a major contributor through one of the following mechanisms: haemoglobin <7g/dL, respiratory distress not associated with evidence of sepsis, ^a acidosis if associated with haemoglobin <7g/dL, splenic rupture, decreased consciousness or malnutrition ^b with two or more documented episodes of vivax malaria in the last year
3	Pure vivax malaria likely to have been a minor contributor to death	Alternative cause(s) more likely to have led to death but vivax malaria a minor contributor through one of the following mechanisms: fever, tachycardia or anaemia (haemoglobin between 7g/dL and the lower limit of normal)
4	Pure vivax malaria unlikely to have contributed to death	No clear pathophysiological mechanism by which vivax malaria could have exacerbated or contributed to the primary cause(s) of death

^a Sepsis conservatively attributed to bacterial coinfection. Evidence of sepsis defined as infiltrates on chest X-ray or any deviation in white cell count from the age-adjusted normal range (birth 20,000-40,000/mm³, 1wk 5,000-21,000/mm³, 2wks 5,000-20,000/mm³, 3mth-12mth 5,000-15,000/mm³, 2yr-5yr 5,000-12,000/mm³, >5yr 4,000-10,000/mm³)

^b Malnutrition defined as documented malnutrition in the notes or a weight-for-age Z-score less than -3, according to the WHO Child Growth Standards [443]

Figure 7.1 *A priori* criteria for classifying cause of death

7.3 Results

7.3.1. The epidemiology of vivax malaria in Mimika District

Between January 2004 and September 2009 there were an estimated 293,763 clinical or subclinical episodes of vivax malaria in Mimika District [1] resulting in 3,495 admissions to hospital with *P. vivax* parasitaemia (Figure 7.2). The number of patients admitted to hospital with malaria showed a bimodal age distribution with peaks between 0-10 and 15-35 years (Figure 7.3). Surveillance data showed manifestations of severity were present in 845 (24%) inpatients with *P. vivax* infection, with severe anaemia present in 19% (n=652), respiratory distress in 5.3% (185) and coma in 1.6% (55). The corresponding figures for the 10,821 patients admitted with *P. falciparum* infection were 13% (1,451, *p* for difference compared to *P. vivax* <0.001), 5.0% (539, *p*=0.5) and 2.8% (298, *p*<0.001) respectively. During the same period there were 311 deaths in hospitalised patients with malaria, 65 (21%) of whom were reported to have had pure *P. vivax* parasitaemia. Based on these data, there was no difference in the age distribution (*p*=0.74) or the proportion of females (*p*=0.48) or non-Papuans (*p*=0.90) in those who died with *P. vivax* parasitaemia versus those who survived.

7.3.2. The death audit

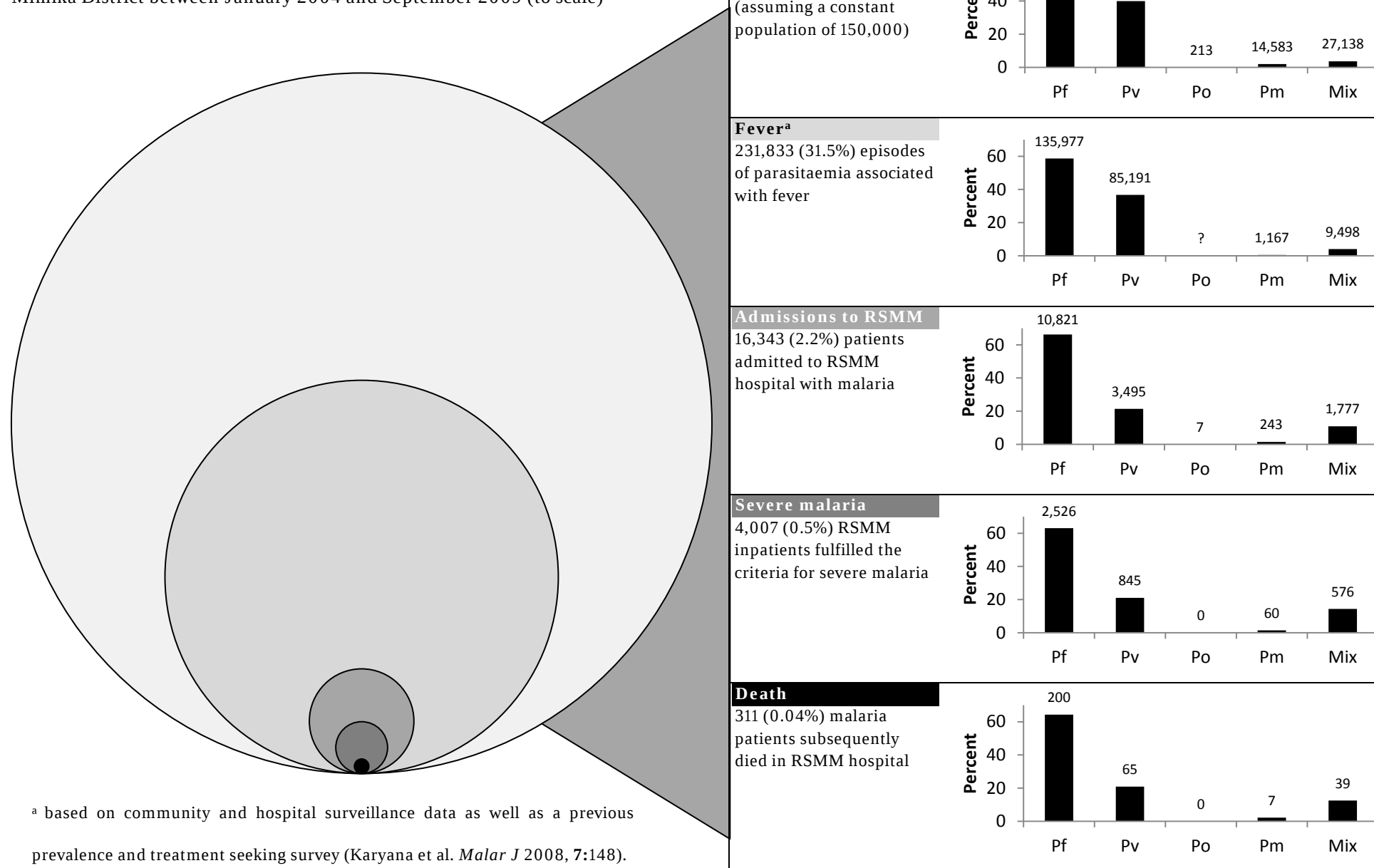
In total 54 (83%) of the 65 relevant clinical charts were available for the death audit (Figure 7.4). The 11 patients for whom charts were not available had similar baseline characteristics to the remaining 54 patients. Two patients were excluded from the audit because they received their parasitological diagnoses outside of the hospital or based exclusively on results from a rapid diagnostic test.

Blood films were available for cross-checking in 19% (10/52) of the remaining cases (Table 7.1) and altered the hospital diagnosis from *P. vivax* monoinfection to mixed *P. vivax* / *P. falciparum* infection in one case. Paracheck Pf® tests were performed at the time of

admission in 19% (10/52) of cases and altered the diagnosis for one further case from *P. vivax* to mixed infection. On review of the notes and the above laboratory findings, a total of 12 of 52 cases (23%) had been incorrectly coded as *P. vivax* infections leaving 40 (77%) patients who, according to the best available evidence, had pure *P. vivax* parasitaemia. Chest X-rays were available for review in 26 of these cases.

Five patients died of causes thought unlikely to be related to *P. vivax* infection (Figure 7.4); two due to road traffic accidents, one due to electrocution, one due to tuberculous meningoencephalitis and one due to severe malnutrition with presumed sepsis. These patients were excluded from further analysis.

Figure 7.2 Distribution of malaria cases, hospital admissions and deaths in Mimika District between January 2004 and September 2009 (to scale)



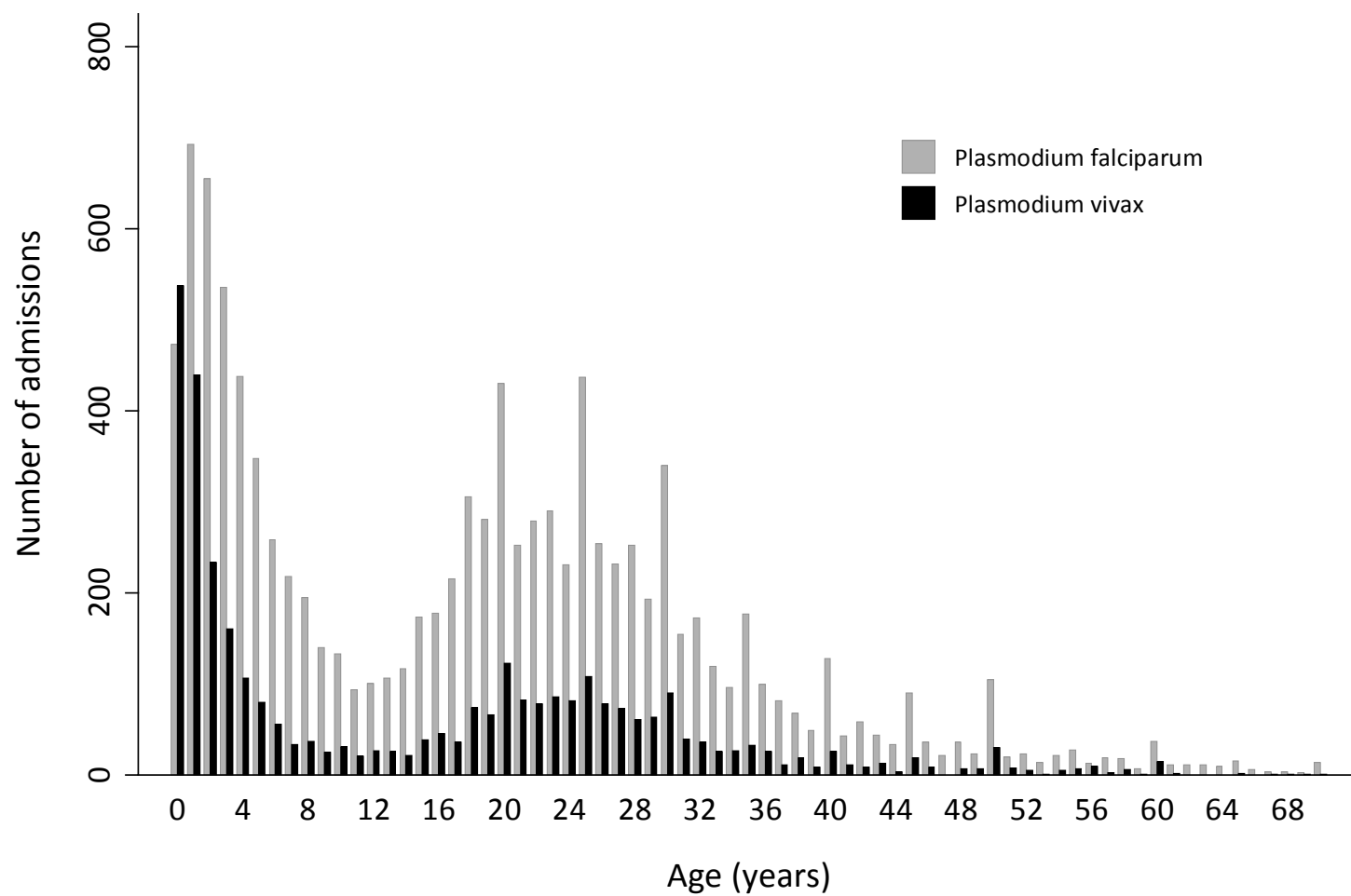


Figure 7.3 Age distribution of patients admitted to Mitra Masyarakat Hospital with malaria between January 2004 and September 2009 by *Plasmodium* species

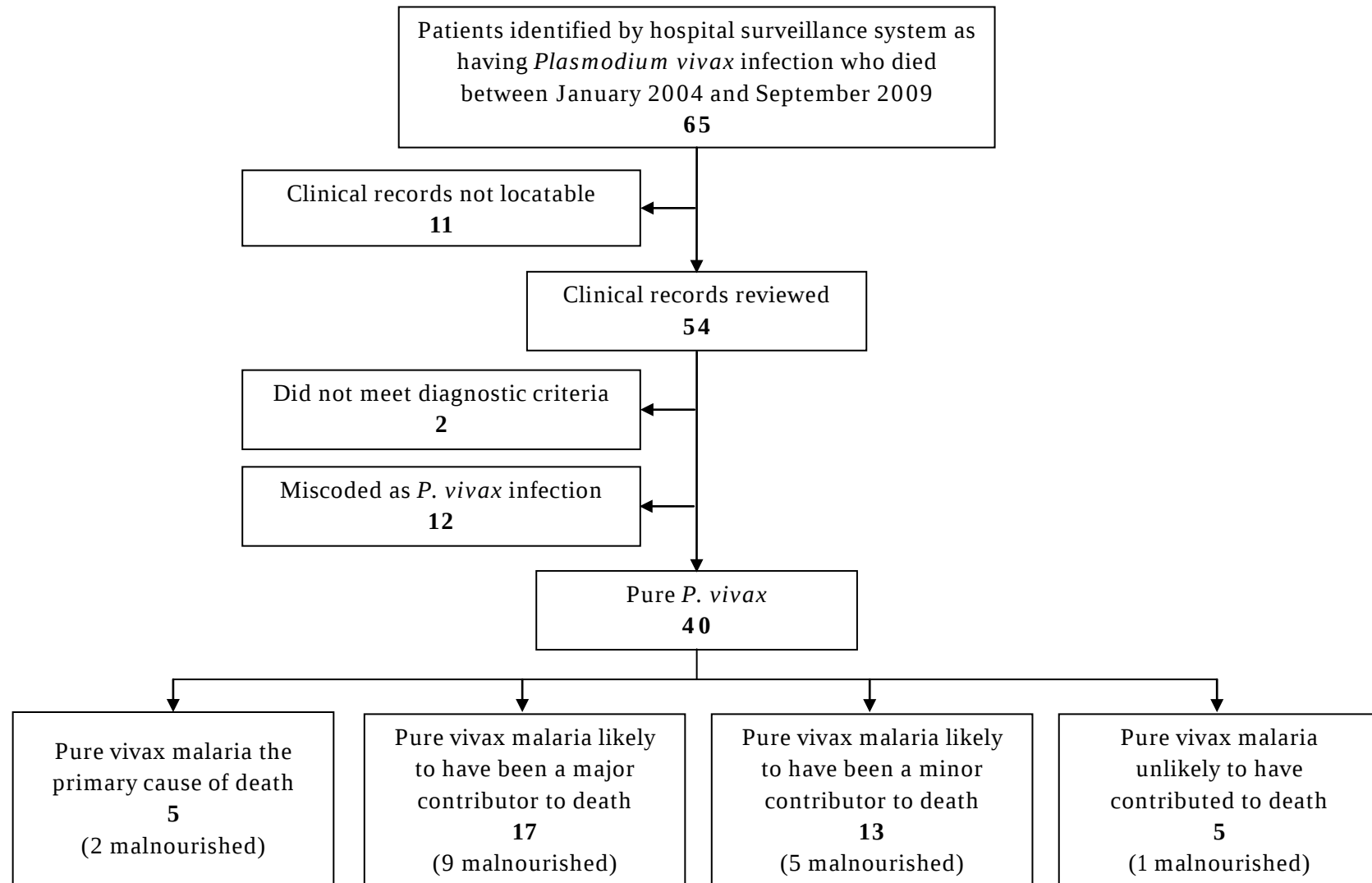


Figure 7.4 Death audit profile

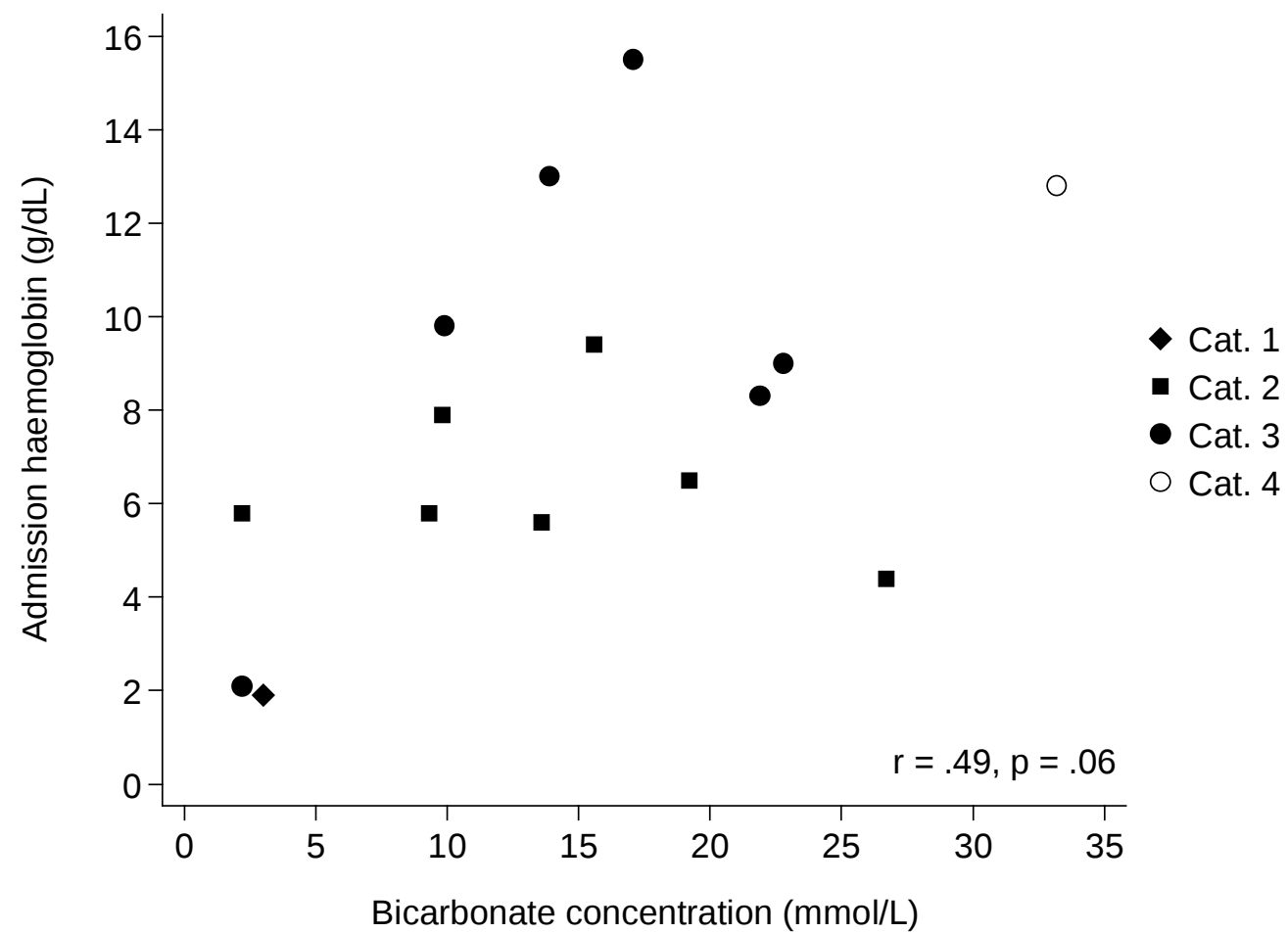


Figure 7.5 Relationship between admission haemoglobin concentration and acidosis in audited individuals with vivax malaria. Categories refer to the contribution of vivax malaria to death as determined by three independent infectious diseases specialists.

Table 7.1 Clinical details of the patients whose deaths were associated with vivax malaria

	Patient number	Sex	Age	Time to death	Weight (kg)	Documented malnutrition	Documented <i>P. vivax</i> in last year	<i>P. vivax</i> parasitaemia	Haemoglobin	White blood cell count per µL	Platelet count per µL	Bilirubin concentration (mg/dL)	Glucose concentration (mg/dL)	CXR	Individual assessors' classifications	Primary cause of death	Indirect causes
1. Primary cause	1	F	19y	1d	40	N	2	++++	2.2	7000	11	7.9	147	-	1,1,1	Vivax malaria with extreme anaemia	
	2 ^a	F	1y7m	1d	7	N	1	++	4.7	8000	142	-	-	-	1,1,1	Vivax malaria	Malnutrition
	3	F	24y	4d	56	N	0	++++	2.6	16100	51	1.7	110	Resolving minor RLL consolidation compared with film 1 month prior	1,1,1	Vivax malaria with extreme anaemia	
	4 ^b	F	10m	3d	7	Y	0	++++	1.9	11800	64	-	244	Day 0 – normal Day 2 – whiteout of R lung and part of L lung. Pulmonary oedema in remainder of L lung	1,1,1	Vivax malaria with extreme anaemia and respiratory distress	Malnutrition
	5	F	1y9m	2d	11	N	0	++++	1.7	20100	139	4.7	101	-	1,1,1	Vivax malaria with extreme anaemia	Possible underlying sepsis

2. Major contributor	6 ^a	F	4y4m	2d	10	Y	0	++++	8.6	14300	13	0.8	87	Normal	2,2,2	Respiratory tract infection	Malnutrition Vivax malaria
	7 ^a	F	3y1m	3h	12	N	0	++	-	-	-	-	-	Normal	2,2,2	Respiratory tract infection	Vivax malaria
	8	M	1y9m	7d	8	Y	3	+++	9.2	11000	163	-	-	-	1,2,2	Malnutrition with sepsis	Vivax malaria
	9	F	1y8m	4d	8	Y	0	++++	5.1	4200	154	-	-	Poor inspiratory effort, unable to assess	2,2,2	Respiratory tract infection	Malnutrition Vivax malaria
	10	F	1y8m	1d	-	Y	0	+++	7.3	13900	13	-	393	Normal	1,2,3	Respiratory tract infection	Vivax malaria Malnutrition
	11 ^a	F	1y3m	9d	6	Y	3	+++	12.5	25300	136	0.3	122	-	2,3,3	Diarrhoeal disease	Malnutrition Vivax malaria
	12	M	1y	7d	6	Y	0	++	7.9	8300	105	-	83	Normal	3,2,2	Diarrhoeal disease	Vivax malaria Malnutrition
	13	F	38y	13d	26	Y	0	+++	6.3	14300	55	5.1	109	Minor chronic changes in LLL	2,2,2	AIDS	Vivax malaria Malnutrition
	14	M	3m26d	5h	-	N	0	+	2.8	29000	123	-	118	Normal	1,2,2	Sepsis	Vivax malaria anaemia
	15	F	2y	2d	12	Y	0	+	4.4	10600	21	1.0	77	Evidence of pneumonia	2,2,2	Respiratory tract infection	Vivax malaria Malnutrition
	16	F	1y5m	17d	5	Y	3	++	6.7	9500	262	-	108	Diffuse changes consistent with pneumonia, R > L	2,2,2	AIDS	Vivax malaria Malnutrition Tuberculosis
	17	F	27y	5d	-	N	1	+	6.5	3800	80	-	-	-	2,2,2	AIDS	Vivax malaria
	18	M	1y23d	6h	9	N	0	+++	5.8	4700	229	-	156	RUL consolidation	2,2,2	Bronchopneumonia	Vivax malaria
	19	F	34y	8h	50	N	0	++	5.8	14100	252	-	88	Bilaterally increased lung markings, R > L, consistent with ARDS or pneumonia	2,2,2	Chronic renal failure	Vivax malaria Respiratory tract infection
	20 ^b	F	61y	6d	50	N	0	+	4.1	3500	9	1.3	95	RML collapse with consolidation, calcification and scarring	2,2,2	Respiratory tract infection	Vivax malaria
	21 ^{a,b}	F	25y	5h	75	N	0	+	6.2	23700	22	-	278	-	2,2,2	GI bleeding secondary to	Vivax malaria

															NSAID		
3. Minor contributor	22 ^b	M	3y	1d	11	N	0	++	9.4	9600	152	-	30	Admission – underexposed but probably normal Later in the day – collapse lung with ETT in R main bronchus. R lung normal	2,2,2	Sepsis	Vivax malaria Trauma
	23	M	2y4m	6d	9	Y	1	++	13.0	12600	231	2.4	124	-	2,3,2	Diarrhoeal disease	Sepsis Malnutrition Vivax malaria
	24	M	59y	3d	50	N	0	+	10.5	35500	173	2.0	75	-	3,3,3	Respiratory sepsis	COPD Vivax malaria Ischaemic heart disease
	25	M	2y10m	5d	12	Y	1	+	9.0	13200	124	-	104	Dense, bilateral patchy consolidation L > R	3,3,3	Tuberculosis with sepsis	Vivax malaria Malnutrition
	26 ^b	F	14y	8d	-	N	0	+++	2.1	4200	89	1.1	-	-	3,2,3	Uraemia secondary to endstage chronic renal failure	Vivax malaria
	27 ^a	M	18y	1d	50	N	0	++	15.5	16100	100	-	206	Normal	3,3,3	Meningitis	Vivax malaria
	28 ^a	F	60y	1d	37	N	0	+	11.5	11800	119	-	102	-	3,3,4	Hepatorenal syndrome	Vivax malaria Possible bacterial infection
	29	F	1y8m	1d	7	N	1	++	9.8	79300	580	-	44	CXR 2 days prior showed infiltrate obscuring L heart border	3,3,3	Respiratory tract infection with overwhelming sepsis	Vivax malaria Malnutrition
	30	F	24y	5d	33	N	2	+	4.7	1500	85	0.4	103	Worsening RML consolidation and cavitation compared with previous films. L mid and lower zone consolidation and cavitation. R mediastinal mass	3,3,3	AIDS with tuberculosis	Vivax malaria
	31	M	5m4d	12d	7	N	0	++	13.0	7200	107	-	-	Infiltrate at R hilum	3,4,3	Bronchopneumonia	Vivax malaria

32	F	1y8m	1d	6	Y	0	++++	10.2	19700	110	-	21	Normal	3,3,3	Diarrhoeal disease with hypovolaemic shock	Vivax malaria Malnutrition
33	F	55y	4d	60	N	0	++++	10.1	19500	55	-	66	Increased lung markings bilaterally	3,3,3	Sepsis	Vivax malaria
34	F	4y	1d	16	N	0	+	11.2	22700	461	-	109	-	3,3,3	GI sepsis	Vivax malaria
35	M	31y	10d	38	Y	1	+	9.9	3700	114	0.2	82	-	3,3,3	HIV associated enteropathy	Malnutrition Vivax malaria

^a Research microscopy done

^b Paracheck Pf[®] done

Abbreviations: F; female, M; male, y; year, m; month, d; day, CXR; chest X-ray, R; right, L; left, RLL; right lower lobe, RML, right middle lobe, RUL; right upper lobe, ETT; endotracheal tube, LLL; left lower lobe, ARDS; acute respiratory distress syndrome, NSAID; non-steroidal anti-inflammatory drug, AIDS; acquired immunodeficiency syndrome, GI; gastrointestinal, HIV; human immunodeficiency virus

The median ages of patients in whom *P. vivax* infection was the prime cause of death (Category 1) or a major contributor (Category 2) were similar (1.8 years [interquartile range (IQR) 1.2-22 years] and 2.0 years [IQR 1.3-26 years] respectively, $p=0.70$), and not significantly lower than the median age of patients for whom *P. vivax* infection was a minor contributor (median 14.3 years [IQR 2.0-43 years], $p=0.15$). There was a predominance of females in all three categories (100% [5/5], 71% [12/17] and 54% [7/13], respectively), although this was also the case in hospitalised patients with vivax malaria as a whole (60% [2,081/3,495]).

Overall the most prominent manifestation of severity was respiratory distress which was present in 74% (25/34) of cases. In total, 17 of 21 (81%) children less than 5 years old had this manifestation compared with 8 out of 13 (62%) of those 5 years or older ($p=0.3$).

Four (13%) of the 31 patients in whom a Glasgow or Blantyre Coma Score was reported fulfilled the criteria for cerebral malaria prior to the periterminal period. None of these cases of coma was deemed to be primarily attributable to vivax malaria.

Overall there was a borderline significant linear association between anaemia and acidosis ($r=0.49$, $p=0.06$; Figure 7.5). Severe anaemia was present in 29% (10/34) and acidosis in 9 (64%) of the 14 patients who had blood gas analyses (three of the latter patients also had severe anaemia and 8 had respiratory distress). Marked thrombocytopenia was relatively common with 6 (18%) of 34 patients having a platelet concentration of less than 30×10^9 per litre. Six patients had an abnormal bilirubin concentration, three of whom succumbed to vivax malaria as a direct cause of death. Hypoglycaemia occurred in 4 cases and a creatinine concentration above the upper limit of normal occurred in 16 (55%) of 29 patients. Only 5 of the latter patients had a concentration above $230 \mu\text{mol/L}$ – the relevant WHO criterion for severe malaria.

Three recurring patterns, and potential mechanisms, of death emerged. The first mechanism was extreme anaemia (haemoglobin <3g/dL) which was deemed to be the cause of death in 4 of the 5 patients in Category 1. The second was a combination of severe malnutrition and vivax malaria, often with additional sepsis with respiratory or gastrointestinal manifestations. Overall, 16 (46%) of the 35 vivax-associated deaths were in patients with malnutrition, of whom 8 had documented evidence of one or more previous episodes of vivax malaria in the last year. The third mechanism was vivax malaria in association with chronic or subacute morbidity such as renal disease (n=2), HIV infection (n=5) or active tuberculosis (n=2).

7.3.3. Overall risks

After correcting for errors in species identification, *Plasmodium vivax* was found to have caused or contributed to a minimum of 35 deaths over the 69-month accrual period, corresponding to a minimum case fatality of 10.0 per 1,000 (35/3,495) hospitalised patients with *P. vivax* infection (Table 7.2). The overall minimum case fatality of *P. vivax* infections in the community was 0.12 per 1,000 (35/293,763) infections. The upper limits for the hospital and community fatality estimates were 16.6 and 0.62 per 1,000 respectively (see Table 7.2 for the assumptions underlying this sensitivity analysis). The case fatality of vivax malaria in children under 5 years of age compared with those 5 years of age or older was 14.2 versus 7.0 per 1,000 in the hospital and 0.25 versus 0.07 per 1,000 in the community as a whole.

Table 7.2 Case fatality per 1,000 patients with vivax malaria

	Hospitalised Patients		Overall Population ^b	
	Minimum (n/D)	Upper limit ^a (n/D)	Minimum (n/D)	Upper limit (n/D)
< 5 years	14.2 (21/1,480*)	18.9 (28/1,480)	0.25 (21/84,028)	1.04 (87.5/84,028)
≥ 5 years	7.0 (14/1,986*)	14.6 (29/1,986)	0.07 (14/209,735)	0.43 (90.6/209,735)
All	10.0 (35/3,495)	16.6 (58/3,495)	0.12 (35/293,763)	0.62 (181.3/293,763)

Abbreviations: n; number, D; denominator

^a Upper limits were calculated assuming the following: 1) all of the patients for whom notes were not available died of causes related to *P. vivax* infection, 2) an equal number of deaths were miscoded as being attributable to *P. falciparum* as were miscoded as being attributable to *P. vivax*

^b Denominators include hospital and community patients with *P. vivax*, estimated from the total number of cases seen in the community surveillance network multiplied by the reciprocal of the proportion who sought treatment at one of the network facilities (40%, established from a house-to-house survey of treatment seeking behaviour [Karyana et al. *Malar J* 2008, 7: 148]). To calculate the upper limits, the same assumptions were made as in the hospitalised patients except the number of deaths was also multiplied by the reciprocal of 32% (the proportion of deaths estimated to occur at the hospital [unpublished data]).

* A small proportion of individuals in the active surveillance database had an unknown age

7.4 Discussion

Over recent years, malariometric surveillance in Southern Papua has identified a significant number of individuals who have died following *P. vivax* infection. A previous publication based on an earlier set of surveillance data reported an overall risk of death in patients admitted with vivax malaria of 1.6%, raising significant concerns that the virulence of this organism had been underestimated [49]. In the current analysis, *a priori* definitions were used to classify the contribution of vivax malaria to those deaths. Individuals with mixed

Plasmodium species infections were excluded and conservative criteria were used to define potential concomitant bacterial sepsis. Our results highlight that over a 5.75-year period, a minimum of 35 deaths could be at least partially attributed to *P. vivax* infection and that in 5 cases, vivax malaria was deemed to be the primary cause of death. This resulted in a case fatality of 1 in 100 for patients admitted to hospital and 1 in 8,393 for the community as a whole. Children under five years old were at a 2 to 4-fold greater risk of death compared to those older than 5 years.

Four of the patients in this study died of extreme anaemia (probably as a result of high output cardiac failure and acidosis), while severe anaemia was a major contributor in 4 other cases. Chapters 5 and 6 have shown that in Timika, the mean reduction in haemoglobin and risk of severe anaemia is greater for infants (<1 year of age) infected with *P. vivax* when compared with *P. falciparum* [49,118]. Thereafter, the haematological impact of *P. falciparum* exceeds that of *P. vivax*. Vivax-associated anaemia has also been shown to be at least as frequent and severe as anaemia caused by falciparum malaria in parts of Latin America [117]. Severe anaemia associated with *P. vivax* infection is likely to be an important comorbidity in young children with malnutrition and other infectious diseases.

Overall, nearly half (46%) of the patients who died of causes related to vivax malaria had evidence of malnutrition and a high proportion of these individuals also had clinical or radiological evidence of possible concomitant bacterial infection. Half of the malnourished patients had had repeated episodes of vivax malaria within the last year, probably as a result of relapsing disease. Chronic and induced *P. vivax* infections have been implicated in a protein-wasting condition akin to kwashiorkor [37,39]. Indeed, the evidence that *P. vivax* causes malnutrition is stronger than that for *P. falciparum* [38]. It is also likely that there is an epidemiological, and possibly a biological, association between malnutrition and a greater

risk of *P. vivax* infection. While this relationship is probably bidirectional, our study supports previous literature suggesting that malnutrition significantly worsens the outcome of infectious disease and implicates vivax malaria not only as a potential cause of malnutrition, but also a subsequent precipitating cause of death in patients with malnutrition.

Over 70% of audited individuals fulfilled the criteria for respiratory distress. Previous work has suggested that vivax-associated lung dysfunction during recovery from malaria may occur from an inflammatory response in the pulmonary microvasculature following commencement of treatment [158]. Severe anaemia and metabolic acidosis are also likely to be key determinants, particularly in children, in whom lung injury is less common than in adults. Eight of the 9 patients with acidosis were found to be in respiratory distress as were all 10 patients with severe anaemia. Only one patient (a 10-month old girl) was documented to have an acute respiratory distress-type picture as described in previous case reports [104,444].

Studies of *P. falciparum* infection in African children have shown high co-prevalence of pneumonia, meningitis and bacteraemia in children with severe malaria [445-447], the largest of which demonstrates a strong association between coinfection and an increased risk of death [447]. Since microbiological data were not available in our study, any deviation in white cell count or an infiltrate on chest X-ray was conservatively regarded as evidence of concomitant bacterial infection. In other studies, albeit without routine blood cultures for bacteraemia, severe and fatal sepsis syndromes have been attributed to vivax malaria [448]. Given the potential for *P. vivax* to cause an intense inflammatory response [171] and / or lung injury / infiltrates [158] it is therefore likely that this analysis has significantly overestimated the contribution of infectious comorbidities and underestimated the contribution of vivax malaria to death in some cases. Nevertheless, our results suggest that as with falciparum

malaria, there may be a potentially fatal interaction between vivax malaria and bacterial infection, particularly pneumonia. To clarify this interaction, prospective studies of severe vivax malaria syndromes need to include systematic investigation for concomitant bacterial infection, including blood cultures. Until then, our findings suggest the need for aggressive antimalarial treatment in patients with evidence of coinfection, and also indicate that apparently mono-infected patients with vivax malaria who show signs of severe disease should be investigated and treated for associated bacterial sepsis.

HIV infection is now recognised as a major contributor to the development of severe and fatal disease in *P. falciparum* malaria [449]. Fourteen percent of patients who died of causes related to *P. vivax* infection were documented to have HIV coinfection, which, with the recent rapid increase in HIV infection in Papua [385] and the retrospective study design, is likely to be an underestimate. While *P. vivax* may act as the final precipitating cause of death in HIV infection, a potential role for HIV in exacerbating severity of vivax malaria, as occurs with *P. falciparum*, requires further investigation.

This study has several important limitations. According to laboratory reports, 12 of the 54 patients in the death audit were actually shown to be suffering from *P. falciparum* or mixed species infections. Since deaths due to *P. falciparum* were not reviewed, it is possible that similar miscoding occurred in the opposite direction. This would have resulted in an underestimation of the true incidence of deaths due to *P. vivax* infection.

Differentiating *P. vivax* from *P. falciparum* using standard microscopy when parasites are still at the early ring phase is fraught with error. Despite relatively high concordance of hospital microscopy results with an independent expert's findings in 2004, it is likely that a degree of misdiagnosis occurred, even in cases where cross-check microscopy was performed.

Fully quantitated parasitaemias were not available to the adjudicating physicians however the semiquantitative data gave an indication of parasite density.

Rapid diagnostic tests were only done in a minority of cases. HRP2-based tests have been shown to be highly sensitive for the detection of falciparum malaria, making a significant biomass of *P. falciparum* unlikely in patients who tested negative [450]. For the remaining patients, it is possible that microscopy may have missed subpatent *P. falciparum* parasitaemias. Siripoon et al found that 13% of patients who had been diagnosed with *P. vivax* mono-infections in Thailand also had subpatent *P. falciparum* infections based on PCR testing [237]. Because PCR diagnostics are still not widely available in endemic areas, this is unlikely to have a bearing on the clinical classification of malaria or its treatment. It does, however, limit our ability to categorically incriminate *P. vivax* mono-infection as a cause of fatal disease.

Previous treatment seeking studies in the area estimate that almost 68% of deaths occur outside of hospital (unpublished data). This crude proportion was incorporated in the sensitivity analysis but it was not possible to make more subtle adjustments based on differences in the demographic and causative distribution of those deaths.

The *a priori* criteria used for categorising deaths in this study were based, as far as possible, on published evidence of clinical syndromes that have been associated with pathological processes in severe *P. vivax* infection. Given that fatal vivax malaria is a newly recognised clinical entity, causal links between these syndromes and death (with the probable exception of splenic rupture) have yet to be firmly established. It is therefore possible that our classification system misrepresented the degree to which at least some of the clinical syndromes contributed to the observed deaths. Post-mortem examination of patients who have died during or following an episode of *P. vivax* parasitaemia would help to solidify

causal links and to exclude other potential causes of death but would still be limited by our incomplete knowledge of the pathogenesis of *P. vivax* infection.

Certain epidemiological methods might be employed to infer a causal relationship between *P. vivax* infection and death. If resources permitted, the most robust of these methods would be to conduct a cluster-randomised trial of the effect of reducing or eliminating transmission of *P. vivax* on all-cause mortality (see Nevill et al for an example in falciparum malaria [409]). The primary advantage of such a randomised study is that it would control for all known and unknown confounders of the relationship between *P. vivax* infection and death.

Observational before-and-after studies, case-control studies and cohort studies could also provide important insights. A reduced all-cause mortality rate in the months to years following removal of vivax malaria from a region would be relatively strong evidence of a causal link provided that there were no other concurrent changes to the underlying disease profile. In a case-control study, patients who died in hospital might be matched by age and sex to hospitalised patients who remained alive in order to compare the odds of prior *P. vivax* infection in the two groups. In a cohort study, individuals would be classified according to their exposure to *P. vivax* infection and would then be followed to establish the relative risk of death in the two groups. In the latter two types of studies, associations between *P. vivax* infection and other diseases would be particularly important sources of confounding to consider. Purely epidemiological studies would be less reliant on clinical assumption than the clinical assessment presented in this chapter and thus more objective. They would not, however, shed any light on the potential mechanisms by which *P. vivax* malaria might lead to death.

In conclusion, this chapter has shown that in Southern Papua, mortality primarily attributable to *P. vivax* infection may occur but that indirect contribution to death in those

with comorbidities such as malnutrition, HIV and possible bacterial sepsis is likely to be a more common scenario. Future research should include post-mortem studies and detailed clinical and microbiological investigations of severe cases of vivax malaria to characterise pathogenic mechanisms and elucidate better therapeutic strategies by which fatal outcomes may be averted. Large-scale epidemiological studies may provide additional, objective evidence for a causal link between *P. vivax* infection and death.

7.5 Acknowledgements

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8. Gametocyte dynamics and the role of drugs in reducing the transmission potential of *Plasmodium vivax*

8.1 Introduction

Having focused on aspects of the haematological morbidity and mortality associated with *P. vivax* infection in Southern Papua, this thesis now turns to the potential of currently available antimalarial drugs to reduce transmission of *P. vivax* in regions that are co-endemic for both falciparum and vivax malaria.

Plasmodium vivax threatens almost half of the world's population and, as shown in the preceding chapters, is associated with significant, relapsing morbidity [49,55,83,84,96,109]. It is set to become the dominant malaria species in the Asia-Pacific region [83]. Transmission of *P. vivax* is dependent upon development of sufficient densities of mature, infectious gametocytes in the peripheral circulation and their subsequent ingestion by competent *Anopheles* mosquito vectors. In order to establish the potential of currently available antimalarial drugs to reduce the chance that transmission occurs, a comprehensive understanding of the biological and epidemiological attributes of *P. vivax* gametocytes is

necessary. This chapter uses data from three large randomised controlled trials, (one on the Thai-Myanmar border [4] and two in Papua, Indonesia [2,3]) to determine and compare the demographic and clinical factors associated with patent gametocytaemia on presentation with vivax malaria and during the 6 to 9 weeks following treatment with one of four antimalarial regimens: artemether+lumefantrine, dihydroartemisinin+piperaquine, artesunate+amodiaquine and chloroquine monotherapy.

8.2 Methods

8.2.1. Study sites

Thailand

The single Thai study in this analysis was conducted at three clinics near Mae Sot on the northwestern border of Thailand [4]. The clinics serve mainly displaced persons and migrant workers of Karen and Burmese ethnicity. Malaria transmission in this region occurs throughout the year with two seasonal peaks between May-July and December-January [51]. In the mid-1990s, the local annual incidence of malaria was approximately one episode per person-year, 53% due to *P. vivax*, 37% due to *P. falciparum* and 10% to mixed infection (according to results of light microscopy) [51,240]. Virtually all *P. falciparum* infections and approximately 90% of *P. vivax* infections were symptomatic [51]. The malaria attributable mortality rate was estimated to be 3 per 1,000 per year (corresponding to 20% of registered deaths) [51]. *Plasmodium vivax* relapses occur approximately three to four weeks following administration of rapidly eliminated antimalarials [229]. Standard treatment for *P. vivax* infections is a three-day course of chloroquine without primaquine. A recent case report suggests that chloroquine-resistant strains of *P. vivax* have emerged in this region [451].

Papua, Indonesia

The two Papuan studies included in this analysis took place at the same two clinics in the municipality of Timika in South-central Papua, Eastern Indonesia [2,3]. The demographics, geography and epidemiology of malaria in this region were described in Chapter 4.

8.2.2. Design of studies

The Thai study was carried out between January 2007 and December 2008 and compared dihydroartemisinin+piperaquine with chloroquine (CQ) for slide-confirmed uncomplicated *P. vivax* monoinfections [4]. Primaquine was not given. Pregnant or lactating women, patients less than one year of age or under 5kg in weight, those with known hypersensitivity to the study medications, intercurrent illness or a haematocrit <20% were excluded.

The first of the two Papuan studies was carried out between July 2004 and June 2005 and compared DHA+PIP with AM+LUM for slide-confirmed uncomplicated malaria due to *P. falciparum*, *P. vivax* or mixed species infection [2]. Unsupervised primaquine at a dose of 0.3mg base/kg per day for 14 days was prescribed to patients with *P. vivax* and mixed species infections at day 28 if they did not have glucose-6-phosphate dehydrogenase deficiency.

The second of the two Papuan studies was carried out between July and December 2005 and compared DHA+PIP with AS+AQ for the treatment of slide-confirmed uncomplicated falciparum, vivax or mixed species malaria [3]. Unsupervised primaquine was offered to G6PD-normal individuals with vivax or mixed species malaria directly after completion of the study regimens. Patients who were pregnant or lactating were excluded from both studies as were those who had a parasitaemia of greater than 4% or who fulfilled World Health Organization criteria for severe malaria [452]. The study comparing DHA+PIP with AL+LUM excluded individuals less than 10kg in weight whereas the study of DHA+PIP versus AS+AQ excluded individuals under 5kg in weight or less than one year of age.

In all studies, patients were followed with daily symptom enquiry and physical examination (including axillary temperature) as well as blood smears until aparasitaemic and afebrile. Thereafter patients were followed weekly for 6 weeks (42 days) in Papua and 9 weeks (63 days) in Thailand. Block randomisation and allocation concealment using sealed opaque envelopes were employed in all studies. Drug administration was open label but microscopists at both sites were unaware of patient allocation.

8.2.3. Laboratory methods

In Thailand, sexual and asexual parasite counts, including the individual densities of trophozoites and schizonts, were expressed per 500 white blood cells or, if parasitaemia was greater than 1%, per 1,000 red blood cells. Slides were declared negative after examination of at least 100 high-power fields. Haematocrit was measured using a micro-centrifuge (Hawksley®) and in this analysis was converted to a haemoglobin concentration in g/dL by multiplying the percentage by a factor of 0.34 [453].

In Papua, asexual and sexual parasite counts were done on Giemsa-stained thick films and reported per 200 white blood cells. Slides were declared negative after examination of at least 100 high-power microscope fields. A thin film was also examined to confirm parasite species and for quantification per 1,000 red blood cells if the parasitaemia was greater than 200 per 200 white blood cells. Haemoglobin was measured using a portable HemoCue® photometer. G6PD status was tested using the fluorescent spot test in both locations.

8.2.4. Statistical analysis

The primary outcomes of interest were the presence or absence of *P. vivax* gametocytaemia at enrolment, time to clearance of *P. vivax* gametocytes and appearance of *P. vivax* gametocytes up to 63 days following enrolment. The following were defined *a priori* as potential risk factors for gametocytaemia at enrolment: sex, age (<5 years, 5 to <15 years, ≥15

years), G6PD status (normal or abnormal), asexual *P. vivax* parasite density ($\log_e$ [parasites / μ L]), anaemia (haemoglobin <9g/dL), fever (temperature >37.5° Celsius), species of infection at enrolment (pure *P. vivax* versus mixed *P. vivax* / *P. falciparum* infection [Papua only]) and stage of infection at enrolment (presence or absence of schizonts [Thailand only]). Risk factors for appearance of *P. vivax* gametocytes during follow-up were as above plus clearance of asexual parasitaemia by day 1 (yes, no) and antimalarial regimen.

Analyses of gametocytaemia at enrolment were done using separate univariable logistic regression models for the two locations. All patient factors were subsequently included in separate multivariable logistic regression models for each location. In Papua, there was no difference in gametocyte carriage following DHA+PIP in the first study (in which primaquine was prescribed at day 28) compared with the second study (in which unsupervised primaquine was prescribed at day 3). Results from the two Papuan studies were therefore pooled in all analyses.

The cumulative incidence of *P. vivax* gametocytaemia in each location between day 7 and the end of follow-up was assessed for each antimalarial regimen using the Kaplan-Meier method and compared using the log-rank test. Clinical and demographic risk factors for recurrent gametocytaemia were examined using univariable and multivariable Cox regression models for each location (the latter stratified by treatment group). Fulfilment of the proportional hazards assumption was assessed by comparing log(cumulative hazard) by time of follow-up curves and subsequently by examination of Schoenfeld's residuals. Patients who had recurrent asexual *P. vivax* infection without concurrent gametocytaemia were retreated with antimalarial medication, and were therefore censored at the time of recurrence. The proportions of individuals who had cleared their gametocytes by days 1 and 2 were examined for each regimen stratified by location and compared using the chi-squared test or Fisher's

exact test. Comparisons of non-normal distributions were done by means of the Mann-Whitney U test or the Wilcoxon signed rank test for matched data. The association between asexual and sexual *P. vivax* parasite density ($\log_e$ transformed) was explored using Pearson's correlation coefficient.

8.3 Results

Overall 492 patients with *P. vivax* monoinfections were evaluable in the Thai dataset and 476 patients with *P. vivax* infections (314 with monoinfections and 162 with concurrent *P. falciparum* infections) were evaluable in the Papuan dataset (Table 8.1). Patients enrolled in the Thai study were slightly older and less anaemic than their Papuan counterparts and had higher asexual parasitaemias (median = 6,565/ μ L versus 2,595/ μ L, $p < 0.001$).

Table 8.1 Characteristics of evaluable patients in the Thai and Papuan studies

Characteristic	Thailand	Papua (monoinfections)	Papua (mixed infections)
n	492	314	162
Sex			
Male	328 (66.7%)	169 (53.8%)	103 (63.6%)
Age			
<5 years	66 (13.4%)	86 (27.4%)	26 (16.0%)
5-<15 years	135 (27.4%)	78 (24.8%)	44 (27.2%)
>15 years	291 (59.1%)	150 (47.8%)	92 (56.8%)
G6PD status			
Normal	463 (94.1%)	231 (73.6%)	104 (64.2%)
Abnormal	28 (5.7%)	45 (14.3%)	12 (7.4%)
Febrile (>37.5°C)	166 (33.7%)	52 (16.6%)	62 (38.3%)
Asexual <i>P. vivax</i> parasitaemia (/µL)	6,565 (193-30,551)*	2,595 (140-27,500)*	606 (38-14,036)*
<i>P. vivax</i> gametocytes detected	415 (84.3%)	209 (66.6%)	92 (56.8%)
<i>P. vivax</i> gametocytaemia (/µL)	266 (33-2,158)*	113 (35-727)*	98 (21-1,205)*
Haemoglobin (g/dL)	12.6 (9.9-15.6)*	10.3 (6.3-14.5)*	9.8 (5.6-14.4)*
Anaemia (Hb <9g/dL)	5 (1.0%)	94 (29.9%)	65 (40.1%)
Stage of infection			
Trophozoites alone	339 (68.9%)	-	-
Trophozoites and schizonts	153 (31.1%)	-	-
Treatment			
Artemether+lumefantrine	0 (0%)	84 (26.8%)	58 (35.8%)
Dihydroartemisinin+piperazine	248 (50.4%)	169 (53.8%)	86 (53.1%)
Artesunate+amodiaquine	0 (0)	61 (19.4%)	18 (11.1%)
Chloroquine	244 (49.6%)	0 (0%)	0 (0%)

Abbreviations: G6PD; glucose-6-phosphate dehydrogenase, n; number

*Median (5th-95th percentiles)

8.3.1. Gametocytaemia on enrolment

Gametocytes were detected on enrolment in 66.6% (209/314) of patients with *P. vivax* monoinfections and 56.8% (92/162) of patients with mixed infections in Papua ($p=0.04$) compared with 84.3% (415/492) of patients in Thailand ($p<0.001$ for comparison between Papuan patients with monoinfection and Thai patients). Thai patients also had a higher median gametocyte density than Papuan patients ($p<0.001$) (Table 8.1 and Figure 8.1).

In univariable analyses, higher $\log_e$ asexual parasite density was associated with a highly statistically significant increase in the risk of gametocytaemia on presentation in both locations (Table 8.2). Presence of schizonts on the admission blood film was associated with a 14-fold increased risk of gametocytaemia in Thailand ($p < 0.001$). In multivariable analyses, the only independent predictors of gametocytaemia on presentation were higher asexual parasitaemia (AOR per $\log_e$ order increase = 2.31 [95% CI 1.86-2.86], $p < 0.001$ in Thailand and AOR = 1.61 [95% CI 1.39-1.87], $p < 0.001$ in Papua) and schizontaemia at enrolment (AOR = 6.31 [95% CI 1.78-22.4], $p = 0.004$ [Thailand only]).

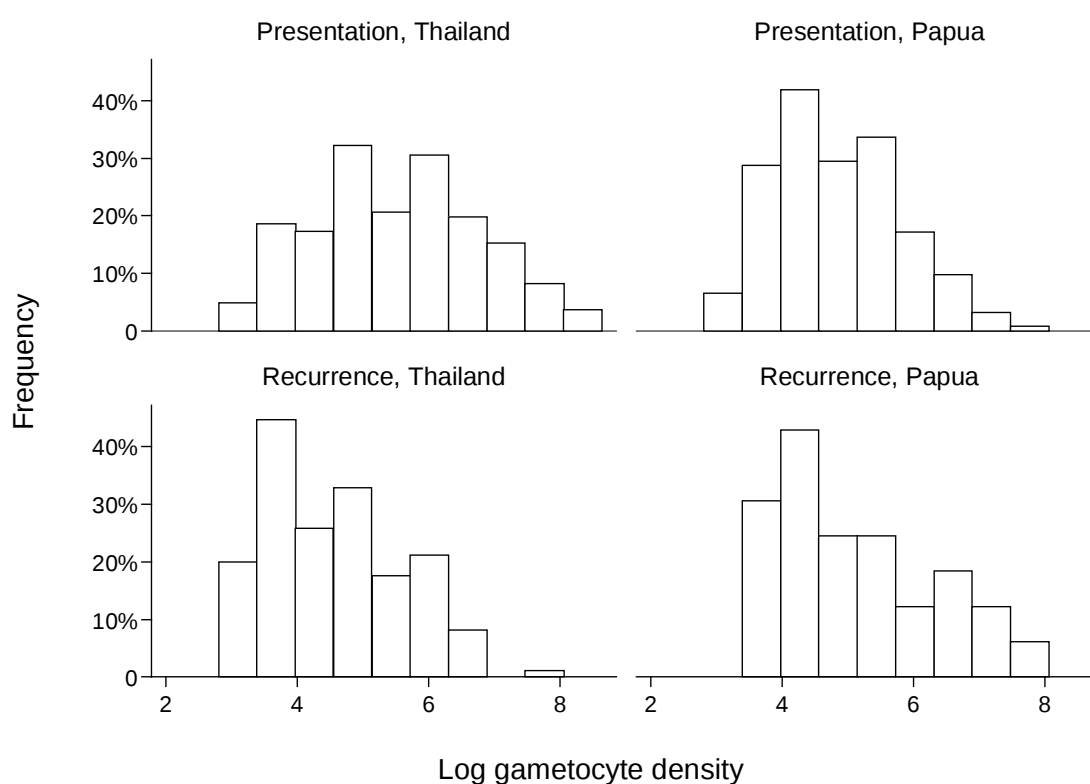


Figure 8.1 Frequency distribution of $\log_e$ gametocyte density for those with *P. vivax* mono-infections on presentation for treatment and at the time of recurrence

8.3.2. Gametocyte clearance

Overall, 42.5% (207/487) of patients in Thailand had cleared their asexual parasitaemia by day 1 versus 90.7% (262/289) of patients with *P. vivax* monoinfections in Papua ($p<0.001$). Of those with *P. vivax* monoinfections and gametocytaemia on enrolment, 58.4% (240/411) had cleared their gametocytaemia by day 1 in Thailand versus 96.4% (270/280) in Papua ($p<0.001$). The proportions for the individual drugs in Thailand were 73.4% (152/207) after DHA+PIP versus 43.1% (88/204) after CQ, $p<0.001$. By day two, 93.5% (245/262) of patients had cleared their gametocytes in Thailand and by this time there was no difference between treatment arms. In Papua, the proportions of patients who had cleared their gametocytaemia by day 1 were 90.2% (37/41) following AM+LUM, 98.1% (101/103) following DHA+PIP and 93.9% (46/49) following AS+AQ ($p=0.03$ for AM+LUM versus DHA+PIP, $p=0.52$ for AM+LUM versus AS+AQ and $p=0.18$ for DHA+PIP versus AS+AQ) (Figure 8.2). No individuals at either site had persistent *P. vivax* gametocytes at day 7. In Thailand 22.1% (17/77) of individuals without gametocytaemia on admission developed *P. vivax* gametocytaemia between day 1 and day 4; the risk being non-significantly greater following chloroquine (30.8%, 12/39) than DHA+PIP (13.2%, 5/38); $p=0.06$. In Papua, no patients (0/103) without gametocytaemia on enrolment subsequently developed gametocytaemia between day 1 and day 4 ($p<0.001$).

Table 8.2 Risk factors for *P. vivax* gametocytaemia at presentation in patients enrolled in the Thai and Papuan treatment trials

	Univariable models						Multivariable models			
	Thailand		Papua (monoinfection)		Papua (mixed infection)		Thailand		Papua	
	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>	AOR (95% CI)	<i>P</i>	AOR (95% CI)	<i>p</i>
Sex										
Male	1		1		1		1		1	
Female	0.85 (0.51-1.42)	0.54	1.03 (0.64-1.66)	0.90	1.20 (0.62-2.31)	0.58	0.97 (0.50-1.88)	0.93	0.91 (0.58-1.44)	0.70
Age										
<5 years	1.16 (0.51-2.60)	0.73	0.86 (0.48-1.54)	0.62	0.76 (0.32-1.84)	0.55	1.22 (0.44-3.45)	0.70	0.82 (0.45-1.49)	0.51
5 to <15 years	0.58 (0.34-0.99)	0.05	0.54 (0.30-0.95)	0.03	0.75 (0.36-1.56)	0.45	0.72 (0.36-1.46)	0.36	0.59 (0.34-1.02)	0.06
≥15 years	1		1		1		1		1	
G6PD status										
Normal	1		1		1		1		1	
Abnormal	0.85 (0.31-2.30)	0.75	1.51 (0.74-3.07)	0.26	1.11 (0.33-3.73)	0.87	1.11 (0.30-4.03)	0.88	1.31 (0.68-2.53)	0.42
Log _e asexual parasite density (per log _e order increase)	2.36 (1.95-2.85)	<0.001	1.49 (1.27-1.74)	<0.001	2.01 (1.57-2.58)	<0.001	2.31 (1.86-2.86)	<0.001	1.61 (1.39-1.87)	<0.001
Anaemia (Hb <9g/dL)	0.75 (0.08-6.76)	0.79	1.89 (1.08-3.28)	0.03	1.35 (0.71-2.57)	0.36	1.26 (0.08-19.6)	0.87	1.39 (0.83-2.34)	0.21
Fever (>37.5°C)	1.10 (0.64-1.87)	0.74	1.59 (0.81-3.13)	0.18	1.11 (0.58-2.11)	0.76	0.53 (0.27-1.05)	0.07	0.89 (0.50-1.58)	0.69
Stage of infection										
Trophozoites alone	1		-		-		1		-	
Trophozoites and schizonts	14.0 (4.33-45.1)	<0.001	-		-		6.31 (1.78-22.4)	0.004	-	
Species of infection										
<i>P. vivax</i> monoinfection	-		-		-		-		1	
Mixed <i>P. vivax</i> / <i>P. falciparum</i>	-		-		-		-		1.03 (0.86-1.24)	0.74

Abbreviations: OR; odds ratio, AOR; adjusted odds ratio, G6PD; glucose-6-phosphate dehydrogenase

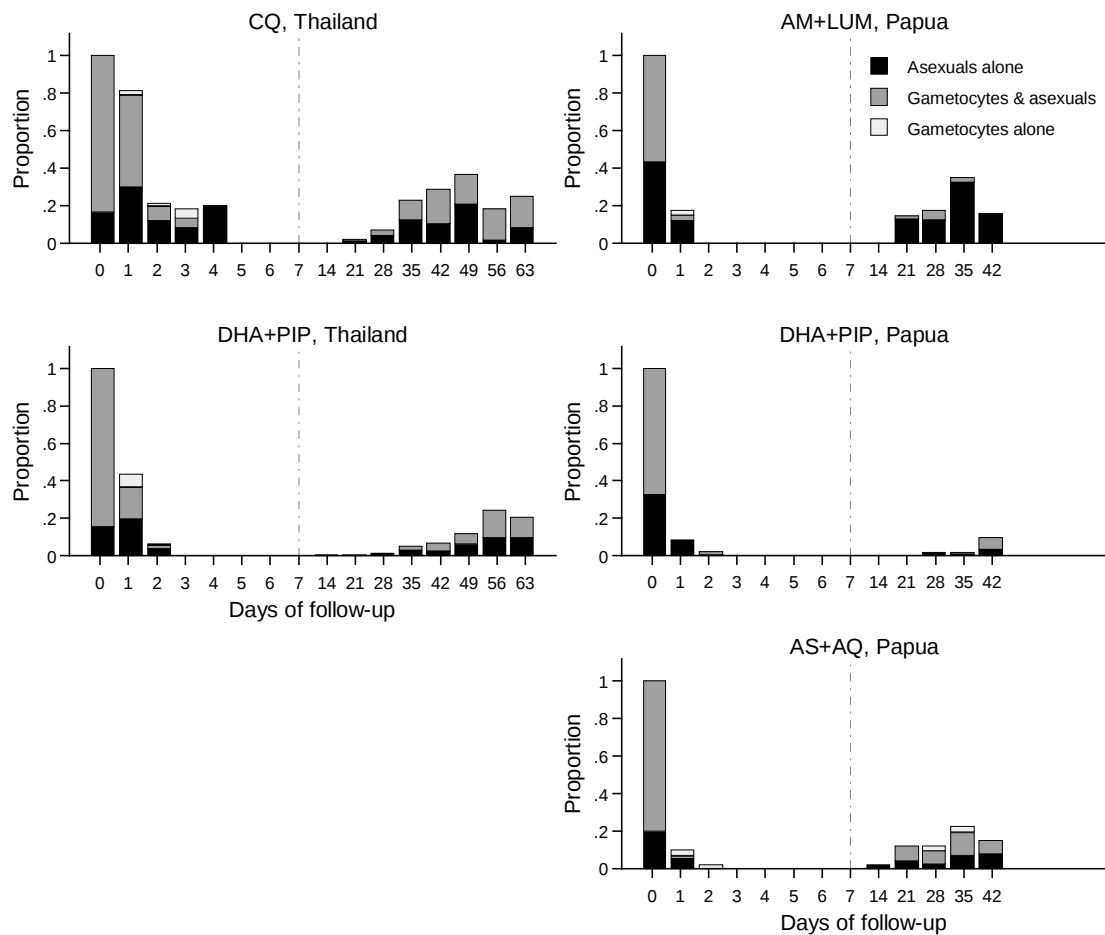


Figure 8.2 Proportion of individuals examined with sexual and / or asexual forms of *P. vivax* from presentation through to the end of follow-up in Thailand and Papua (excludes patients with mixed infection on presentation in Papua)

8.3.3. Gametocytaemia during follow-up

Overall, 146 of 492 (29.7%) participants had appearance of *P. vivax* gametocytaemia between day 7 and 63 in Thailand and 28 of 314 (8.92%) participants with *P. vivax* mono-infections had appearance of *P. vivax* gametocytaemia between day 7 and 42 in Papua (see Table 8.3). Of all 174 appearances of gametocytaemia during follow-up, only two (1.15%) were not associated with concurrent asexual stage infection; both of these individuals had been treated with AS+AQ. In Thailand, 54.2% (147/271) of patients had patent gametocytaemia at the time of *P. vivax* asexual recurrences compared to 33.8% (26/77) following *P. vivax* mono-infection in Papua ($p=0.002$).

In Thailand, the day 63 cumulative risk of gametocyte carriage was lower following DHA+PIP (32.9% [95% CI 26.5-40.4%]) than following CQ (57.9% [95% CI 49.4-66.6%], $p<0.001$). The cumulative percentage gametocyte carriage by day 42 following *P. vivax* monoinfections in Papua was greatest for AS+AQ (33.6% [95% CI 21.6-49.8%]) and lowest for DHA+PIP (6.80% [95% CI 3.46-13.2%], $p<0.001$) (Table 8.3). There was no difference in the day 42 cumulative risk of gametocytaemia following DHA+PIP between the Thai and Papuan studies (Table 8.3, $p=0.87$).

In univariable models, risk factors for appearance of gametocytes during follow-up included higher initial asexual parasite density in both locations and presence of gametocytaemia on enrolment as well as persistence of asexual parasitaemia on day 1 in Thailand (Table 8.3). Persistent asexual parasitaemia on day 2 was rare in Papua and in Thailand was not associated with recurrent gametocytaemia in a univariable model (Hazard Ratio = 1.47 [95% CI 0.87-2.49], $p=0.15$). The only independent predictors of gametocytaemia during follow-up after adjustment for all factors in the multivariable models were higher asexual parasite density on enrolment (Adjusted Hazard Ratio [AHR] = 1.18 [95% CI 1.02-1.35], $p=0.02$ in Thailand and 1.58 [95% CI 1.25-1.98], $p<0.001$ in Papua) and mixed as opposed to pure *P. vivax* infection on enrolment in Papua (AHR= 2.76 [95% CI 1.26-6.04], $p=0.01$) (Table 8.4).

The relationship between $\log_e$ transformed asexual and sexual stage density at enrolment and during follow-up is presented in Figure 8.3. In patients with *P. vivax* monoinfections at enrolment at either location, the median ratio of gametocyte to asexual stage density was 0.028 (IQR 0.004-0.083) at presentation and 0 (IQR 0-0.11) at the time of asexual stage failure ($p=0.89$ for matched comparison limited to patients who had asexual stage recurrence).

Table 8.3 Cumulative percentage gametocyte carriage by treatment

		Cumulative percentage gametocyte carriage (95% confidence interval)					<i>p</i> values			
		AM+LUM	DHA+PIP	AS+AQ	CQ	All	AM+LUM v DHA+PIP	AM+LUM v AS+AQ	DHA+PIP v AS+AQ	DHA+PIP v CQ
Day 7-42	Thailand	-	6.92 (4.23-11.2)	-	29.1 (23.0-36.5)	16.9 (13.5-21.0)	-	-	-	<0.001
	Papua (pure)	7.42 (3.14-17.0)	6.80 (3.46-13.2)	33.6 (21.6-49.8)	-	12.1 (8.50-17.2)	0.39	<0.001	<0.001	-
	Papua (mixed)	17.5 (9.12-32.1)	4.76 (1.54-14.2)	34.7 (14.4-68.7)	-	12.3 (7.55-19.7)	0.01	0.39	0.001	-
Day 7-63	Thailand	-	32.9 (26.5-40.4)	-	57.9 (49.4-66.6)	43.7 (38.4-49.4)	-	-	-	<0.001

Abbreviations: AM+LUM; artemether+lumefantrine, DHA+PIP; dihydroartemisinin+piperaquine, AS+AQ; artesunate+amodiaquine, CQ; chloroquine

Table 8.4 Risk factors for gametocytaemia during follow-up

	Univariable models						Multivariable models			
	Thailand Day 7-63		Papua (monoinfection) Day 7-42		Papua (mixed infection) Day 7-42		Thailand Day 7-63		Papua Day 7-42	
	HR (95% CI)	<i>p</i>	HR (95% CI)	<i>p</i>	HR (95% CI)	<i>p</i>	AHR (95% CI)	<i>p</i>	AHR (95% CI)	<i>p</i>
Female sex	0.93 (0.66-1.31)	0.69	2.21 (1.02-4.78)	0.05	1.16 (0.41-3.27)	0.77	0.91 (0.62-1.32)	0.62	1.62 (0.83-3.18)	0.16
Age										
<5 years	1.53 (0.98-2.39)	0.06	2.28 (0.96-5.40)	0.06	0.93 (0.20-4.36)	0.92	1.54 (0.95-2.49)	0.08	1.41 (0.58-3.42)	0.45
5 to <15 years	0.78 (0.53-1.16)	0.23	1.39 (0.52-3.74)	0.51	1.29 (0.42-3.95)	0.65	0.89 (0.58-1.35)	0.57	1.92 (0.82-4.52)	0.13
≥15 years	1		1		1		1		1	
G6PD status										
Normal	1		1		†		1		1	
Abnormal	1.05 (0.49-2.25)	0.90	1.56 (0.66-3.67)	0.31	†		0.95 (0.43-2.09)	0.90	1.84 (0.74-4.53)	0.19
Enrolment log _e asexual parasite density (per log _e order increase)	1.21 (1.08-1.36)	0.001	1.48 (1.14-1.91)	0.003	1.72 (1.30-2.27)	<0.001	1.18 (1.02-1.35)	0.02	1.58 (1.25-1.98)	<0.001
Gametocytes on enrolment	1.82 (1.07-3.11)	0.03	2.15 (0.87-5.31)	0.10	2.22 (0.71-6.98)	0.17	1.22 (0.68-2.22)	0.50	1.31 (0.54-3.16)	0.55
Persistent asexual parasitaemia on day 1	1.45 (1.04-2.03)	0.03	2.12 (0.73-6.17)	0.17	1.78 (0.39-8.01)	0.46	0.84 (0.57-1.25)	0.39	1.13 (0.42-3.01)	0.81
Anaemia on enrolment (Hb <9g/dL)	0.54 (0.08-3.86)	0.54	1.46 (0.68-3.17)	0.33	1.68 (0.59-4.81)	0.33	0.85 (0.11-6.45)	0.88	1.26 (0.60-2.62)	0.55
Fever on enrolment (>37.5°C)	1.33 (0.94-1.87)	0.11	0.40 (0.09-1.67)	0.21	1.12 (0.40-3.15)	0.83	1.22 (0.84-1.77)	0.29	0.45 (0.17-1.20)	0.11
Schizonts on admission blood film	1.23 (0.87-1.73)	0.24	-		-		1.05 (0.70-1.57)	0.81	-	
Species at enrolment										
<i>P. vivax</i> monoinfection	-		-		-		-		1	
Mixed <i>P. vivax</i> / <i>P. falciparum</i>	-		-		-		-		2.76 (1.26-6.04)	0.01

Abbreviations: G6PD; glucose-6-phosphate dehydrogenase, HR; hazard ratio, AHR; adjusted hazard ratio, 95% CI; 95% confidence interval

Multivariable models stratified by treatment group.

†No patients with mixed infection and an abnormal glucose-6-phosphate dehydrogenase status had a recurrence of *P. vivax* gametocytaemia between day 7 and 42.

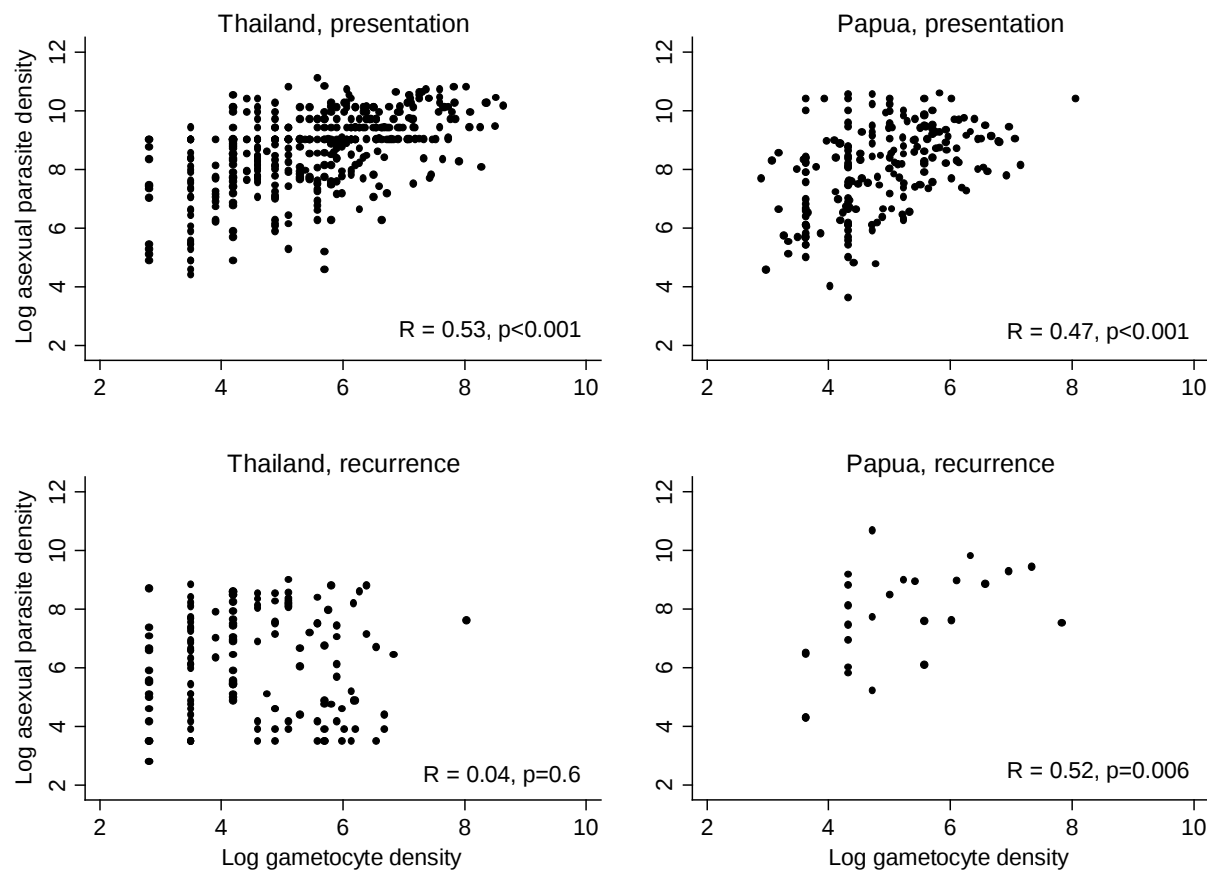


Figure 8.3 Correlation between the density of asexual and sexual stages of *P. vivax* at presentation for treatment and at the time of recurrence after treatment (analyses limited to those with *P. vivax* mono-infections at enrolment)

8.4 Discussion

This analysis of three large clinical drug trials from Thailand and Indonesia highlights several fundamental properties of *P. vivax* transmission dynamics, some of which have been given little consideration since early studies of neurosyphilitics and military personnel in the first half of the 20th century [287,454-462].

First, *P. vivax* gametocytes appear early in the course of blood stage infection and are cleared rapidly by schizontocidal drugs. Second, the relationship between asexual and sexual stage parasitaemia does not differ significantly between acute and recurrent infections and therefore the factors that determine *P. vivax* transmissibility are those that determine asexual stage parasite dynamics. Third, there are significant differences in the effects of modern antimalarial regimens on the risk of recurrent parasitaemia and gametocytaemia.

In symptomatic falciparum malaria, gametocytes usually appear in the peripheral circulation after the onset of symptoms [286]. Rapidly effective blood schizontocidal drugs can therefore have a profound impact on overall gametocyte carriage and transmission potential [279]. The artemisinin derivatives are highly potent antimalarials that reduce the biomass of asexual parasites rapidly whilst also exerting strong gametocytocidal activity against early stage sexual forms [279,463-467]. When combined with a slowly eliminated partner drug, they minimise the risk of recrudescence and reduce *P. falciparum* transmissibility [356].

The dynamics of gametocyte carriage in vivax malaria are notably different. Sexual stages appear early in the course of infection [287,454-456,460,468,469] together with the rise in asexual parasitaemia and thus transmission often occurs before antimalarial treatment. Unlike *P. falciparum* gametocytes, *P. vivax* sexual forms are susceptible to all blood schizontocidal medications [328]. *Plasmodium vivax* can be transmitted comparatively efficiently at subpatent gametocyte densities [460,470-473] suggesting that microscopic

examination such as used in this analysis may underestimate the total period of infectiousness following therapy. Nevertheless, the relative transmission-blocking benefit of drugs with greater potency, such as the artemisinin derivatives, that reduce *P. vivax* parasitaemia more rapidly than others, is likely to be minimal. Far more important will be the potential of a drug to prevent recurrent infections.

The ideal antimalarial regimen with the greatest potential to block transmission of *P. vivax* will therefore include one or more highly active blood schizontocidal medication(s) that facilitate complete eradication of blood stages and thus prevent recrudescence in combination with a hypnozoitocidal medication for prevention of future relapses. Unfortunately, toxicity concerns and poor adherence to two-week regimens continue to hamper the safe and effective use of primaquine, the only currently licensed hypnozoitocidal medication [255]. Where primaquine is not used or has been shown to be ineffective, slowly eliminated blood schizontocides that suppress the first relapse may have benefits over regimens with shorter elimination half-lives [108], though whether they reduce the total number of relapses and overall transmission potential is unknown.

Artesunate+amodiaquine has consistently been associated with higher *P. falciparum* recrudescence and, as shown in this analysis, higher *P. vivax* recurrence rates than either AM+LUM or DHA+PIP [3,79]. This is likely to be attributable to the relatively short terminal elimination half-life of amodiaquine and declining parasite susceptibility to this drug [3]. Gametocyte carriage was higher following AM+LUM (half-life ~4 days) than DHA+PIP although this only reached significance in patients treated for mixed infections (Table 8.3).

Chloroquine is highly potent against susceptible *P. vivax* strains and has a terminal elimination half-life of 1-2 months [80]. It therefore has the potential to limit recrudescence and suppress the first and possibly even second *P. vivax* relapse. In Thailand, chloroquine

was associated with greater gametocyte carriage during follow-up than DHA+PIP (elimination half-life ~28 days) suggesting declining chloroquine susceptibility of local strains. This scenario is likely to be mirrored in other regions where chloroquine has been used as the mainstay of vivax malaria treatment for many years [108].

High asexual parasite density was shown to be a strong risk factor for gametocyte carriage during follow-up, independent of age and other potential confounders. There are two likely explanations for this finding. First, high asexual parasitaemia increases the risk of parasite recrudescence (as shown in falciparum malaria) [299,356,474-476]. Second, high parasite density reflects poor immunity which has been associated with a greater risk of patent relapse [221]. Since *P. vivax* gametocytaemia mirrors asexual infection, a higher risk of asexual recurrence, whether due to recrudescence or relapse, will result in a higher risk of gametocyte carriage.

This analysis has limitations. Follow-up was three weeks longer in Thailand as compared with Papua and thus conclusions drawn for the period 42 to 63 days were based on Thai data only. A 42-day follow-up period is insufficient to capture first relapses that follow administration of slowly eliminated antimalarial drugs. Parasite counts were done against 200 white blood cells (WBC) in Papua whereas in Thailand they were done against 500 WBC. This will have increased the likelihood of gametocyte detection in Thailand relative to Papua and may partially explain the shorter gametocyte clearance times in Papua. Even high quality microscopy is well known to underestimate the proportion of gametocytaemic individuals and thus the true transmission potential of *P. vivax*, particularly since *P. vivax* is so efficiently transmitted at subpatent gametocyte densities [460,470-473]. However these limitations would not negate the differences observed between drug regimens within each location.

In conclusion, this chapter shows that *P. vivax* gametocytaemia closely mirrors asexual stage carriage. Persistence of gametocytaemia following eradication of asexual stages does not occur. These results emphasise that the most important means of blocking *P. vivax* transmission is to prevent future relapses, especially in patients with high asexual parasite density. Optimal prescribing practices that maximise patient adherence to primaquine are needed and, given the limitations of this drug, very high priority must be given to the development of new hypnozoitocidal agents.

8.5 Acknowledgments

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9. *Plasmodium vivax* recurrence following falciparum and mixed species malaria: risk factors and effect of antimalarial kinetics

9.1 Introduction

In South-east Asia, *Plasmodium vivax* infection follows treatment of falciparum malaria with substantially greater frequency than entomological inoculation rates would predict [2,79,236,241,252,294,477]. The reasons for this are not clear. One postulate is that contemporaneous inoculation of *P. vivax* and *P. falciparum* occurs relatively frequently and that acute *P. falciparum* suppresses *P. vivax* parasitaemia below levels detectable by light microscopy [236,478]. According to this hypothesis, most recurrent *P. vivax* infections following treatment of falciparum malaria are relapses from simultaneously acquired hypnozoites [236,478]. An alternative theory is that either *P. falciparum* infection or its treatment somehow precipitate blood-stage relapse from dormant, previously acquired hypnozoites [478].

Whatever the underlying mechanism, *P. vivax* recurrence following falciparum malaria carries significant morbidity, impairing clinical and haematological recovery [2,3] and worsening the socioeconomic burden of malaria [83]. Since asexual *P. vivax* parasitaemia

after blood-stage treatment is frequently associated with concurrent gametocytaemia (as shown in Chapter 8) [2,3,285], it is also likely to have an important role in sustaining transmission of *P. vivax* [108]. The efficacy of antimalarial treatment for preventing *P. vivax* recurrence after falciparum malaria is therefore an important consideration for malaria control strategies.

This chapter uses pooled data from a large series of clinical trials conducted at Shoklo Malaria Research Unit on the Thai-Myanmar border between 1991 and 2005 to establish the effect of demographic and clinical factors as well as antimalarial elimination kinetics on the risk of *P. vivax* recurrence following *P. falciparum* or mixed *P. vivax* / *P. falciparum* malaria.

9.2 Methods

9.2.1. Study sites

The studies included in this analysis were carried out at camps for displaced persons of the Karen ethnic minority and border clinics serving mainly Burmese migrant workers along the northwestern border of Thailand. The characteristics of this region were introduced in the previous chapter. Standard treatment of uncomplicated falciparum malaria in this region was mefloquine monotherapy (25mg base/kg total dose) between 1991 and 1994 and thereafter mefloquine (25mg base/kg) plus artesunate (12mg/kg over three days) [479].

9.2.2. Design of the studies

This analysis includes 24 studies that investigated 25 different antimalarial treatment regimens. None included routine administration of primaquine (Table 9.1). Sixteen of the studies were randomised controlled trials of different treatments for uncomplicated falciparum malaria, with or without concomitant *P. vivax* infection; the remainder were single-arm clinical trials conducted to assess drug efficacy or safety. None included children

under 5kg or pregnant women. Two studies restricted recruitment to children aged ≤ 15 years and one study restricted recruitment to children < 5 years (Table 9.1).

Patients with severe disease according to World Health Organization criteria [452] were excluded though the studies of intravenous quinine plus mefloquine and of the 5 and 7 day courses of artesunate in combination with mefloquine included patients with uncomplicated hyperparasitaemia ($> 4\%$ parasitised red blood cells) (Table 9.1). Follow-up was standardised for all studies and lasted 28 days (6 studies; 1,398 patients), 42 days (11 studies; 5,354 patients) or 63 days (7 studies; 3,797 patients). Patients were seen every day until fever and parasite clearance and then weekly thereafter. In the event of illness in between these visits, patients were asked to return to the clinic for treatment.

9.2.3. Study data

Basic demographic and clinical details were recorded at enrolment including age, sex, parasitaemia, temperature and, in most cases, haematocrit and white blood cell count. Symptoms, temperature and parasite count were assessed at follow-up visits. Diagnosis of *Plasmodium* infection and subsequent species identification was established by examination of Giemsa stained thick and thin blood films. Parasitaemia was reported as the number of asexual parasites per 500 WBC or per 1,000 red blood cells (RBC) and subsequently converted to a count per microlitre using the patient's WBC count or haematocrit if available. Population means or assumed values of 8,300 WBC/ μL and 35% respectively were used if necessary. Asexual parasite densities in mixed infection were given as a summed total in the majority of studies, and separately for both species in a minority. For this analysis the summed total was used.

Patients were censored and deemed to have failed if there were signs of early treatment failure due to either malaria parasite species [480], if asexual *P. falciparum* or *P. vivax* parasitaemia persisted beyond 7 days, or if either species reappeared in the circulation up to

63 days following initial clearance. Patients who did not fail were censored on the date of their last negative blood smear.

9.2.4. Statistical analysis

The primary outcome for this analysis was recurrence with *Plasmodium vivax* up to 63 days following treatment for *P. falciparum* or mixed *P. falciparum* / *P. vivax* infection. Potential risk factors examined were species of infection at enrolment (*P. falciparum* or mixed infection), age, sex, initial log_e parasite density, baseline haematocrit and *P. falciparum* gametocytaemia at enrolment (yes, no). Non-parametric continuous data were compared using the Kruskal-Wallis test, unpaired proportions using the chi-squared test and paired proportions using McNemar's test. The impact of antimalarial drugs was assessed in two separate comparisons. Firstly I examined outcomes for all antimalarial drugs or combinations grouped by their terminal elimination half-lives ($t_{1/2}$) (see Table 9.1; short = $t_{1/2}$ <1 day; intermediate = $t_{1/2}$ >1 day and <1 week; long = $t_{1/2}$ >1 week). Secondly I compared outcomes between individual artemisinin combination therapies. The Kaplan-Meier function and log-rank test were used for univariable analyses. Multivariable analyses were done using the Cox proportional hazards model with gamma frailty to account for heterogeneity of results between studies [481] (examined using the Wald test for significance of interaction terms in preliminary models). Fulfilment of the proportional hazards assumption was assessed by comparing log(cumulative hazard) by time of follow-up curves for each of the model covariables.

9.3 Results

Between 1991 and 2005, 10,549 patients (4,960 children aged <15 years and 5,589 adults) were treated for falciparum malaria of whom 9,385 (89.0%) had *P. falciparum* monoinfections and 1,164 (11.0%) had mixed infections. Overall, 2,925 patients (27.7%) had recurrence of parasitaemia, 1,570 (53.7%) with pure *P. vivax*, 1,269 (43.4%) with pure *P.*

falciparum and 86 (2.9%) with mixed infections. The median time to recurrence with *P. falciparum* monoinfection was 28 days, with *P. vivax* monoinfection was 35 days, and with mixed infection was 33 days (p for overall difference =0.0001). The number and characteristics of individuals receiving each of the treatment regimens are shown in Table 9.1. According to Kaplan-Meier analyses the cumulative proportion of patients failing with any species by day 63 was 45.6% (95% CI 44.1-47.0%), the proportion failing with *P. falciparum* (either pure or mixed) was 21.5% (95% CI 20.3-22.8%) and with *P. vivax* (either pure or mixed) was 31.5% (95% CI 30.1-33.0%). Overall, 3.5% (36/1,024) of recurrences with asexual *P. falciparum* were associated with patent *P. falciparum* gametocytaemia. Gametocyte data for *P. vivax* recurrences were not available.

Haematocrit data were available for 90.7% (9,565/10,549) of patients at enrolment and 58.9% (1,724/2,925) at the time of failure. In total, 14.5% (1,382/9,565) of patients were anaemic (haematocrit <30%) at enrolment to the studies. Of those who did not have parasitological failure, 13.5% (925/6,869) were anaemic at baseline versus 4.0% (192/4,755) at the last time of follow-up ($p<0.0001$). The corresponding figures at baseline and at the time of recurrence were 14.2% (169/1,189) versus 11.3% (78/692) for those who failed with *P. falciparum* ($p=0.1$) and 18.7% (296/1,586) versus 7.2% (78/1,091) for those who failed with *P. vivax* ($p<0.0001$). Patients who had recurrent *P. falciparum* monoinfection, *P. vivax* monoinfection or mixed infection were anaemic at the time of failure in 11.9% (75/633), 7.3% (75/1,032) and 5.1% (3/59) of cases respectively (p for overall difference =0.004).

Table 9.1 Details of treatment regimens and characteristics of patients

Code	Total treatment dose (total regimen duration, total number of doses)	Year(s) studied	t _½	n	Male (%)	Age (years)*	Parasitaemia/μL*
AAP	Artesunate 12mg/kg (3 days, 3 doses) + atovaquone 45mg/kg (3 days, 3 doses) + proguanil 24mg/kg (3 days, 3 doses)	1998-2000	Int	526	353(67)	20 (7-41)	4,408 (176-86,219)
AM7	Artemether 12mg/kg (7 days, 7 doses)	1993-1996	Short	206	114 (55)	15 (2-33)	4,850 (273-73,853)
AP	Atovaquone 45mg/kg (3 days, 3 doses) + proguanil 24mg/kg (3 days, 3 doses)	1998-2000	Int	528	354 (67)	20 (7-43)	3,841 (142-66,870)
AS3	Artesunate 12mg/kg (3 days, 3 doses)	1992-1994	Short	5	3 (60)	14 (1-25)	105,278 (4,428-151,926)
AS5	Artesunate 12mg/kg (5 days, 5 doses)	1992-1995	Short	153	86 (56)	5 (1-25)	13,842 (424-430,713)
AS7	Artesunate 12mg/kg (7 days, 7 doses)	1992-1996	Short	452	245 (54)	10 (2-29)	6,972 (331-149,142)
AS7T7	Artesunate 12mg/kg (7 days, 7 doses) + tetracycline 112mg/kg (7 days, 7 doses)	1993-1995	Short	20	12 (60)	14 (9-39)	9,396 (1,065-205,230)
COA4	Artemether 6.8mg/kg (3 days, 4 doses) + lumefantrine 48mg/kg (3 days, 4 doses)	1995-1997	Int	387	265 (68)	21 (9-41)	4,529 (278-88,957)
COA6a	Artemether 10.2mg/kg (60hrs, 6 doses) + lumefantrine 72mg/kg (96hrs, 6 doses)	1996-1998, 2000-2002	Int	1,115	757 (68)	20 (7-45)	6,414 (489-88,297)
COA6b	Artemether 10.2mg/kg (96hrs, 6 doses) + lumefantrine 72mg/kg (96hrs, 6 doses)	1996-1997,	Int	87	62 (71)	22 (11-41)	5,460 (1,023-78,561)
DP+	DHA 6.3mg/kg (3 days, 4 doses) + piperaquine 51.3mg/kg (3 days, 4 doses) + either artesunate 400mg (3 days, 4 doses) or extra DHA to achieve total dose of 12mg/kg (3 days, 4 doses)	2002-2003	Long	174	125 (72)	20 (6-45)	16,830 (415-105,630)
DP3	DHA 6.3mg/kg (3 days, 3 doses) + piperaquine 51.3mg/kg (3 days, 3 doses)	2003-2004	Long	170	104 (61)	21 (6-43)	11,304 (496-75,360)
DP4	DHA 6.3mg/kg (3 days, 4 doses) + piperaquine 51.3mg/kg (3 days, 4 doses)	2002-2004	Long	340	216 (64)	22 (7-44)	13,816 (802-94,878)
M25	Mefloquine 25mg/kg (1-2 days, 1-2 doses)	1991-1994	Long	949	543 (57)	14 (4-38)	3,818 (213-36,754)
MA	Artesunate 10mg/kg (1 day, 3 doses) + mefloquine 15mg/kg (1 day, 1 dose)	1991	Long	323	190 (59)	15 (3-38)	3,486 (249-23,652)

MAM1	Artemether 4-10mg/kg (1 day, 2-3 doses) + mefloquine 25mg/kg (1 day, 1 dose)	1992	Long	19	10 (53)	20 (11-50)	6,739 (253-228,592)
MAM3	Artemether 12 mg/kg (3 days, 3 doses) + mefloquine 25mg/kg (1 day, 1 dose)	1993-1994	Long	180	86 (48)	16 (5-42)	5,299 (326-78,442)
MAS1	Artesunate 4mg/kg (1 day, 1 dose) + mefloquine 25mg/kg (1 day, 1 dose)	1992	Long	152	94 (62)	16 (4-35)	4,847 (315-26,892)
MAS3	Artesunate 12mg/kg (3 days, 3 doses) + mefloquine 25mg/kg (1-2 days in 1-2 doses)	1992-2005	Long	4,106	2,533 (62)	14 (5-39)	7,300 (349-93,085)
MAS5	Artesunate 12mg/kg (5 days, 5 doses) + mefloquine 25mg/kg (1 day, 1 dose)	1992-1995	Long	57	29 (51)	6 (2-23)	326,874 (14,472-707,962)
MAS7	Artesunate 12mg/kg (7 days, 7 doses) + mefloquine 25mg/kg (1 day, 1 dose)	1993-1995	Long	139	82 (59)	7 (3-12)	270,957 (162,778-597,555)
MASF	Artesunate 12mg/kg (3 days, 3 doses) + mefloquine 25mg/kg (3 days, 3 doses) in fixed combination	2004-2005	Long	247	170 (69)	20 (6-45)	14,469 (342-92,547)
MQIV	Quinine 40mg/kg (1 day, 3 doses) + mefloquine 25mg/kg (1 day, 1 dose)	1993	Long	31	18 (58)	9 (4-29)	309,177 (150,850-562,186)
Q7	Quinine 210mg/kg (7 days, 7 doses)	1992-1993	Short	28	16 (57)	5 (2-8)	3,819 (130-26,158)
Q7T7	Quinine 210mg/kg (7 days, 7 doses) + tetracycline 112mg/kg (7 days, 7 doses)	1992-1994	Short	155	97 (63)	15 (9-34)	4,284 (294-79,409)
Total		1991-2005		10,549	6,564 (62)	15 (5-40)	6,586 (328-101,284)

* Median and 90% range are presented

Abbreviations: $t_{1/2}$; elimination half-life category, n; number, int; intermediate, DHA; dihydroartemisinin. Table reproduced with permission from Douglas NM, et al. *Plasmodium vivax* recurrence following falciparum and mixed species malaria: risk factors and effect of antimalarial kinetics. *Clin Infect Dis* 2011; **52**: 612-20.

Symptomatology data were available at the time of parasitological failure for 68.3% of study participants (1,997/2,925). Recurrences with pure *P. falciparum*, pure *P. vivax* and mixed infections were associated with symptoms in 65.5% (537/820), 44.3% (495/1,118) and 71.2% (42/59) of cases respectively (p for overall difference <0.001). At the time of recurrence, the proportion of patients who were febrile (temperature >37.5 degrees Celsius) or had a history of fever within the last 24 hours was 51.7% (455/880) for *P. falciparum* monoinfections, 33.6% (386/1,148) for *P. vivax* monoinfections and 61.4% (35/57) for mixed infections (p for overall difference <0.001).

Of patients who had recurrent *P. falciparum* monoinfection, *P. vivax* monoinfection or mixed infection, 41.2% (523/1,269), 30.5% (479/1,570) and 58.1% (50/86) respectively presented outside of routine weekly follow-up and therefore presumably of their own volition (p for overall difference <0.001). *Plasmodium vivax* recurrences following treatment with short, intermediate and long half-life combinations were symptomatic in 58.3% (158/271), 42.7% (230/539) and 40.6% (149/367) of cases respectively (p for trend <0.001).

9.3.1. Risk factors for *Plasmodium vivax* recurrence

The cumulative risk of *P. vivax* recurrence by day 63 following *P. falciparum* monoinfection was 29.4% (95% CI 27.9-30.9%) and following mixed infection was 49.3% (95% CI 44.3-54.5%); AHR = 2.47 (95% CI 2.15-2.85), $p<0.001$ (Table 9.2 and Table 9.3). Univariable analyses showed a statistically significant increase in the risk of *P. vivax* recurrence following pure *P. falciparum* infection with decreasing age, low haematocrit ($<30\%$), increasing log_e asexual parasite density and presence of *P. falciparum* gametocytaemia (Table 9.2). Males were significantly more likely to have recurrent *P. vivax* after both pure falciparum malaria and mixed infections (Table 9.2 and Table 9.3, AHR = 1.27 [95% CI 1.14-1.41], $p<0.001$).

Table 9.2 Baseline risk factors for *P. vivax* recurrence

Initial species	<i>P. falciparum</i>				Mixed <i>P. falciparum</i> / <i>P. vivax</i>			
	n	Failure*	95% CI	<i>p</i>	n	Failure*	95% CI	<i>P</i>
Age group								
<5 years	802	39.4	33.7-45.6	<0.0001†	204	56.2	43.1-70.1	0.6†
5-15 years	3,347	35.4	32.7-38.3		607	52.9	45.9-60.1	
>15 years	5,236	24.6	22.8-26.5		353	41.4	33.7-50.1	
Sex								
Male	5,925	30.3	28.4-32.3	0.03	639	53.7	47.0-60.7	0.02
Female	3,460	27.8	25.5-30.3		525	43.7	36.7-51.5	
Haematocrit %								
<30	1,252	38.6	34.4-43.1	<0.0001	130	47.2	35.1-61.2	0.4
≥30	7,318	27.9	26.3-29.6		865	50.4	44.9-56.2	
Log _e parasitaemia								
<25 th centile (~1,400/μL)	2,454	23.3	20.7-26.2	<0.0001†	158	39.3	28.2-53.0	0.005†
25 th -50 th centile (~1,400-6,600/μL)	2,223	26.8	23.7-30.1		391	50.0	41.1-59.6	
50 th -75 th centile (~6,600-35,900/μL)	2,184	29.4	26.4-32.5		429	40.1	32.0-49.3	
>75 th centile (>~35,900/μL)	2,524	36.9	33.9-40.0		186	69.4	59.5-78.8	
<i>P. falciparum</i> gametocytaemia at enrolment								
No	8,847	28.7	27.2-30.3	<0.0001	1,116	48.3	43.2-53.6	0.008
Yes	437	41.4	34.4-49.2		43	69.1	48.5-87.4	
Total	9,385	29.4	27.9-30.9		1,164	49.3	44.3-54.5	

* Kaplan-Meier cumulative failure estimates at day 63

† Log-rank test for trend

Abbreviations: n; number, 95% CI; 95% confidence interval. Table reproduced with permission from Douglas NM, et al. *Plasmodium vivax* recurrence following falciparum and mixed species malaria: risk factors and effect of antimalarial kinetics. *Clin Infect Dis* 2011; **52**: 612-20.

Table 9.3 Multivariable Cox proportional hazards models showing the effect of baseline factors and antimalarial drugs on risk of *P. vivax* recurrence

	Recurrence with <i>P. vivax</i>		
	AHR	95%CI	<i>p</i>
All drugs			
Drug half-life			
Short ($t_{1/2} < 1$ day)	1	-	-
Intermediate ($t_{1/2}$ 1-7 days)	0.43	0.29-0.63	<0.001
Long ($t_{1/2} \geq 7$ days)	0.12	0.08-0.18	<0.001
Species at enrolment			
Pure <i>P. falciparum</i>	1	-	-
Mixed <i>P. falciparum</i> / <i>P. vivax</i>	2.47	2.15-2.85	<0.001
Age (per year increase)	0.98	0.97-0.98	<0.001
Sex			
Female	1	-	-
Male	1.27	1.14-1.41	<0.001
Hct (per percentage point increase)	0.98	0.97-0.99	<0.001
Log _e parasite density (per log _e order)	1.09	1.07-1.12	<0.001
<i>P. falciparum</i> gametocytaemia			
No	1	-	-
Yes	1.38	1.14-1.69	0.001
Artemisinin combination therapies*			
Artesunate+mefloquine combinations	1	-	-
DHA+piperaquine combinations	1.12	0.79-1.58	0.5
Artemether+mefloquine combinations	0.80	0.42-1.51	0.5
Artemether+lumefantrine	3.57	2.91-4.37	<0.001
Artesunate+atovaquone+proguanil	4.20	2.79-6.31	<0.001

Abbreviations: AHR; adjusted hazard ratio, 95%CI; 95% confidence interval, Hct; haematocrit, DHA; dihydroartemisinin

* Model also includes species at enrolment, age, sex, haematocrit, log_e parasite density and *P. falciparum* gametocytaemia at enrolment. Table reproduced with permission from Douglas NM, et al. *Plasmodium vivax* recurrence following falciparum and mixed species malaria: risk factors and effect of antimalarial kinetics. *Clin Infect Dis* 2011; **52**: 612-20.

9.3.2. Effect of antimalarial drugs on risk of *Plasmodium vivax* recurrence

The median times to *P. vivax* recurrence following treatment with short, intermediate and long half-life regimens were 28, 29 and 49 days respectively (p for overall difference = 0.0001; Figure 9.1). Treatment with more slowly eliminated antimalarials was associated with a significant trend to decreasing risk of *P. vivax* recurrence up to 63 days after both pure falciparum malaria and mixed infection (p for trend <0.0001 in both cases; Figure 9.2). The cumulative proportion of patients treated with a rapidly eliminated antimalarial who had a recurrence of *P. vivax* following pure falciparum malaria was 53.8% (95% CI 48.5-59.3%) compared to 21.1% (95% CI 19.5-22.9%) in those treated with slowly eliminated regimens (p <0.0001). All patients with mixed species infections who were treated with a rapidly eliminated antimalarial had a recurrent infection within 49 days of follow-up. The adjusted hazard ratios for *P. vivax* recurrence following either falciparum or mixed infection for patients receiving long or intermediate half-life regimens were 0.43 (95% CI 0.29-0.63), p <0.001 and 0.12 (95% CI 0.08-0.18), p <0.001 respectively when compared to those receiving rapidly eliminated antimalarials (Table 9.3).

The median times to *P. vivax* recurrence following artesunate+atovaquone+proguanil, artemether+lumefantrine, artesunate+mefloquine, dihydroartemisinin+piperazine and artemether+mefloquine were 28, 29, 49, 49, and 56 days respectively (p for overall difference =0.0001). Of the artemisinin combination therapies, those regimens containing mefloquine or piperazine appeared to be equally effective at preventing *P. vivax* recurrence in both univariable and multivariable analyses (Figure 9.3 and Table 9.3). The shorter-acting combinations, artemether+lumefantrine and artesunate+atovaquone+proguanil, were associated with 3.6 and 4.2 fold increases in risk of *P. vivax* recurrence respectively when compared with artesunate+mefloquine (p <0.001 in both cases) (Table 9.3).

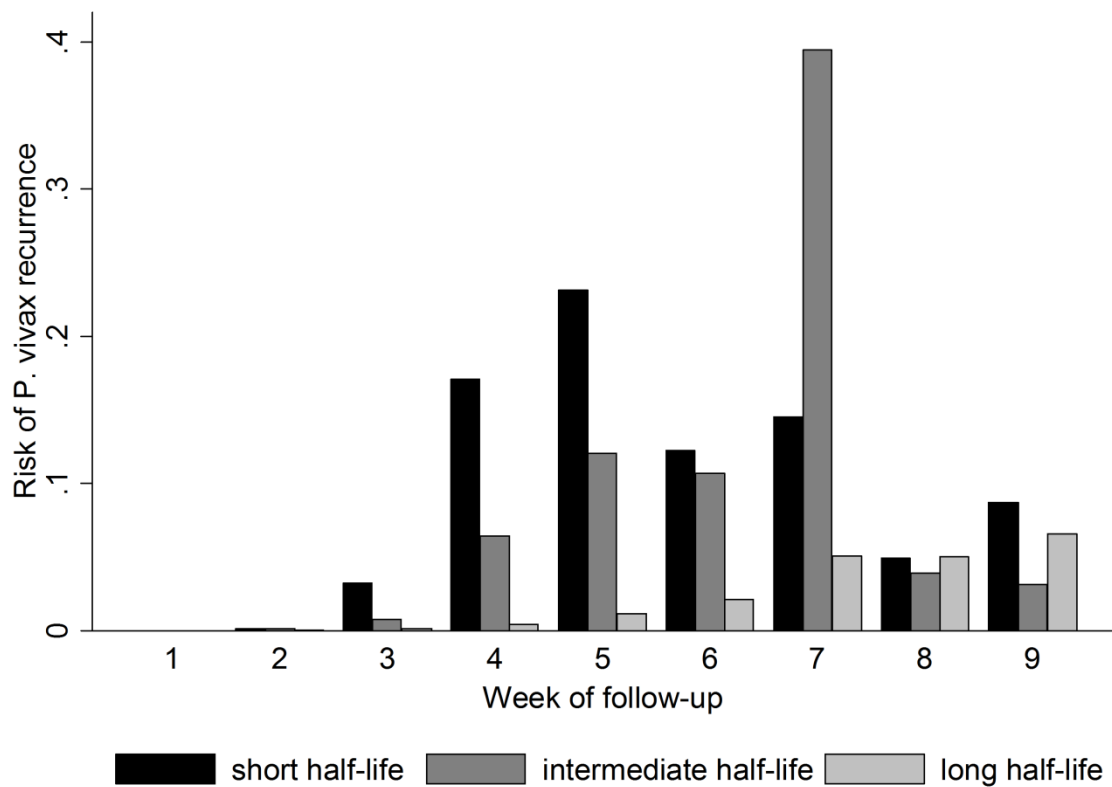


Figure 9.1 Risk of *P. vivax* recurrence following *Plasmodium falciparum* monoinfection or mixed *P. vivax* / *P. falciparum* malaria by week of follow-up and antimalarial half-life. Reproduced with permission from Douglas NM, et al. *Plasmodium vivax* recurrence following falciparum and mixed species malaria: risk factors and effect of antimalarial kinetics. *Clin Infect Dis* 2011; 52: 612-20.

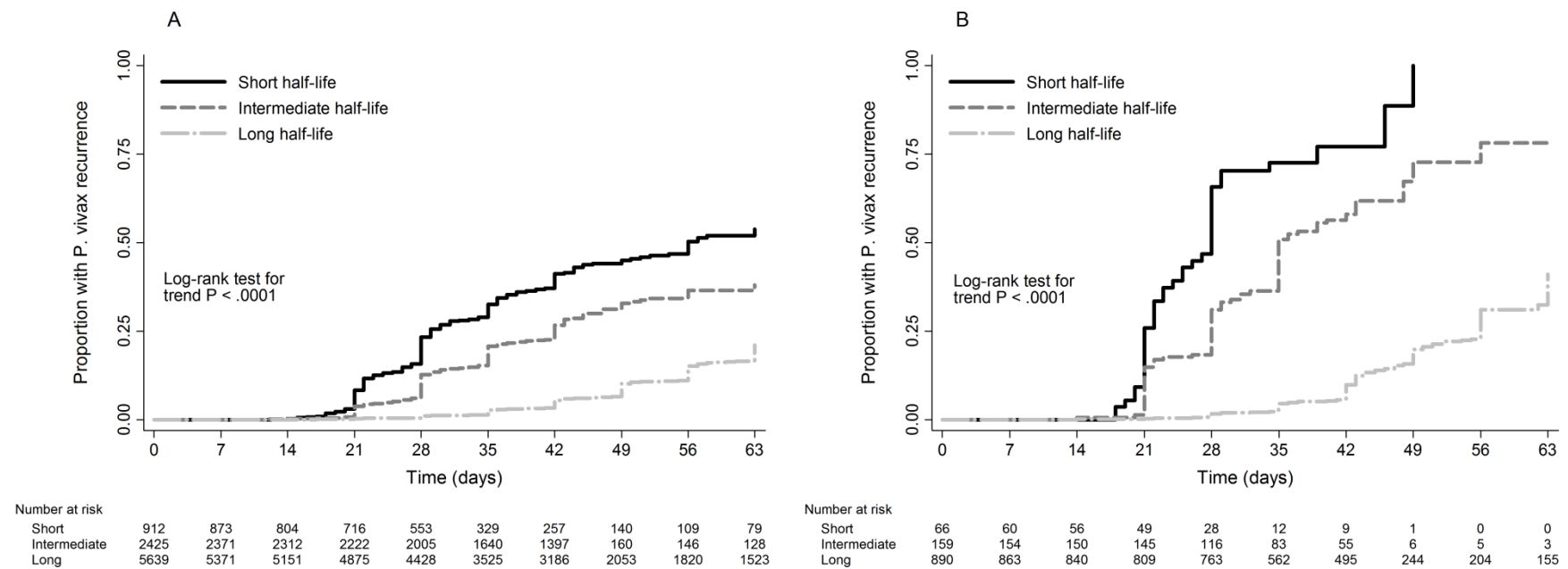


Figure 9.2 Kaplan-Meier failure estimates for the cumulative risk of *Plasmodium vivax* recurrence following *P. falciparum* infection (A) and following mixed *P. falciparum* / *P. vivax* infection (B) by antimalarial half-life. Reproduced with permission from Douglas NM, et al. *Plasmodium vivax* recurrence following falciparum and mixed species malaria: risk factors and effect of antimalarial kinetics. *Clin Infect Dis* 2011; **52**: 612-20.

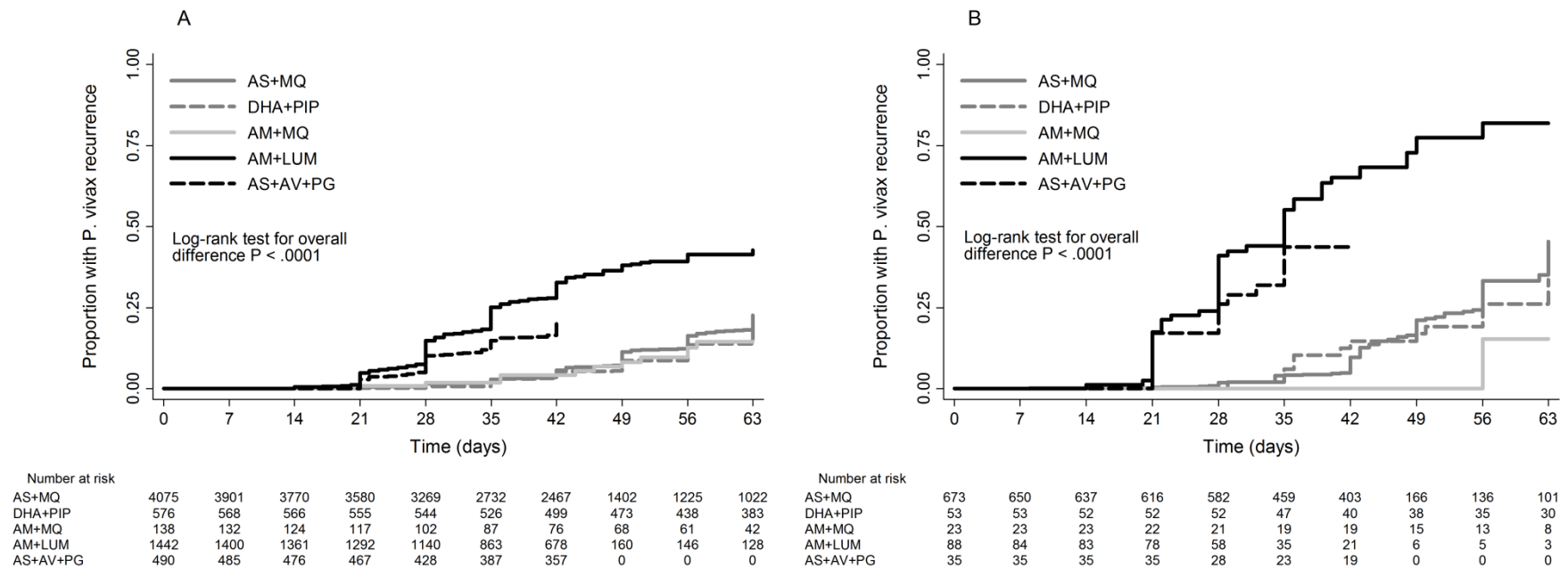


Figure 9.3 Kaplan-Meier failure estimates for the cumulative risk of *Plasmodium vivax* recurrence following *P. falciparum* infection (A) and following mixed *P. falciparum* / *P. vivax* infection (B) for artemisinin combination therapies. Reproduced with permission from Douglas NM, et al. *Plasmodium vivax* recurrence following falciparum and mixed species malaria: risk factors and effect of antimalarial kinetics. *Clin Infect Dis* 2011; 52: 612-20.

Abbreviations: AS+MQ; artesunate+mefloquine, DHA+PIP; dihydroartemisinin+piperaquine, AM+MQ; artemether+mefloquine, AM+LUM; artemether+lumefantrine, AS+AV+PG; artesunate+atovaquone+proguanil.

9.4 Discussion

In a large series of clinical trials conducted on the Thai-Myanmar border, *Plasmodium vivax* infection accounted for substantially more malaria recurrences within 63 days of treatment for falciparum or mixed malaria than *P. falciparum*. Since *P. vivax* is more frequently associated with gametocytaemia [2,3,285] and is more transmissible at low parasite densities [287], the most commonly transmitted parasite following treatment for falciparum malaria paradoxically was not *P. falciparum* but *P. vivax*.

Statistically significant baseline risk factors for *P. vivax* recurrence following acute falciparum malaria included initial mixed species infection, male sex, younger age, higher total asexual parasitaemia, lower haematocrit and presence of *P. falciparum* gametocytaemia. Slowly eliminated antimalarial regimens, such as those containing mefloquine or piperazine, were associated with a markedly lower risk of *P. vivax* recurrence when compared to rapidly eliminated drugs.

High asexual *P. falciparum* parasitaemia is a well-recognised risk factor for subsequent *P. falciparum* recrudescence [299,474-476,482]. In the present analysis it was also shown to increase the risk of *P. vivax* recurrence. One potential explanation for this phenomenon is that higher *P. falciparum* density, lower haematocrit and younger age are proxy markers of malaria naivety and hence poor immunity to both *P. falciparum* and *P. vivax* infections. If this is true, relapses from *P. vivax* hypnozoites acquired at or around the same time as the index *P. falciparum* infection would have a greater chance of reaching patency. Simultaneous, or near simultaneous, infection with *P. falciparum* and *P. vivax* is probably relatively common. Mason and colleagues showed that 10.5% of patients treated for vivax malaria in Bangkok subsequently had recurrence of *P. falciparum* within 28 days [483]. Since *P. falciparum* does not have a dormant form, and there is no local malaria transmission in Bangkok, these parasites are most likely to have been acquired at the same time as the *P. vivax* infections.

An alternative, but potentially complimentary, hypothesis is that high parasitaemia and low haematocrit are indicators of greater disease severity and hence pathophysiological and immunological derangement; a consequence of which may be stimulation of *P. vivax* relapse and / or failure to suppress growth of recurrent blood stage infection. This mechanism would be equally plausible regardless of whether the relapsing *P. vivax* hypnozoites had been acquired at the same time or prior to the index *P. falciparum* infection. Since the excess risk of *P. vivax* recurrence is seen even after slowly eliminated therapies, these putative factors would either have to be long-lasting or induce a prolonged stream rather than a single pulse of relapsing merozoites from the liver.

Highly sensitive PCR-based assays typically reveal a much higher prevalence of concurrent mixed species infection than light microscopy [237,238,241,368,484]. This suggests that a sizeable proportion of patients with microscopically confirmed *P. falciparum* mono-infections in co-endemic regions actually have subpatent *P. vivax* parasitaemia. In our study, patients presenting with falciparum gametocytaemia were at 1.38 times the risk of early recurrence with *P. vivax* compared to those without gametocytaemia. Presence of gametocytes is more likely in patients with chronic, asymptomatic infections and may therefore be suggestive of multiple previous exposures to both *Plasmodium* species and thus a greater risk of subpatent vivax infection at enrolment.

This pooled meta-analysis included a large number of individuals treated with multiple different antimalarial regimens. The individual trials were conducted in similar physical environments helping to ensure comparability of their results. Nevertheless, several sources of inter-study heterogeneity remain. Some of these could be partially addressed in multivariable models by controlling for differences in the age structure and median parasite density of study participants. Other known and unknown sources of heterogeneity such as differences in dosing schedules for individual regimens and temporal differences in local

malaria incidence could not be controlled for. The Cox models with gamma frailty present an averaged effect of specific regimens across the different studies [481].

The long-term benefits of prolonged post-treatment prophylaxis against recurrent parasitaemia have yet to be determined. With the exception of the antifolate drugs, antimalarial compounds active against *P. falciparum* have excellent efficacy against the blood stages of *P. vivax* and thus the drug regimens included in this analysis should have cleared initial subpatent *P. vivax* infections [308]. The risk of *P. vivax* reinfection in this region is low (less than 5% during a 42 day period) [51,485]. One can therefore assume that most of the observed *P. vivax* recurrences were relapses from dormant liver-stage forms. Hypnozoites have the potential to seed multiple relapses and, as stated in the previous chapter, it is not known whether prevention of just one of these by use of a slowly eliminated antimalarial will reduce the total number of relapses (and therefore reduce transmission potential) or simply delay the occurrence of the next one. Regardless, a greater period of post-treatment prophylaxis against recurrence with any *Plasmodium* species should facilitate fuller haematological and clinical recovery [2,3].

These speculative benefits must be weighed against potential disadvantages. Drugs with long terminal elimination half-lives will be present in the bloodstream at sub-therapeutic concentrations for longer than rapidly eliminated drugs and will therefore provide a more powerful force for the spread of drug-resistant parasites [75,108,486]. The combination of mefloquine and artesunate has been used for *P. falciparum* malaria along the northwestern border of Thailand both in trials and routine practice since 1994. Recent studies have revealed an increase in the prevalence of *pvm-dr1* gene amplification in local *P. vivax* isolates, a polymorphism associated with reduced susceptibility to mefloquine [370]. Although post-hoc exploratory analyses (not presented), showed that the risk of *P. vivax* recurrence following artesunate+mefloquine has increased slightly with time, it is unclear whether this is due to emerging mefloquine resistance or variation in background endemicity.

In this series of clinical trials, *P. vivax* was the most common cause of parasitological failure and almost certainly the most frequently transmitted parasite following *P. falciparum* and mixed infection. The risk of *P. vivax* recurrence in the 9 weeks following initial falciparum or mixed malaria is inversely correlated with antimalarial half-life. Slowly eliminated regimens should facilitate full clinical recovery and if used on a large scale, may reduce transmission of both *P. falciparum* and *P. vivax*. Although further work is required to establish the risk and deleterious effects of *P. vivax* recurrence in other regions, our study suggests that there is a coherent argument for safe provision of a sterilising course of antirelapse therapy (currently 14 days of primaquine) for all patients with malaria in co-endemic regions.

9.5 Acknowledgements

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10. Effectiveness of unsupervised primaquine for preventing *Plasmodium vivax* relapses

10.1 Introduction

The 8-aminoquinoline primaquine is the only licensed agent known to be active against *Plasmodium vivax* hypnozoites [290]. As emphasised in previous chapters, it is a critically important drug for preventing relapses and thus reducing, or eliminating, the global burden of vivax malaria. The World Health Organization recommends a dose of 0.25-0.5mg/kg primaquine daily for 14 days (based on “very low quality evidence”) for radical treatment of vivax malaria in those who are not deficient in the glucose-6-phosphate dehydrogenase enzyme [78]. Outside of study environments, adherence to this long regimen is commonly, though anecdotally, believed to be poor [255]. Adherence data from clinical trials are inconsistent with some studies suggesting supervision of therapy improves its effectiveness [284,487,488] but another study showing no impact of supervision [489]. Despite widespread use since the 1960s, there have not been any rigorous studies of the effectiveness of primaquine prescribing in routine practice in vivax-endemic regions. The potential perils of unevaluated use are starkly demonstrated by events in India, Pakistan and other regions of

Asia where a 5-day unsupervised course was used for many years until randomised controlled trials showed that this regimen had no effect on local relapse patterns [391,490,491].

Early work by Alving and others demonstrated that concurrent administration of primaquine with either quinine or chloroquine was associated with lower risk of subsequent vivax relapse (5% and 26% respectively) than serial administration of quinine followed by primaquine (79%, $p<0.001$ and $p<0.01$ respectively) [364]. Subsequent research reviewed by Schmidt and others has suggested that the degree of potentiation of primaquine might vary depending on the particular blood schizontocide administered [255,365,391]. Chloroquine remains the mainstay of treatment for *P. vivax* infections in most endemic countries. However four regions (Indonesian Papua, Papua New Guinea, Vanuatu and the Solomon Islands) have adopted artemisinin combination therapies for first-line treatment of vivax malaria and with worsening chloroquine resistance, this strategy is likely to gain wider acceptance [108]. Although the artemisinins and their common partner drugs are known to be highly active against the blood stages of *P. vivax* [2,80,252], there are no data on whether they potentiate the antirelapse effect of primaquine. The presence or absence of this property will have a major bearing on the desirability of a unified ACT-based treatment strategy for all *Plasmodium* species in regions with co-endemicity.

Using individualised, prospective clinical and pharmacy surveillance data collected between April 2004 and May 2009 at Mitra Masyarakat Hospital in Southern Papua, Indonesia, this analysis aimed to establish: first whether unsupervised primaquine at a dose of 0.25-0.5mg/kg per day for 14 days compared with no primaquine reduces the risk of re-presentation to hospital due to vivax malaria, second whether a dose of 0.5mg/kg of primaquine per day for 14 days provides greater benefit than 0.25mg/kg per day for 14 days

and third whether co-administration of dihydroartemisinin+piperaquine potentiates the action of primaquine to the same extent as co-administration of oral quinine.

10.2 Methods

10.2.1. Hospital treatment protocols

The geography, climate and demographics of Mimika District as well as the characteristics of Mitra Masyarakat Hospital were introduced in Chapter 4 [1,49]. Between 2004 and 2009, protocols for the treatment of uncomplicated *P. vivax* infections at RSMM changed from oral quinine plus 0.25mg/kg of unsupervised primaquine per day for 14 days to dihydroartemisinin+piperaquine plus 0.5mg/kg of unsupervised primaquine per day for 14 days. The switch from oral quinine to DHA+PIP occurred over a period of one month in March 2006 whereas the change in dose of primaquine occurred much more gradually (Figure 10.1). Although primaquine has been recommended consistently, its administration has been relatively inconsistent due to individual variation in clinical practice and interruptions in the supply chain. Testing for glucose-6-phosphate dehydrogenase deficiency is not done routinely.

10.2.2. Laboratory and data collection procedures

This analysis uses data from the routine surveillance system operated by staff of Mitra Masyarakat Hospital. Every patient presentation to RSMM, whether to the outpatients department, the emergency department or the inpatient wards, is recorded by dedicated hospital administrators and linked to a unique patient identifier. Details of all prescriptions filled at the hospital pharmacy (including drug name, total dose prescribed and date of prescription) are also recorded and linked indirectly to the same unique identifying number. Strict hospital protocols require that all patients presenting with fever or other symptoms potentially compatible with malaria have blood taken for a malaria film.

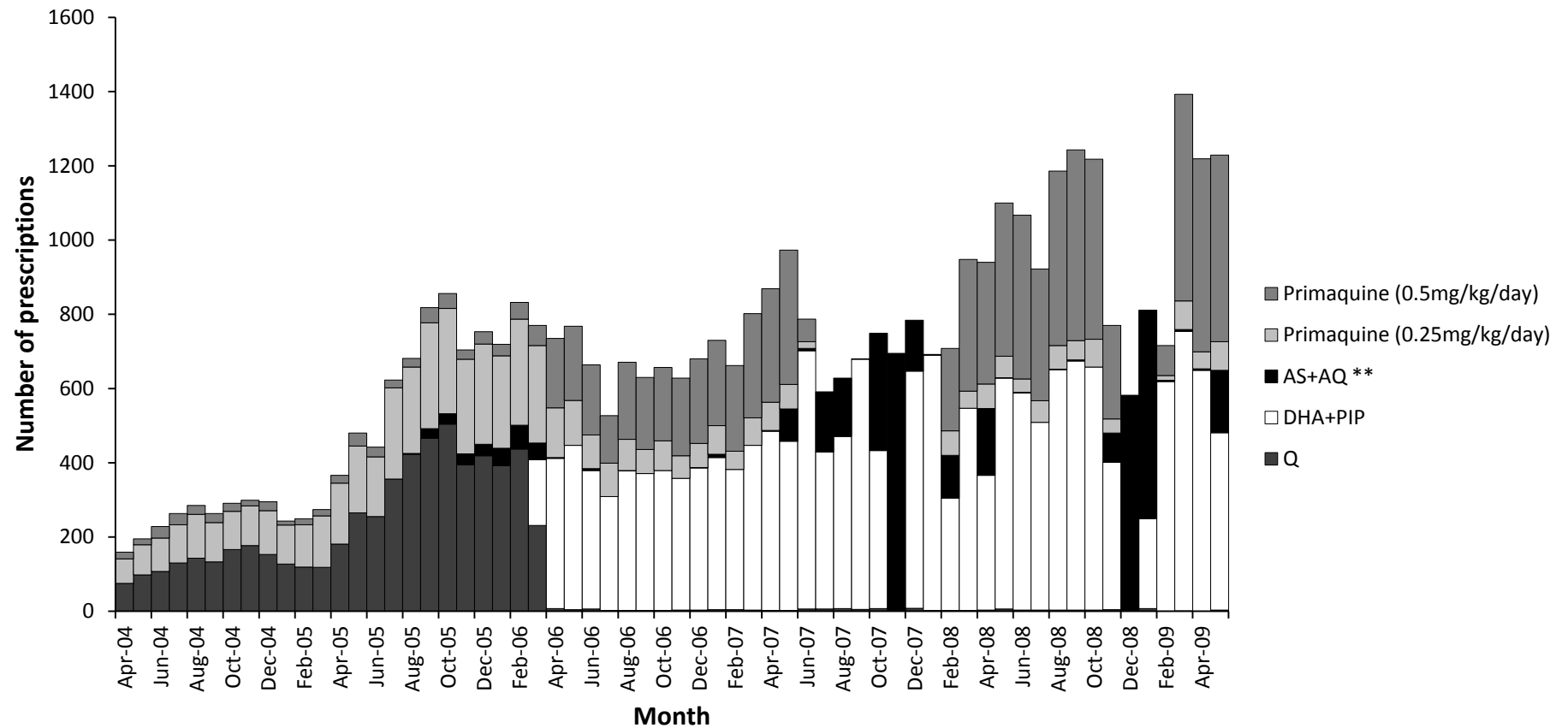


Figure 10.1 Timeline of antimalarial prescriptions for patients with *P. vivax* infection presenting to Rumah Sakit Mitra Masyarakat between April 2004 and May 2010. ** Patients treated with artesunate+amodiaquine were not included in this analysis.

Abbreviations: AS+AQ; artesunate+amodiaquine, DHA+PIP; dihydroartemisinin+piperaquine, Q; quinine

10.2.3. Data merging and statistical analysis

Clinical events (n=497,983) were merged with antimalarial prescriptions (n=186,038) by forming all pairwise combinations for each individual's hospital number. Of the antimalarial prescriptions, 182,285 (98.0%) matched to a corresponding hospital number in the clinical database and of these 165,961 (91.0%) fell between the date of admission and discharge (the same day for outpatient visits). Antimalarial prescriptions outside of these dates were dropped (n=16,324, 9.0%). Total primaquine doses were estimated in milligrams per kilogram per day over a 14-day period using predicted mean weights for age and sex obtained from the house-to-house survey of 5,255 individuals performed in 2005 [1]. Doses were then categorised as: (a) no primaquine (n=14,915 events [9,915 individuals], (b) 0.25mg/kg/day (taken as 0.15 to <0.4mg/kg/day, n=6,049 events [4,863 individuals]), (c) 0.5mg/kg/day (taken as >0.4mg/kg/day, n=9,352 events [7,001 individuals]) or (d) doses less than 0.15mg/kg/day but greater than zero (excluded from analyses, n=2,092 events [1,938 individuals]).

Kaplan-Meier failure curves for the risk of re-presentation to hospital with vivax malaria were plotted stratified by baseline characteristics, blood schizontocidal treatment and dose of primaquine administered. The log-rank test for overall difference or trend (as appropriate) was used to compare the hazard of re-presentation by age group (<1 year, 1-<5 years, 5-<15 years and ≥15 years), sex, ethnicity (non-Papuan, Highland Papuan and Lowland Papuan), year of initial presentation (2004 to 2009), species of initial infection (pure *P. vivax* or mixed *P. vivax* / *P. falciparum*), presentation number (1st-5th presentation), admission status (inpatient or outpatient), blood schizontocidal treatment (oral quinine or dihydroartemisinin+piperaquine) and dose of primaquine.

Cox proportional hazards regression was used for multivariable analyses. A single model inclusive of all patients treated with either oral quinine or DHA+PIP was used to establish

the effectiveness of primaquine as well as the relative benefit of the higher versus the lower dose. To examine the relative effectiveness of primaquine when combined with oral quinine as opposed to DHA+PIP, separate models (adjusting for the same confounders) were constructed for these two schizontocides then the resultant hazard ratios were compared according to the method of Altman and Bland [492]. The robustness of the results to the potential confounding effects of age and year of presentation was examined by constructing *a priori* subgroup models for the age groups 1 to <5 years, 5 to <15 years and ≥ 15 years and for the year 2006 (when there were frequent prescriptions of both oral quinine and dihydroartemisinin+piperazine).

In order to determine the most appropriate duration of follow-up that would capture most of the period of excess risk of re-presentation due to relapse but not result in excessive influence of background reinfection rates, I constructed graphs of the first and second derivatives of the overall failure curve (which can be interpreted as the non-cumulative risk of re-presentation by time and the rate of change in risk of re-presentation by time respectively; Figure 10.2). The point of inflexion of the first derivative and the point at which the second derivative settled at zero occurred at approximately one year of follow-up, after which the rate of change in risk of re-presentation remained static, reflecting background re-presentation rates. Follow-up data were therefore censored at one year for all analyses.

Children under one year of age were excluded from all models since primaquine is contraindicated in this age group [493] and was rarely prescribed. Data on pregnancy status were not available. The proportional hazards assumption was assessed for each covariable by comparing visually the log(cumulative hazard) by time of follow-up curves for each covariable category and subsequently by fitting and comparing models (by means of the likelihood ratio test) with and without time of follow-up interaction terms. Year of

presentation and blood schizontocidal treatment were found to violate the proportional hazards assumption. I therefore stratified the Cox models for these two factors.

A substantial minority of patients in the database (n=6,406, 35.2%) had multiple presentations to hospital with vivax malaria. To avoid problems with non-independence of the data, Kaplan-Meier analyses were restricted to time between the 1st and 2nd presentations. In the Cox models, up to 5 presentations per individual were included and the variance-covariance matrices were corrected for intra-subject correlation (giving robust standard errors). Since number of presentations was predictive of a further presentation, this was also included as a covariable in the final models.

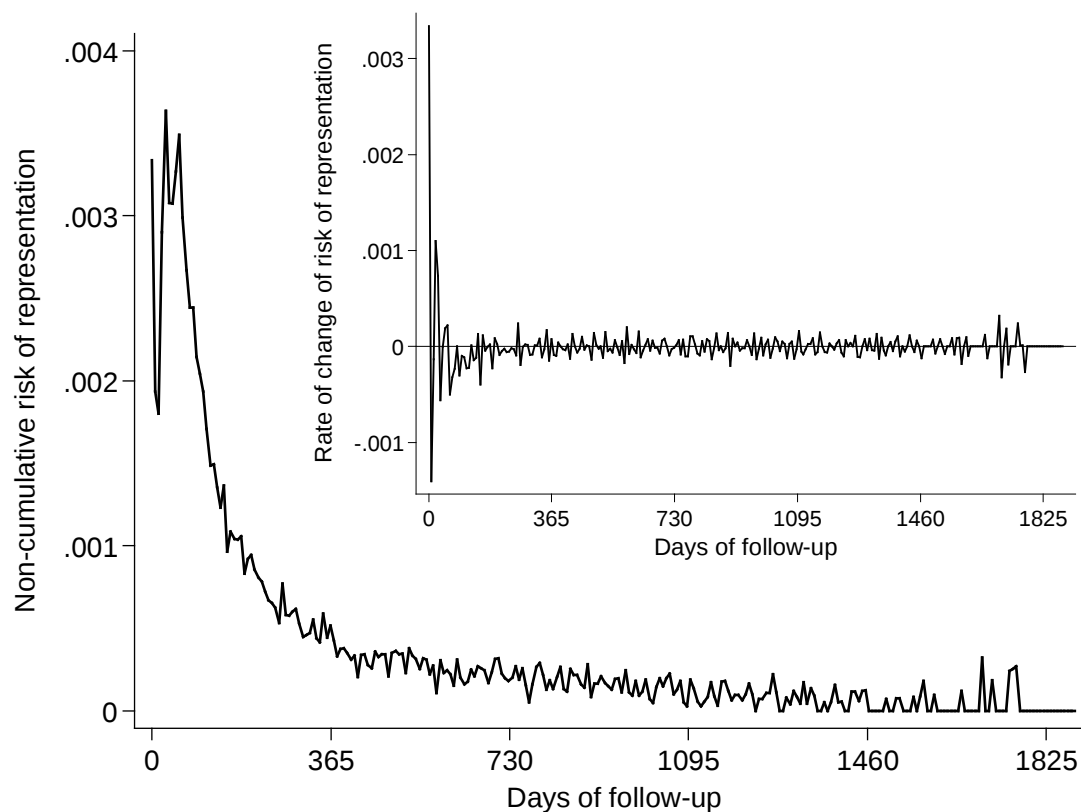


Figure 10.2 Non-cumulative risk of re-presentation to hospital with vivax malaria following initial *P. vivax* infection (main figure) and rate of change of risk of re-presentation (inset)

10.3 Results

Between April 2004 and May 2009, 103,500 individuals presented to Rumah Sakit Mitra Masyarakat a total of 497,983 times. During that period 18,194 individuals had a total of 32,408 presentations with either pure *P. vivax* malaria (n = 26,395, 81.4%) or mixed *P. vivax* / *P. falciparum* infection (n = 6,013, 18.6%). Of these, 5,293 (16.3%) presentations resulted in admission to hospital. The maximum number of presentations with vivax malaria for any one individual was 10 (IQR 1-2). The median age for those presenting with pure *P. vivax* infections was 9 years (IQR 2.4-23.6 years) and for those presenting with mixed infection was 13.1 years (IQR 3.2-24.2). Overall, 6,004 (18.5%) cases were treated with oral quinine, of whom 2,818 (46.9%) also received primaquine at a dose of 0.25mg/kg/day for 14 days and

491 (8.2%) received 0.5mg/kg/day. In total, 17,783 (54.9%) cases were treated with dihydroartemisinin+piperaquine of whom 1,918 (10.8%) received the lower dose of primaquine and 8,235 (46.3%) the higher dose (Table 10.1). Prescription of oral quinine was rare beyond 2006 and no dihydroartemisinin+piperaquine was prescribed before 2006.

10.3.1. Baseline risk factors for re-presentation with vivax malaria

After one year of follow-up, the cumulative proportion of individuals representing to RSMM at least once with vivax malaria following initial *P. vivax* or mixed infection was 31.7% (95% CI 31.0-32.42%). The median time to re-presentation was 86 days (IQR 41-201 days). Assuming that the risk of re-presentation between one and three years of follow-up reflects the background rate of re-presentation due to reinfection and that recurrence of *P. vivax* is equally likely to result in re-presentation to hospital whether it is due to recrudescence, relapse or reinfection, 9.63% of re-presentations by one year of follow-up were estimated to be due to reinfection.

In univariable analyses, increasing age was associated with a statistically significant trend to decreasing risk of re-presentation with individuals aged over 15 years having less than half the risk of infants under one year of age (1-year cumulative risk of re-presentation = 24.1% [95% CI 23.2-25.0%] and 53.4% [95% CI 51.0-55.9%] respectively, p for trend <0.0001) (Figure 10.3). Gender had negligible effects on the risk of re-presentation whereas Highland Papuans were at much greater risk (35.3% [95% CI 34.5-36.2%]) than both non-Papuan Indonesians (20.9% [95% CI 19.3-22.7%]) and Lowland Papuans (21.8% [95% CI 19.9-23.8%]), p <0.0001). Overall 32.5% (95% CI 31.8-33.3%) of individuals with pure *P. vivax* infection had a recurrence within one year compared to 28.1% (95% CI 26.5-29.7) of patients with mixed *P. vivax* / *P. falciparum* infections (p <0.0001).

Table 10.1 Distribution of risk factors for re-presentation with vivax malaria by treatment group

	Oral Quinine, number of patients (%)				Dihydroartemisinin+piperaquine, number of patients (%)			
	No PQ	PQ 0.25mg/kg daily	PQ 0.5mg/kg daily	Total*	No PQ	PQ 0.25mg/kg daily	PQ 0.5mg/kg daily	Total*
Species								
Pure <i>P. vivax</i>	1,523 (83.5)	2,181 (77.4)	433 (88.2)	4,722 (78.6)	5,272 (79.4)	1,543 (80.4)	6,694 (81.3)	14,266 (80.2)
Mixed <i>P. vivax</i> / <i>P. falciparum</i>	302 (16.5)	637 (22.6)	58 (11.8)	1,282 (21.4)	1,365 (20.6)	375 (19.6)	1,541 (18.7)	3,517 (19.8)
Age								
<1 year	643 (35.2)	14 (0.5)	34 (6.9)	705 (11.7)	1,385 (20.9)	3 (0.2)	36 (0.44)	1,429 (8.0)
1-5 years	598 (32.8)	549 (19.5)	305 (62.1)	1,835 (30.6)	1,881 (28.3)	217 (11.3)	3,090 (37.5)	5,597 (31.5)
5-15 years	117 (6.4)	455 (16.1)	143 (29.1)	894 (14.9)	876 (13.2)	404 (21.1)	1,595 (19.4)	3,048 (17.1)
>15 years	467 (25.6)	1,800 (63.9)	9 (1.8)	2,570 (42.8)	2,495 (37.6)	1,294 (67.5)	3,514 (42.7)	7,709 (43.4)
Ethnicity								
Highland Papuan	1,433 (78.5)	2,376 (84.3)	393 (80.0)	4,913 (81.8)	5,357 (80.7)	1,518 (79.1)	6,761 (82.1)	14,461 (81.3)
Lowland Papuan	252 (13.8)	189 (6.7)	68 (13.8)	605 (10.1)	551 (8.3)	115 (6.0)	598 (7.3)	1,346 (7.6)
Non-Papuan	120 (6.6)	231 (8.2)	27 (5.5)	434 (7.2)	702 (10.6)	276 (14.4)	854 (10.4)	1,911 (10.7)
Sex								
Male	843 (46.2)	1,528 (54.2)	226 (46.0)	3,067 (51.1)	3,223 (48.6)	907 (47.3)	4,425 (53.7)	9,041 (50.8)
Female	982 (53.8)	1,290 (45.8)	265 (54.0)	2,937 (48.9)	3,414 (51.4)	1,011 (52.7)	3,810 (46.3)	8,742 (49.2)
Year								
2004	552 (30.2)	322 (11.4)	125 (25.5)	1,182 (19.7)	0 (0)	0 (0)	0 (0)	0 (0)
2005	931 (51.0)	1,865 (66.2)	270 (55.0)	3,627 (60.4)	0 (0)	0 (0)	0 (0)	0 (0)
2006	240 (13.2)	630 (22.4)	95 (19.3)	1,091 (18.2)	596 (9.0)	873 (45.5)	1,736 (21.1)	3,565 (20.0)

2007	54 (3.0)	1 (0.0)	1 (0.2)	56 (0.9)	3,563 (53.7)	342 (17.8)	1,385 (16.8)	5,494 (30.9)
2008	37 (2.0)	0 (0)	0 (0)	37 (0.6)	1,516 (22.8)	507 (26.4)	3,616 (43.9)	5,983 (33.6)
2009	11 (0.6)	0 (0)	0 (0)	11 (0.2)	962 (14.5)	196 (10.2)	1,498 (18.2)	2,741 (15.4)
Admission status								
Outpatient	1,060 (58.1)	2,202 (78.1)	382 (77.8)	4,258 (70.9)	5,573 (84.0)	1,616 (84.3)	7,411 (90.0)	15,420 (86.7)
Inpatient	765 (41.9)	616 (21.9)	109 (22.2)	1,746 (29.1)	1,064 (16.0)	302 (15.7)	824 (10.0)	2,363 (13.3)
Presentation number								
1	1,172 (64.2)	1,887 (67.0)	313 (63.7)	3,938 (65.6)	3,478 (52.4)	1,195 (62.3)	4,236 (51.4)	9,451 (53.1)
2	369 (20.2)	500 (17.7)	89 (18.1)	1,130 (18.8)	1,381 (20.8)	336 (17.5)	1,537 (18.7)	3,446 (19.4)
3	147 (8.1)	217 (7.7)	48 (9.8)	477 (7.9)	765 (11.5)	163 (8.5)	857 (10.4)	1,879 (10.6)
4	81 (4.4)	111 (3.9)	17 (3.5)	242 (4.0)	424 (6.4)	93 (4.8)	557 (6.8)	1,118 (6.3)
5	33 (1.8)	47 (1.7)	13 (2.6)	106 (1.8)	238 (3.6)	47 (2.5)	382 (4.6)	715 (4.0)
Total	1,825 (100)	2,818 (100)	491 (100)	6,004 (100)	6,637 (100)	1,918 (100)	8,235 (100)	17,783 (100)

Abbreviation: PQ; primaquine

* Totals include patients who received doses of primaquine greater than 0mg/kg/day but less than 0.15mg/kg/day

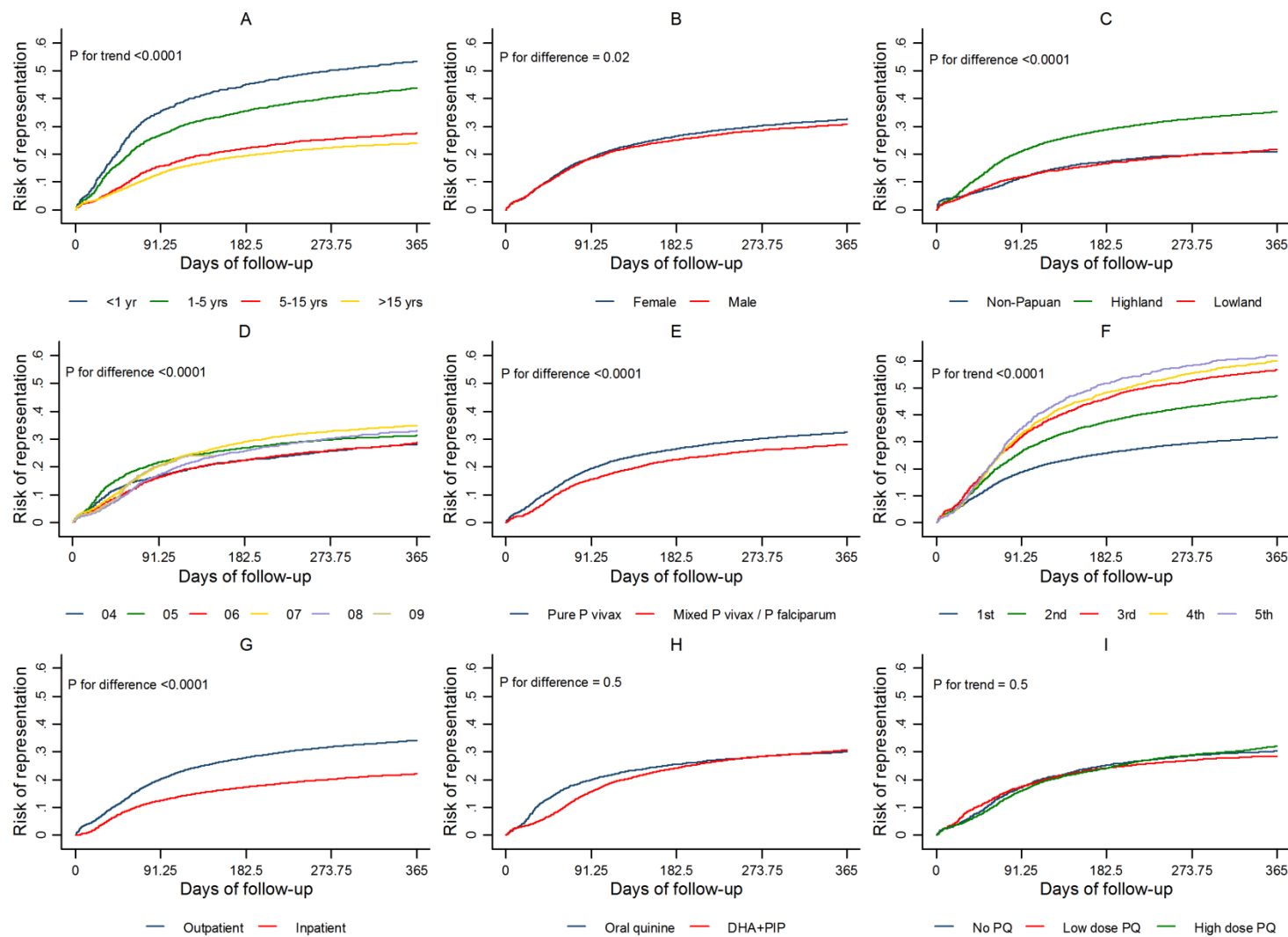


Figure 10.3 Cumulative risk of re-presentation to hospital with vivax malaria following initial *P. vivax* infection by age group (A), sex (B), ethnicity (C), year of initial presentation (D), species of initial infection (E), presentation number (F), admission status (G), schizontocidal treatment (excludes children under 1 year old) (H) and dose of primaquine (excludes children under 1 year old) (I)

10.3.2. Impact of primaquine on the risk of re-presentation

In univariable analyses the one year cumulative risk of re-presentation following administration of blood schizontocidal treatment alone was 30.4% (95% CI 28.9-32.1) compared to 28.5% (95% CI 26.9-30.2%) for patients receiving primaquine at a dose of 0.25mg/kg/day and 32.1% (95% CI 30.6-33.6) for patients receiving 0.5mg/kg/day of primaquine (p for trend 0.5; Figure 10.3).

After adjusting for known confounders, the overall Cox model similarly showed no benefit of unsupervised primaquine at a dose of 0.25 mg/kg/day (AHR = 1.09 [95% CI 1.01-1.19], $p=0.03$) or a dose of 0.5mg/kg/day (AHR = 1.01 [0.95-1.07], $p=0.7$) (Table 10.2). When the dose of primaquine was modelled as an unrestricted continuous variable, the hazard ratio for every 0.1mg/kg/day increase in dose was 1.01 (95% CI 1.00-1.02) $p=0.2$. There was no evidence of differential potentiation of primaquine by oral quinine versus dihydroartemisinin+piperaquine (ratio of AHRs for oral quinine $\pm$ 0.5mg/kg/day primaquine and DHA+PIP $\pm$ 0.5mg/kg/day primaquine = 0.92 [95% CI 0.71-1.04], $p=0.1$).

Table 10.2 Results of Cox proportional hazards models for the effect of primaquine on the risk of re-presentation to hospital with vivax malaria

	Primaquine dose	AHR	95% confidence interval	<i>p</i>
Overall model				
All ^a	None	1	-	-
	0.25mg/kg/day	1.09	1.01-1.19	0.03
	0.5mg/kg/day	1.01	0.95-1.07	0.7
Subgroup analyses				
Quinine ^b	None	1	-	-
	0.25mg/kg/day	1.08	0.94-1.24	0.3
	0.5mg/kg/day	0.89	0.74-1.06	0.2
DHA+PIP ^b	None	1	-	-
	0.25mg/kg/day	1.09	0.98-1.21	0.1
	0.5mg/kg/day	1.03	0.96-1.10	0.4
1-5yrs ^c	None	1	-	-
	0.25mg/kg/day	1.07	0.93-1.22	0.4
	0.5mg/kg/day	1.01	0.92-1.10	0.9
5-15yrs ^c	None	1	-	-
	0.25mg/kg/day	1.13	0.92-1.38	0.2
	0.5mg/kg/day	0.97	0.82-1.13	0.7
>15yrs ^c	None	1	-	-
	0.25mg/kg/day	1.13	0.99-1.28	0.07
	0.5mg/kg/day	1.12	1.01-1.24	0.04
2006 ^d	None	1	-	-
	0.25mg/kg/day	1.07	0.87-1.32	0.5
	0.5mg/kg/day	1.07	0.88-1.30	0.5

Abbreviations: DHA+PIP; dihydroartemisinin+piperazine, AHR; adjusted hazard ratio.

^a Cox model stratified by year and blood schizontocidal treatment (oral quinine or dihydroartemisinin+piperazine). Other covariables include: admission status (outpatient or inpatient), age, age squared, ethnicity (non-Papuan, Highland Papuan and Lowland Papuan) and presentation number.

^b Cox models stratified by year. Other covariables include: admission status, age, age squared, ethnicity and presentation number.

^c Cox models stratified by year and blood schizontocidal treatment. Other covariables include: admission status, ethnicity and presentation number.

^d Cox model stratified by blood schizontocidal treatment. Other covariables include: admission status, age, age squared, ethnicity and presentation number.

10.4 Discussion

The World Health Organization recommends a 14-day course of primaquine for terminal eradication of *P. vivax* infections. Several comparative trials have shown such regimens can be highly efficacious at reducing the risk of *P. vivax* relapse [249,255,261,284,290,494-496]. The main determinant of efficacy is the total dose given rather than the treatment duration with most recent studies suggesting that 7mg/kg is required for reliable cure [394,497]. In adults, this is usually given as 30mg per day for a minimum of 14 days (approximately 0.5mg/kg/day), although there has also been success with 60mg per day for 7 days [393,494] (the same dose over 3 days has not been shown to be efficacious) [495]. Adherence to the 14-day regimen is thought to be poor but trial data are inconsistent [284,487-489]. There is also evidence that the total dose of primaquine required may vary depending on local parasite strains [498,499]. The Chesson strain, first isolated in Papua New Guinea in 1944, [388] has long been held to be relatively tolerant to primaquine [392-394,500,501]. Whether this reflects acquired drug resistance or an intrinsic lack of drug response is the subject of some debate.

This chapter presents a large-scale comparative assessment of the anti-relapse effect of unsupervised primaquine in a real-world setting. After controlling for key confounders, our results provide no evidence that addition of 14 days of unsupervised primaquine at a dose of either 0.25 or 0.5mg/kg/day to oral quinine or dihydroartemisinin+piperaquine reduces the risk of re-presentation with vivax malaria in Southern Papua. This finding is consistent with either poor adherence to primaquine or tolerance / resistance of local *P. vivax* parasites in Papua. If local strains are indeed relatively primaquine-tolerant or resistant one would expect that supranormal doses of primaquine should at least partially overcome this lack of treatment response. In separate analyses (not shown), there was no benefit of primaquine even when given at very high doses, suggesting that poor adherence to the 14-day regimen rather than reduced drug susceptibility was more likely to be the prime determinant of the lack of effectiveness.

Around the mid-20th century, several authors posited that the 8-aminoquinolines, primaquine and pamaquine, may be potentiated by co-administration of quinine and chloroquine [364,394,502]. Alving suggested that this might relate to similarities in “chemical structure and pharmacologic action” of the drugs. Little by way of mechanistic explanation has been added since. Even very high doses of quinine and chloroquine have no demonstrable effect on hypnozoites. Therefore, if potentiation does indeed occur, it is likely to be due to facilitation of increased hypnozoitocidal activity of primaquine in the presence of the blood schizonticide (perhaps via increased concentration in the hepatocyte) rather than due to any hypnozoitocidal activity of the blood schizonticide. A potential concern is that drugs with different structures and mechanisms of action, such as many of the individual components of commonly-used ACTs, may not exhibit this synergistic property. Since this study did not show any significant antirelapse effect of primaquine, it was not possible to demonstrate any difference in the degree of potentiation provided by the two blood

schizontocides. Definitive evidence for a difference in potentiation will require large, randomised studies with supervised drug administration and long-term follow-up.

Our pragmatic study has some important strengths. Due to very large numbers, the estimates of the risk of re-presentation are unlikely to be attributable to chance variation. Microscopy services at RSMM have performed relatively well in quality assurance procedures (see Chapter 4) [1] and thus parasitological diagnoses are comparatively accurate when considered in the context of field laboratory services. Crucially, patients included in this analysis received their medications according to normal hospital procedures and were free from the powerful biases and coercive forces associated with study involvement. Although this is an important strength, it is also a notable limitation. In the absence of randomised and contemporaneous allocation, our data are susceptible to the effects of unknown confounders as well as attrition bias. These factors may explain the borderline significant association between low-dose primaquine prescription and an increased risk of subsequent re-presentation with vivax malaria overall. As is typical of routinely collected data sources, some degree of non-differential misclassification of both treatment and outcome may also have occurred. If present, this will have tended to dilute any associations towards the null. Although there was little temporal overlap in the prescription of oral quinine and dihydroartemisinin+piperaquine, restriction of the Cox model to the year 2006 (when substantial numbers of patients were treated with both oral quinine and DHA+PIP) did not materially change our findings suggesting that year of presentation was unlikely to have been an important confounder.

The analyses in this chapter assume that the risk of re-presentation to RSMM with vivax malaria is reflective of the overall risk of recurrence and, therefore, a reasonable outcome measure of the effectiveness of primaquine. Acquired immunity against *P. vivax* develops

quickly and will reduce the chance that a given infection becomes symptomatic. Therefore this assumption is likely to be most robust for young children. Dihydroartemisinin+piperaquine is rapidly parasitocidal and suppresses the first liver stage-relapse, [2,252] both properties that may limit the development of immunity when compared with oral quinine. Although this suggests that relapses following DHA+PIP may be more likely to be symptomatic than those following oral quinine (and therefore more likely to lead to re-presentation), this analysis has focused on proportionate rather than absolute changes in the risk of re-presentation in subgroup models, and thus should have accounted for any differential effects on immunity.

In summary, our study shows no evidence for any benefit of the currently recommended primaquine treatment regimen for terminal elimination of vivax malaria in Papua, Indonesia. Two urgent courses of action now need to be taken. First, the efficacy of primaquine regimens needs to be demonstrated in areas endemic for the Chesson strain of *P. vivax* in large randomised comparative trials with supervised administration. Second, public health measures aimed at increasing adherence to primaquine regimens, either through supervision or more practical dosing regimens, need to be implemented. In the meantime, it is critically important that high priority is given to development of alternative, effective, safe and pragmatic tissue schizontocides.

10.5 Acknowledgements

A modified version of this chapter is soon to be submitted to a peer-reviewed journal. The co-authors are Daniel A Lampah,¹ Enny Kenangalem,^{1,2} Paulus Sugiarto,³ Julie A Simpson,⁴ Yati Soenarto,⁵ Jeanne R Poespoprodjo,^{1,2} Nicholas M Anstey^{6,7} and Ric N Price.^{6,7,8}

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11. General discussion

This thesis has drawn on data from an extensive malariometric surveillance system, multiple clinical trials in both Papua and Thailand and a cross-sectional house-to-house survey. It set out to address two primary aims: first, to describe the burden of anaemia and direct and indirect mortality attributable to vivax malaria in Southern Papua and second, to determine the potential of currently available antimalarial drugs to reduce transmission of *P. vivax* in co-endemic areas. The following are the salient findings relevant to those aims.

Symptomatic *P. vivax* mono-infection in patients presenting for treatment in Southern Papua results in a similar overall mean reduction in haemoglobin compared to *P. falciparum* malaria and is proportionately just as likely to cause severe anaemia. The severity of haematological impairment associated with vivax malaria is greatest in the first year of life, an age group that also suffers the highest proportionate incidence of infection with *P. vivax*. Overall, vivax malaria is responsible for 6% of cases of severe anaemia at the hospital and 5% of moderate-to-severe anaemia in the community. In infancy, the corresponding population attributable fractions are 23% and 28% - substantially greater than for falciparum malaria (15% and 8% respectively).

In Southern Papua, patent coinfection with *P. vivax* and *P. falciparum* is associated with substantially greater haematological impairment than mono-infection with either species alone. The additional haematological insult is particularly noticeable after about three years of age.

Death directly attributable to vivax malaria, in particular vivax-associated extreme anaemia, does occur in Southern Papua but is relatively rare. More commonly, vivax malaria indirectly contributes to death in those with comorbidities such as malnutrition and / or chronic infectious diseases such as HIV and tuberculosis. The minimum case fatality of vivax malaria in hospitalised patients in Southern Papua is estimated to be 10 per 1,000 infections and in the community is 0.12 per 1,000 infections.

The transmissible sexual forms of *P. vivax* are short-lived and only appear in the presence of asexual forms. Adequate blood schizontocidal treatment of acute vivax malaria therefore results in rapid disappearance of gametocytes. Recurrent asexual infection, whether due to relapse, recrudescence or reinfection, is commonly associated with patent gametocytaemia, even in its early stages. Patients with high asexual parasitaemia at presentation are at increased risk of subsequent appearance of gametocytes during follow-up but this risk is most dependent on the antimalarial regimen used for acute treatment. All other things being equal, drugs or combinations with long terminal elimination half-lives result in a lower risk of gametocytaemia during 9 weeks of follow-up than rapidly eliminated drugs.

In a large series of clinical trials conducted on the Northwestern Thai-Myanmar border, *P. vivax* parasitaemia was the most common cause of parasitological failure following treatment of falciparum malaria – occurring in more than 50% of patients treated with rapidly eliminated drugs by 9 weeks of follow-up. A high proportion of the patients who had recurrent *P. vivax* infection are assumed to have been concomitantly or previously infected with *P. vivax* as opposed to newly infected during follow-up. Treatment with slowly eliminated antimalarials such as those containing mefloquine or piperazine was associated with a markedly reduced risk of *P. vivax* recurrence at 9 weeks. On the contrary, young

children, those with high asexual *P. falciparum* parasite density and those with lower haemoglobin concentrations were at increased risk of *P. vivax* recurrence.

Primaquine remains the only licensed drug for eradicating *P. vivax* hypnozoites and therefore the only feasible chemotherapeutic means of preventing future *P. vivax* relapses. In Southern Papua, prescription of a 14-day unsupervised course of primaquine according to WHO guidelines does not lead to a demonstrable reduction in the risk of re-presentation to hospital with vivax malaria, irrespective of whether the primaquine is given in combination with oral quinine or dihydroartemisinin+piperazine.

11.1 Project strengths

The project described in this thesis has important site-specific and methodological strengths. Malaria remains a significant scourge on Southern Papuan populations causing frequent presentation to health care facilities. Consequently the local surveillance systems have accrued very large numbers of cases of malaria over a relatively short period of time. Assessments of the haematological effects of malaria derived from these surveillance systems are therefore highly precise (as distinct from necessarily accurate). Large numbers of cases allowed the use of fractional polynomial models to depict the complex evolution of haematological changes associated with malaria infection throughout life. The local incidence of *P. vivax* infection is similar to falciparum malaria enabling meaningful comparisons of the burden of these two species. Moreover, a significant proportion of the population is aparasitaemic at any point in time and therefore attributing adverse haematological effects to parasitaemia in both the house-to-house survey and hospital datasets was less fraught with difficulty than it would have been in hyper- or holoendemic regions of Africa.

Use of surveillance data is a pragmatic means of assessing the patterns and impact of disease free from some of the important biases associated with a more intensive study environment.

For example, patients included in the analysis of the effectiveness of primaquine in Chapter 10 received their medication according to every-day procedures and had no additional study-related incentives to adhere to their prescribed regimens. Although other important sources of bias must be considered, such assessments offer a rare insight into the effectiveness of such interventions in the “real world”.

Unique identification of individuals within the hospital surveillance systems provided a powerful opportunity to conduct disaggregated and time-to-event analyses. Examples include the assessment of adjusted fractions of severe anaemia attributable to vivax malaria in Chapter 5 and the assessment of the effectiveness of primaquine in Chapter 10. Hospital malariometric surveillance was carried out relatively consistently during the accrual period. This can be attributed to a range of factors including strict hospital protocols regarding blood film examination and haematological assessment, automated data entry from the hospital pharmacy and the laboratory Coulter Counter and permanent employment of data entry clerks at both the hospital and the research facility.

Using multiple different data sources from Papua increased the internal validity of certain results and ensured a broad picture of the burden of vivax malaria in the region. Taken alone, relatively little weight could be given to the finding that a much higher proportion of infantile anaemia in the community is attributable to vivax malaria as opposed to falciparum malaria. However, replication of this observation in the hospital setting with much larger numbers significantly increases its credibility. Consistency of the effects of DHA+PIP and clinical factors on the risk of recurrent gametocyte carriage in Papua and Thailand similarly increased the internal validity of these findings. Given the different transmission dynamics in these two locations, it also suggests that these results may be generalisable to other regions with endemic vivax malaria.

11.2 Project limitations

Some important general limitations of this work should also be considered. The analyses in this thesis relied heavily upon data from routine surveillance systems and independent epidemiological studies. Analytical compromises had to be made that may not have been necessary had the studies been designed and initiated prospectively. For example a more robust analysis of the haematological effects of vivax malaria in the hospital might have been possible if data on nutritional status (as assessed by height and weight), intestinal worm infestation and haemoglobin and red blood cell variants were collected. Such intensive data collection would only have been practicable as part of a targeted observational study with much smaller numbers of patients. In the series of Thai trials used in the analysis of *P. vivax* recurrence following falciparum malaria, information on the presence or absence of *P. vivax* gametocytes at the time of recurrence would have given a better indication of the transmission potential of recurrent vivax infections.

Routinely collected surveillance data are susceptible to several forms of bias. Misclassification of *Plasmodium* species will have occurred in the hospital surveillance systems as a result of both microscopy and data entry errors. In 2004, concordance between hospital microscopists and research microscopists was 81.7% for a selection of slides initially read as *P. vivax* (3.8% were reclassified as *P. falciparum* infections) and 95.3% for a selection of slides initially read as *P. falciparum* (0.8% were reclassified as *P. vivax* infections). This mildly differential misclassification (in combination with presumably non-differential data entry error) will have tended to dilute the apparent difference in haematological effects of *P. vivax* and *P. falciparum*, in particular by making the effects of *P. vivax* seem slightly more severe. Microscopy will not identify very low density infections and it was thus impossible to exclude concurrent subpatent *P. falciparum* infections in the *P. vivax* death audit presented in Chapter 7. The audit was reliant on clinical judgement rather than scientific evidence of

the contribution of vivax malaria to the observed deaths. Alternative epidemiological methods might have provided a more objective means of inferring causality but would not have given any indication of the likely mechanisms of death. Incomplete ascertainment of haemoglobin status at the hospital has occurred and may have biased the fractions of severe anaemia attributable to *Plasmodium* parasitaemia towards zero. A substantially smaller proportion of aparasitaemic individuals had a matched haemoglobin measurement in the hospital database indicating that the clinical threshold for ordering a full blood count was higher in these individuals. The haemoglobin concentrations available for aparasitaemic individuals are therefore likely to be more heavily skewed to the left than the concentrations available for parasitaemic patients.

Ascertainment bias will also have reduced the ability to generalise results from the hospital to the community setting. Patients who present to the hospital, and who are therefore detected by hospital surveillance, have a more severe distribution of malarial disease than patients who remain in the community. Simple extrapolation of findings from the hospital to the community will therefore overestimate the regional impact of vivax malaria. Passive, hospital-based follow-up of patients included in the assessment of primaquine will not have detected individuals with recurrent asymptomatic parasitaemia who did not represent to hospital for medical review. Although this incomplete ascertainment will have decreased the apparent risk of recurrent *P. vivax* infection, there seems little reason to suspect that the effects of this bias would have been different in those who received primaquine as opposed to those who did not.

An important focus of Chapters 8 and 9 is the effect of blood schizontocide elimination kinetics on the transmission potential of *P. vivax* malaria. Strong evidence has been provided for a beneficial effect of slowly eliminated regimens on the risk of recurrent gametocytaemia

following vivax and mixed species infections and on recurrent asexual parasitaemia following falciparum malaria. However the trials included in these analyses had a maximum follow-up duration of 9 weeks and censored individuals at the point of first recurrence. As a result, the long-term transmission-blocking benefits of slowly eliminated blood schizontocidal drugs (in the absence of curative hypnozoitocidal therapy) remain speculative.

Several unique aspects of *P. vivax* epidemiology in New Guinea may limit the ability to generalise the results of the analyses in this thesis to other parts of the world. High-grade chloroquine resistance is much more prevalent amongst local *P. vivax* strains than elsewhere [108]. There is evidence that *P. vivax* in New Guinea is more resistant to the effects of primaquine than in other regions and therefore requires the higher 0.5mg/kg/day dosing regimen as opposed to the lower 0.25mg/kg/day regimen for radical cure [498,503]. Recent population-based reports also suggest that disease caused by *P. vivax* is particularly severe in this part of the world [49,50,96,118]; though whether this is due to greater virulence of circulating *P. vivax* strains or greater host-susceptibility remains unclear. Extrapolating the adverse haematological effects of vivax malaria in Papua is likely to overestimate the burden *P. vivax* infections in certain other regions. Similarly, imperfect adherence to primaquine in regions where *P. vivax* is highly sensitive to this medication may have less of an impact on effectiveness than it is assumed to have had in Papua.

11.3 Interpretation, implications and future priorities

Considering the forgoing strengths and limitations, the following offers speculative interpretation of the findings of this thesis and outlines their implications for malaria control efforts and future research priorities.

The entomological inoculation rate for *P. vivax* in Southern Papua is lower than for *P. falciparum* (unpublished data), but the force of infection due to vivax malaria is substantially

higher. This is because each inoculation with *P. vivax* has the potential to cause multiple future relapses. The greater comparative parasite exposure from these relapses contributes substantially to the more rapid acquisition of immunity and therefore a much younger age distribution of vivax malaria in Timika. Beyond early childhood, robust immunity is likely to have developed in a high proportion of individuals, even in regions with relatively low-level endemicity. This will not be the case in areas where there is substantial immigration of working age people from non-endemic areas [504], such as in Papua.

In non-immune individuals, *P. vivax* causes a similar degree of red blood cell removal as *P. falciparum* although some of the mechanisms differ. In particular, there is a much greater proportionate removal of uninfected red blood cells in vivax malaria [154], presumably, at least partially, due to the greater non-specific immune response for a given parasitaemia with this species [170-172]. Early development of strain-specific immunity will drastically reduce this non-specific immune response and therefore reduce total red cell loss. A further factor that may accentuate the haematological impact of vivax malaria in young infants is that cells that are susceptible to infection by this species (namely those that have been released from the bone marrow within the last 14 days [147]) are likely to be proportionately more numerous, allowing development of higher parasitaemias.

Previously, authors have suggested that, due to the protective effect of concurrent *P. vivax* on *P. falciparum* infection, a reduction of vivax malaria may result in unpredictable and potentially adverse effects on falciparum malaria [239,505]. In Timika, mixed *P. vivax* / *P. falciparum* infections cause substantially greater haematological morbidity than infection with either species alone. This has also been shown to be the case across the border in Papua New Guinea [96]. Various explanations for these diametrically opposing results are presented in Chapter 5. Given that the greater severity of mixed infections is not apparent until after

about three years of age, the most promising of these relates to the effects of greater transmission intensity on the development of immunity. In regions with very low malaria endemicity, severe anaemia associated with *falciparum* malaria is likely to result from a single fulminant infection in a non-immune individual. In this circumstance, any immunomodulatory effect of concomitant *vivax* infection will be proportionately important. In regions such as Papua and Papua New Guinea where transmission is more intense, partial immunity to malaria will typically have developed by an early age and therefore severe anaemia is more likely to represent chronic or repetitive infection in which case the haematological effects of both species are likely to be additive and any immunomodulatory effect of *P. vivax* on *P. falciparum* will be proportionately negligible. Whatever the cause, the results of the analyses in this thesis suggest reassuringly that reducing the burden of *vivax* malaria in Papua will cause total malaria morbidity to fall rather than rise. This is also likely to be true in other areas with similar transmission dynamics such as Papua New Guinea.

This thesis emphasises that, at least in Southern Papua, *P. vivax* parasitaemia is associated with significant haematological morbidity and almost certainly both direct and indirect mortality. These findings alone provide a strong argument for a greater focus on *P. vivax* in malaria control and elimination efforts. Nevertheless, a substantial proportion of the adverse impact of this species is likely to have been missed by this, and most other studies, because of their reliance on current parasitaemia to define those expressing morbidity associated with this species. In fact, morbidity due to *P. vivax* infection persists for some time after eradication of parasites [2,3] and may well accumulate with repetitive infections [166]. Elucidating the total impact of exposure to *P. vivax* infection, in particular its developmental, social and economic effects (about which virtually nothing is known), is an important priority for future research. On an individual level, the best way of achieving this is to commit to a long-term and detailed cohort study in which factors such as overall mortality, school

attendance and performance are related to total exposure to *P. vivax* infection, as established by regular active parasitological surveillance from birth. Cohort studies such as this are underway in Papua New Guinea [120] and are planned in Timika. They should also be replicated in other vivax-endemic regions with different transmission intensity. Of course these studies will still miss the impact of vivax malaria on the prosperity of entire populations – an outcome that is virtually impossible to measure directly but may be intimated by ecological comparisons.

Given that the incidence and morbidity of *P. vivax* infection is heavily skewed to very young children, it is important that measures to reduce the burden of vivax malaria and transmissibility of this species are active right from birth. Several potential strategies are worthy of investigation in vivax-endemic regions. These include: provision of long-lasting insecticide-treated bed nets at the time of birth (though as mentioned in Chapter 3, there is some evidence that this may not be as effective at preventing *P. vivax* infection as *P. falciparum* infection [291]) and presumptive treatment with antimalarial medication during infancy and childhood (potentially incorporated into the package of care provided as part of the Expanded Programme on Immunization [506]). Since the symptoms and signs of malaria can be difficult to detect in infancy, a policy of routine parasitological testing of all infants presenting to health care institutions in vivax-endemic regions may also be advisable in certain circumstances. For the latter control mechanisms to be effective, safe and efficacious antimalarial medications must be available. Unfortunately, young infants are rarely included in trials of antimalarial medications and therefore lack of safety data precludes the use of important drugs such as primaquine and artemisinin combination therapies in this age group. The World Health Organization states that primaquine is contraindicated in “children less than 4 years of age” and that “the available evidence in young infants (<5kg) is insufficient for confident recommendations for any of the ACTs, to the extent that many of

the drugs carry label restrictions that they should not be used” [78]. Establishing the safety of these medications in early infancy is clearly critically important for future vivax malaria control efforts.

The analyses presented in Chapter 9 show that a high proportion of patients in co-endemic areas will have a recurrence of *P. vivax* malaria shortly after treatment of supposedly pure *P. falciparum* infection [229]. The two major potential explanations for this are that *P. falciparum* infection stimulates relapse of previously acquired hypnozoites and / or that concurrent subpatent blood stage infection with *P. vivax* is more frequent than previously thought [229,236,507]. Both may well be important. There is certainly strong evidence to suggest that concurrent subpatent infection with either *P. falciparum* or *P. vivax* is relatively common in the setting of patent *Plasmodium* mono-infection [237,483]. Irrespective of the cause for this phenomenon, it suggests strongly that individuals presenting with malaria in co-endemic regions should be assumed to have mixed infection (whether patent or not) and therefore that treatment should be unified, highly effective and radically curative against both species.

A recent World Health Organization assessment of chloroquine for the treatment of falciparum malaria showed that median failure rates were “high to extremely high (19.8%-100%)” in all 30 countries assessed other than Honduras, Malawi and Nicaragua (0-1.3%) [508]. Therefore, chloroquine cannot be regarded as a viable option for unified blood schizontocidal therapy. Furthermore, chloroquine is undoubtedly becoming less effective against *P. vivax* (as demonstrated in the review presented in Chapter 3 [108] and more recently published studies [451,509,510]); a trend that will continue wherever this drug is used.

Artemisinin combination therapies are highly potent against both *P. falciparum* and *P. vivax* and are therefore prime candidate blood schizontocidal regimens for unified treatment strategies in co-endemic regions. The greater potency and spectrum of gametocytocidal activity of the artemisinin derivatives substantially reduces *P. falciparum* gametocyte carriage and therefore transmissibility when compared with standard antimalarials [74,279,466]. Chapter 8 highlights that although ACTs reduce *P. vivax* gametocytaemia more rapidly than chloroquine, the transmission-blocking benefit of this is likely to be minimal since *P. vivax* gametocytes are short-lived and most individuals are gametocytaemic prior to receipt of treatment. The most important means of reducing transmission of *P. vivax* infection by chemotherapeutic means is to prevent recurrence, in particular that due to relapse. The prevailing view is that this can be achieved by prescription of a 14-day course of primaquine [78]. If malaria treatment is to be truly unified in co-endemic regions, then this also demands prescription of primaquine to patients with *P. falciparum* mono-infections as detected by microscopy. At the least this would sterilise circulating *P. falciparum* gametocytes which, although of no individual benefit, will reduce transmission of falciparum malaria [79,511]. In a situation where a drug is being provided for community rather than individual benefit, it is imperative that the safety of the drug is ensured. The current lack of a reliable, cheap and rapid test for glucose-6-phosphate dehydrogenase deficiency is a huge hindrance to safe prescription of primaquine. It is very important that current efforts to develop such a test are scaled up substantially [512-514].

Of course any recommendations on the use of hypnozoitocidal courses of primaquine are contingent upon the effectiveness of this drug. Chapter 10 highlights that in Southern Papua neither the low or high dose unsupervised primaquine regimens currently recommended by the WHO have any clinically important effect on reducing the risk of re-presentation to hospital with a *P. vivax* relapse. The most likely explanation for this is that patients are not

fully adhering to the two-week treatment course and are therefore receiving an insufficient total dose of the drug to eradicate all hypnozoites. This mandates two courses of action, first, the efficacy of the drug must be proved in Papua, and ideally elsewhere and secondly, measures to improve patient adherence must be explored. A good means of achieving both of these aims, and one for which plans are already afoot in Papua, is to conduct a large, factorial randomised controlled trial in which individuals are allocated to supervised or unsupervised primaquine and to a 14-day 0.5mg/kg/day or 7-day 1.0mg/kg/day course. A small number of trials of a 7-day, high-dose primaquine course have already been conducted and show promising results as a potential means of improving patient adherence without causing excessive additional safety concerns [249,393,494]. In order to establish the likely impact of poor adherence in such a trial, great care would need to be taken to avoid biases associated with study involvement. Despite these potential biases, a recent trial by Takeuchi et al demonstrated that a 14-day course of directly observed primaquine was significantly more effective than the equivalent unsupervised regimen [488]. Efforts to develop an effective and safe alternative to primaquine are urgently needed [95].

Until an effective, cheap and rapid diagnostic test for G6PD deficiency becomes available and questions regarding the effectiveness of primaquine are answered, many regions will opt, as is currently the case, to omit hypnozoitocidal primaquine therapy from malaria treatment protocols altogether. In these circumstances, optimising blood schizontocidal therapy becomes the primary objective. All currently available ACTs rapidly reduce asexual *P. falciparum* and *P. vivax* parasitaemia and, with the probable exception of artesunate+amodiaquine [3,79], are associated with very low risk of recrudescence. The elimination kinetics of the partner drug is therefore the main differentiating feature of ACTs relevant to the transmissibility of *P. vivax* infections. A major premise of this thesis, demonstrated in Chapters 8 and 9, is that slowly eliminated ACTs can suppress at least the

first *P. vivax* relapse in tropical regions and therefore may provide a transmission-blocking benefit over rapidly eliminated combinations. This is likely to depend on the transmission dynamics of the given locality and whether or not suppressing the first relapse reduces or delays subsequent relapse and associated gametocyte carriage following a primary infection. Given that prolonged drug elimination may enhance selective transmission of resistant parasites [486], it is important that this potential transmission-blocking benefit is either confirmed or refuted.

Two general methodological approaches are likely to be particularly useful in this endeavour. The first of these is to conduct large randomised trials with long-term follow-up in which participants are randomised to repeatedly receive either a slowly eliminated or rapidly eliminated ACT for all future episodes of vivax malaria. The primary outcome of interest would be total number of events over a given period of time as opposed to time to the first (and only) event; the latter being the norm for current therapeutic trials. This approach is currently being employed in trials of ACTs for falciparum malaria in Uganda [515-517]. Randomising villages or communities as opposed to individuals would allow assessment of the combined individual and herd benefits of using these regimens.

The second main methodological approach to determining the impact of different treatments on *P. vivax* transmission is to monitor and assess opportunistically the effects of policy changes in endemic regions (as has been done for falciparum malaria [89,276,277,518,519]). A planned continuation of the work in this thesis is to assess the effect of introducing dihydroartemisinin+piperazine for treating all uncomplicated malaria in Timika. This will involve use of survival techniques to determine and compare the risk of re-presentation to hospital with malaria of the same parasitological cause following treatment with oral quinine or dihydroartemisinin+piperazine and the use of aggregated surveillance data to construct

time-series of the relative incidence of malaria and malaria-associated morbidity and mortality before and after policy change. These analyses will be subject to many forms of bias, particularly related to changes in treatment seeking behaviour and immunity. Some of these will be addressed by the repeat house-to-house survey that is planned for the second half of 2011.

Given the emphasis that this discussion has placed on the potential benefits of a unified treatment strategy for malaria in co-endemic regions, a final important question is how infrequent one *Plasmodium* species would have to be in relation to the other before a separate treatment strategy for *P. vivax* and *P. falciparum* would be acceptable, or even advisable? Further analyses of the risk of “heterologous” *Plasmodium* recurrence such as that presented in Chapter 9 may inform decisions regarding this issue but ultimately this is likely to come down to a judgement based on local parasite susceptibility profiles, cost and relative safety of the two strategies. Assuming that ACTs retain efficacy against *P. falciparum* in most parts of the world, the spread of chloroquine-resistant *P. vivax* suggests that this judgement is increasingly likely to fall in favour of unified ACT-based therapy.

12. Conclusions

Plasmodium vivax should not be neglected. In Papua, it is an important cause of haematological morbidity at all ages but in particular during early life. *Plasmodium vivax* rarely causes death directly but rather indirectly contributes to mortality through exacerbation of comorbid conditions. This process is likely to be substantially more common than previously appreciated. At least in Papua, concomitant *P. vivax* infection does not attenuate the haematological effects of *P. falciparum* and therefore reducing transmission of this species should not have adverse effects on haematological morbidity associated with *falciparum* malaria.

In regions where *P. falciparum* and *P. vivax* cohabit, a substantial proportion of patients treated for *falciparum* malaria will have a recurrence of *vivax* malaria within two months. This, in combination with inaccuracies in current diagnostic modalities, argues for a unified antimalarial treatment strategy for malaria of any parasitological cause in co-endemic regions. *Plasmodium falciparum* is resistant to chloroquine in most endemic regions and although slower to appear, chloroquine-resistant *P. vivax* strains are now also spreading throughout the world. Artemisinin combination therapies remain highly efficacious against both species (except for parts of Cambodia) and are therefore prime candidates for unified treatment. All currently available ACTs rapidly reduce *P. vivax* gametocytaemia but the transmission-blocking benefit of their remarkable potency is likely to be minimal. Slowly eliminated ACTs can suppress the first relapse of tropical strains of *P. vivax* but whether this

reduces transmission in the long term remains to be proven. The most important potential chemotherapeutic means of interrupting transmission of vivax malaria will be radical cure and thus prevention of all future relapses by using hypnozoitocidal medication. Primaquine is recommended for this purpose but this thesis provides no evidence that standard regimens have any effect on re-presentation to hospital with vivax malaria in Papua. High priority must be given to new hypnozoitocidal drug discovery. In the interim, optimising the safety and effectiveness of primaquine through development of rapid diagnostic tests for G6PD deficiency and improving drug adherence will be crucial for reducing transmission of vivax malaria.

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