Clinical studies to determine the optimal treatment for drug resistant malaria in Timika, Indonesia

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Thesis is submitted for the degree of
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Menzies School of Health Research and
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DECLARATION

I hereby declare that the work herein, now submitted as a thesis for the degree of Master by research of the Charles Darwin University, is the result of my work and was implemented under the supervision of Ric Price and Nick Anstey. I wrote all chapters and analysed all data. I hereby certify that the work embodied in this thesis has not already been accepted in substance for any degree, and is not being currently submitted in candidature for any other degree.

Hadjar Siswantoro

6 July 2006

Dated

DEDICATION

I dedicated this for my people and my country, the Republic of Indonesia, in particular the Eastern Indonesian region where malaria remains a serious public health problem.

ABSTRACT

Multidrug resistant strains of *Plasmodium falciparum* and *P. vivax* pose a significant challenge to Indonesian communities. In southern Papua, Indonesia we conducted a series of studies to determine the efficacy of the existing antimalarial regimens and to compare the safety and efficacy of two fixed dosed artemisinin combination therapies (artemether-lumefantrine/AL and dihydroartemisinin-piperaquine/DP). In the first study, consecutive patients with malaria due to *P. falciparum, P. vivax, P. ovale* or *P. malariae* presenting to a rural clinic were enrolled and treated with supervised CQ+SP (*P. falciparum*) or CQ (non-*P. falciparum*) and followed for 28-42 days. In the second study, patients with symptomatic infections with *P. falciparum* and or *P. vivax* were randomized to receive either coartemether (AL) or artekin (DP). Patients with vivax recurrence within 42 days were retreated with amodiaquine monotherapy. The first study was completed in August 2005 and enrolled 207 patients (88 *P. falciparum*, 40 *P. vivax*, 15 mixed infections, 50 *P. malariae* and 14 *P. ovale*). Early treatment failures occurred in 4 of 86 (5%) patients with falciparum malaria, 6 of 37 (16%) patients with vivax malaria and none of those with *P. ovale* or *P. malariae* infections. The failure rate by day 28 for *P. vivax* was 73% (22/30). After correcting for reinfections the day 42 recrudescence rate for falciparum malaria was 52% [95%CI: 39-64] and in 29% (63/103) of cases this was in the presence of chloroquine levels above 30ng/ml. Retreatment with unsupervised quinine ± doxycycline resulted in further recurrence of malaria, however by day 28 57% [95%CI: 33-79] had had a further recurrence of *P. falciparum* infections. None of the patients with *P. ovale* or *P. malariae* had treatment failures within 28 days.

In the second study, a total of 774 patients (474 *P. falciparum*, 176 *P. vivax*, 112 mixed infections, 12 *P. malariae* and *P. ovale*) were enrolled. The overall day 42 failure rates were 41.6% (122/293) for AL and 16.1% (44/273) for DP (RR=2.58 [95%CI 1.91-3.50]) p<0.001. After correcting for reinfections, the day 42 recrudescence rate for *P. falciparum* was 1.4% [95%CI 0-5] after AL and 1.1% [95%CI 0-4] after DP with no difference between regimens. However recurrence of vivax occurred in 57% [95%CI 47-65] of patients treated with AL compared to 10% [95%CI 6-17] treated with DP, p<0.001. Patients receiving DP were 2.1 fold [95%CI 1.2-3.8] less likely to be anaemic at the end of the study and 6.6 fold [95%CI 2.8-16] less likely to carry vivax gametocytes.

We conclude that there is a high prevalence of antimalarial drug resistance of *P. falciparum* and *P. vivax* to the existing antimalarial drugs in this region and that chloroquine and SP therapy can no longer be advocated. DP and AL are safe and highly effective for the treatment of multidrug resistant uncomplicated malaria. However DP's prolonged therapeutic activity reduces significantly the rates of falciparum reinfection and vivax relapse, and decreases the risk of anaemia and *P. vivax* gametocyte carriage. In this area DP has become the preferred treatment option for uncomplicated malaria.

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I acknowledge the contributions to this work made by others:

- The PCR genotyping was performed by Alan Brockman at Menzies School of Health Research. Alan Brockman provided the results of the genotyping. I analysed the data.
- The levels of chloroquine and its metabolite, desethyl-chloroquine were measured by high performance liquid chromatography by Mike Edstein at QMIR, Brisbane. I analysed the data.
- The protocol to be submitted to the ethics panels was written by Dr Ric Price, and Professor Nick Anstey. Dr Emiliana Tjitra attended meetings of the Indonesian ethics committee. Dr Ric Price also performed power calculations for the proposed sample size. Dr Julie Simpson provided the randomization sequence for the comparative trial.
- The data and safety monitoring committee consisted of Dr Liliana Kurniawan (NIHRD), Dr Julie Simpson, Dr Allen Cheng (MSHR), Dr Bob Taylor (WHO, Geneva). They provided a report both on the pilot study and the comparative study.
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- The malaria slides were read in the clinic by Rosmini and Buhari. The slides were reread by Ferryanto Chalfein. For quality assurance 10% slides were reread by experienced microscopists in Jakarta.
- Data was entered, using Epidata software vs 3.0 (Epidata Association,

Odense Denmark), by Natalia Dwi Haryanti and Sri Hasmunik.

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- Dr Ric Price helped with the analysis and provided editorial comment on the draft manuscript.

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LIST OF ABBREVIATIONS

ACR	Adequate Clinical Response
ACT	Artemisinin Combination Therapy
AFRO	African Regional Office
AL	Artesunate-Lumefantrine
API	Annual Parasite Incidence
AMI	Annual Malaria Incidence
ART	Artesunate
BW	Body weight
CQ	Chloroquine
DCQ	Desethylchloroquine
DHFR	Dihydrofolate reductase
DP	Dihydroartemisinin-Piperaquine
DHPS	Dihydropteroate synthase
Ditjen	Direktorat Jenderal (Directorate General)
ETF	Early Treatment Failure
FCT	Fever Clearance Time
Hb	Hemoglobin
KLB	Kejadian Luar Biasa (an outbreak)
LCF	Late Clinical Failures
LPF	Late Parasitological Failure
LTF	Late Treatment Failure
MALCON	Malaria Control
MDR	Multi-Drug Resistance
SEARO	South Office East Asia Regional Office
SP	Sulfadoxine-Pyrimethamine
PCR	Polymerase Chain Reaction
PH	Public Health
P. falciparum	Plasmodium falciparum
P. vivax	Plasmodium vivax
P. ovale	Plasmodium ovale
P. malariae	Plasmodium malarie

QHS	Qinghaosu
Surkesnas	Survey Kesehatan Nasional (National Health Survey)
WHO	World Health Organisation

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1 Introduction

1.1 Indonesia

Indonesia stretches along the equatorial line between two continents, Asia and Australia. It is an archipelago with five main islands (Java, Sumatra, Kalimantan, Sulawesi and Papua) and 13,767 smaller islands. The country consists of 33 provinces, 349 regencies and 91 municipalities, 5,277 sub-districts and 69,858 villages with a total population of over 218 million people. The total area is about 1.9 million square kilometres. Java is the most densely populated island with around 59 percent of the population. Meanwhile, Maluku and Papua which have area of 24 percent of the total area of Indonesia are inhabited by only 2 percent of total population. These figures indicate the uneven population distribution and natural resources among provinces in Indonesia [1].

Indonesia has two seasons, dry season (June to September) and rainy season (December to March). The relative humidity is usually high. In 2003, it ranged from 62% to 81%. The temperature varies, with the monthly average temperature ranging from 24^0C to 31°C.

The national health development strategy is aimed to increase the awareness, intention, and ability of the people to live healthily so that an optimum public health status can be achieved. Since 1999 reform in the health sector has been carried out under the health development vision **"Healthy Indonesia by 2010"**. The objective of this vision is that people will be proactive in maintaining and increasing health, prevent the risk, protect them selves from contracting disease, and actively take part in a healthy existence. To assist these aims it is expected that

people are able to access a qualified health service without barriers, either economic or non-economic [2].

The most common diseases in Indonesia are still communicable diseases. The common infectious diseases are acute respiratory infection, gastroenteritis, tuberculosis, and malaria particularly in Eastern Indonesia. Overall the mortality and morbidity due to malaria has decreased in the last five years. However since the burden of malaria in the Eastern Provinces remains high and can still cause outbreaks of disease elsewhere, the government still regards malaria as a threat to health status especially of the rural population[3].

1.2 Malaria situation

Malaria is caused by the development of *Plasmodium* spp. parasites within host red blood cells. Patients with malaria commonly present with non-specific symptoms such as periodic fever, chills, headache, pains in the neck, back limbs or joints, malaise, anorexia, nausea, vague abdominal pain, vomiting or mild diarrhoea. The physical findings include prostration, postural hypotension, jaundice and hepato-spleenomegaly. Although infections with *P. vivax, P. ovale, or P. malariae* are rarely fatal, in non-immune individuals infections with *P. falciparum,* can progress very rapidly to severe life-threatening malaria unless appropriate treatment is started [4, 5].

Worldwide malaria remains one of the most important diseases infecting man. The estimated annual, global malaria burden is 300 – 500 million cases [6, 7]. *Plasmodium falciparum* is predominant species and, together with *P. vivax,* accounts for most of the world's malaria. Approximately 1.7 – 3 million deaths occur annually, mostly in African children with *P. falciparum*, the form of malaria that

causes severe and complicated disease [7]. Recent studies have shown that the burden of malaria in Asia is greatly under appreciated, with recent estimates suggesting that 45% of the global burden is in the South East Asia Regional Office (SEARO) region [8].

In Indonesia, malaria is a major health problem, particularly in high risk groups such as babies, children and pregnant women. In 1998, it was reported that 46% of the total 211 million Indonesian population lived in malaria endemic areas and among these, 56 million living in moderate to high risk areas. The highest rates of malaria are reported from Eastern Indonesia, such as Papua, East Nusa Tenggara, North Maluku and South-East Sulawesi. In other provinces such as West Kalimantan, Bangka - Belitung, South Sumatra, Bengkulu and Riau, the number of malaria cases is also high ranging from 53% to 71% during 1986-1990 [9]. Over the last five years the number of malaria cases has declined according to national statistics. In Java-Bali the Annual Parasite Incidence (API) fell from 0,81% in 2000 to 0,15% in 2004. In regions outside Java-Bali, Annual Malaria Incidence (AMI) was generally higher but also decreased from 31% to 20% in the year 2004[3]. According to the National Health Household Survey in 2001[10], there were around 15 million malaria cases every year and approximately 1,2% (23,483 deaths) of all deaths were caused by malaria. Between 2004 and 2005 several outbreaks malaria (KLB Malaria) occurred, causing up to 2,986 cases and 81 deaths in 15 villages [11].

The national malaria control program aims to decrease the mortality and morbidity of malaria by focusing on early diagnosis, optimizing treatment protocols, surveillance and vector control. Up until 2004, chloroquine remained the first line therapy but more recently Indonesia has adopted the combination of artesunate with amodiaquine as the first line therapy for uncomplicated malaria, however the

possibility of cross resistance of amodiaquine with highly resistance of chloroquine particularly in Papua Province and the emergence of multi-drug resistance has lead to an urgent need to test and determine the safety and efficacy of various artemisinin derivative combination therapies (ACT).

1.3 Available antimalarial drugs

Despite the huge need for antimalarial compounds the number of antimalarial drugs in use is relatively low, a reflection that this is a disease of tropical countries and especially developing nations with low health expenditure. Nearly all currently available antimalarial drugs fall into four broad groups: the quinolines, the aryl-amino alcohol drugs, the antifolates, and other novel agents.

1.3.1 The quinolines

1.3.1.1 The 4-aminoquinolines: Chloroquine and Amodiaquine

Chloroquine (4-aminoquinoline) was first synthesized in 1934 [12] and acts only against those stages of the malaria life cycle which actively digest haemoglobin within the erythrocyte [13]. It is administered once daily for the treatment of all species of malaria (10mg/kg on days one and two, 5mg/kg on day three) [14]. The drug is rapidly absorbed with peak plasma concentrations reached within 2 hours [15, 16] following oral administration. The drug is then metabolized in the liver to desethyl-chloroquine and bisdesethyl-chloroquine and excreted in part through the kidneys and bile, for this reason it should not be used in individuals with serious liver and kidney diseases. Therapeutic blood concentration persists for 6-10 days after a single dose, but the terminal elimination half-life is 1-2 months [17]. Serious side effect are uncommon, but when plasma concentrations exceed 250µg/ml,

unpleasant symptoms such as dizziness, headache, diplopia, disturbed visual accommodation, dysphagia, nausea and malaise may develop[16, 17]. In Africans, Haitians and dark-skinned Asian, pruritus of the palms, soles and scalp is a frequent problem. Rare toxic effects include photoallergic dermatitis, aggravation of psoriasis, skin pigmentation, leucopenia, bleaching of the hair and aplastic anaemia. Chloroquine can also exacerbate epilepsy [17]. **Amodiaquine** (4-aminoquinoline) is thought to have a similar mode of action to that of chloroquine. It has been used since the 1940s with a recommended dosage of 10mg/kg base once daily for three days. Amodiaquine is rapidly and extensively converted to the biologically active metabolite desethylamodiaquine [18]. The parent compound has an elimination half-life of approximately 10 hours [19], but desethylamodiaquine, like chloroquine, is extensively distributed and eliminated slowly. Another metabolite, a quinoneimine, is probably responsible for toxic hepatitis and potentially lethal agranulocytosis, which occurred in 1 in 2000 of taking amodiaquine prophylactically [17]. Therefore, amodiaquine must not be used as prophylaxis as it can cause fatal liver and bone marrow toxicity. However when used to treat *P. falciparum* it has been shown to be safe and well tolerated [20].

1.3.1.2 The aryl-amino alcohols: Quinine, mefloquine, halofantrine, lumefantrine

Quinine is used for the treatment of uncomplicated, drug-resistant *P. falciparum* and severe falciparum malaria. It is a bitter powder obtained from the bark of cinchona tree. Like chloroquine, quinine interferes with parasite metabolism of haemin, a toxic product of haemoglobin digestion. It is possible that quinine opposes the polymerization of haemin into inert crystals of malarial pigment (haemozoin) [17]. As monotherapy, quinine has to be given 10 mg/kg/dose, 3 times

a day, for 7 days to achieve cure. A consequence of these complicated dosing regimens is generally poor compliance which limits its usefulness unless fully supervised [14, 21]. It is generally well absorbed after oral or intramuscular administration for malaria treatment [22-25]. Peak levels are usually reached within 4 hours (more rapidly if the intramuscular injections are diluted) [21] and then metabolized and eliminated with an elimination half-life of approximately 18-20 hours in cerebral malaria, 16 hours in uncomplicated malaria and 11 hours in health [24, 26, 27]. The therapeutic range has not been well defined but total plasma concentrations of between 8 and 15 mg/l appear safe and effective [28]. Approximately 80% of the administered drug is eliminated by hepatic biotransformation, and the remaining 20% is excreted unchanged by the kidney [24]. Quinine usually produces unpleasant adverse effects ('cinchonism'), such as; tinnitus, high-tone hearing impairment, nausea, dysphoria and vomiting.

Mefloquine, a fluorinated 4-quinoline methanol is effective against the asexual stages of malaria including chloroquine resistant strains [29]. The recommended treatment dose of mefloquine is either 15 mg/kg as one dose or preferably a split dose of 25 mg/kg; the 10 mg/kg dose is given after 6-24 hours [30].

Mefloquine is moderately well absorbed, extensively distributed, and slowly eliminated [23, 31-37]. The long half-life (approximately 180 hours) allows a single dose to be sufficient for the treatment and weekly administration for prophylaxis. Mefloquine is cleared principally by hepatic biotransformation to inactive metabolites [21]. Toxicity is relatively common and includes nausea, vomiting, dizziness, weakness and sleep disturbances [38-40]. Rates of vomiting of mefloquine within one hour of administration are about 8%, but can reach as high as 30% in children under 2 years of age [40].

Halofantrine is a 9-phenanthrene methanol**.** The mechanism of action of halofantrine is similar to that quinine and mefloquine, but it is intrinsically more potent than quinine or mefloquine [40]. It is thought to act by binding to haemin and prevention of haemin polymerization to relatively inert malaria pigment [17]. It is poorly and erratically absorbed with levels significantly elevated after a fatty meal [21, 41]. Peak plasma concentrations occur after about 6 hours, and the apparent terminal half-life is 1-2 days. Peak concentrations of the major metabolite, N-desbutylhalofantrine, occur at about 12 hours after ingestion, and the metabolite has an apparent terminal elimination half-life of 3-5 days [42]. The dosage in adults is 500mg at 6-hourly intervals for three doses (total first course dose 1500mg), and in children weighing >10kg is 8mg base/kg at 6-hourly intervals for three doses. Its use is restricted to patients with no pre-existing heart disease because it carries a significant risk of sudden death, which has been attributed to ventricular tachyarrhytmias [14, 43]. Mefloquine and halofantrine, both aryl amino alcohol derivatives of quinine were introduced into areas where quinine resistance already existed, such as Southeast Asia, and cross-resistance between quinine and the aryl amino alcohols may exist [44], and this may account for why resistance to these compounds developed quickly [38, 45-47].

Lumefantrine, formerly called benflumetol, has structural similarities to halofantrine. It was developed by Chinese scientist. The mode of action of lumefantrine is unknown, but it is thought to accumulate in the food vacuoles of parasites and bind to haemin, forming toxic complexes [17]. Lumefantrine is lipophilic and hydrophobic. Its absorption is considerably increased by taking the drug together with fatty food (a 16-fold increase with a fatty meal) [21, 48]. Absorption is reduced in the acute phase of malaria, but then increases considerably

as symptoms resolve and the patient starts to eat [48]. Therefore, the patient should be encouraged to take the drug with food. The elimination half-life is 3-4 days [49]. It is now available only in a fixed tablet combination with artemether known as Coartemether™. The combination is very effective against multi-drug-resistant falciparum malaria, free of adverse effects and appears to be well tolerated [50]. Although sharing structural similarities with halofantrine there has been no evidence of cardiotoxicity following administration of coartemether-lumefantrine [51, 52]. There is no experience in pregnancy and therefore the drug can not yet be advocated in pregnant women. There is no paediatric formulation.

1.3.1.3 The 8-aminoquinoline: Primaquine

Primaquine was synthesized in the 1940s. Since 1950, it has been the drug of choice for the radical cure of *P. vivax* and *P. ovale* infections. The site of action is thought to be in the mitochondria, possibly by competitive inhibition of dihydro-orotate dehydrogenase involved in pyrimidine synthesis [17]. Primaquine is active against exo-erythrocytic schizonts (causal prophylaxis) and is gametocytocidal (to prevent transmission) for all species of human malaria parasite [14] and hypnozoitocidal (to prevent relapse) for *P. vivax* and *P. ovale.* Primaquine is either prescribed for terminal eradication of *P. ovale* or *P. vivax* (0,5mg/kg bw/day for 14 days) or a single dose of 45 mg to decrease gametocyte carriage [53]. The drug is readily absorbed from the gastrointestinal tract with a peak concentration approximately 3 hours after ingestion and half-life of approximately 6 hours [16, 17]. It is then rapidly metabolized in the liver with the main metabolite being carboxyprimaquine. Although it is well absorbed, gastrointestinal effects are common and include nausea, headache, vomiting and abdominal discomfort particularly if higher doses (>30mg) are taken on an empty stomach [21]. A more serious complication involves the ability

of primaquine to induce haemolysis in patients with G6PD deficiency as well as met-haemaglobinopathy [53]. Primaquine is contraindicated in pregnant women and in children bellow 4 years of age due to fear of hemolysis [16].

1.3.2 The antifolates drugs

The antifolate drugs used for malaria chemoprophylaxis can be used either as single drug formulation or as fixed combination

1.3.2.1 Single drugs

Sulphonamides (e.g. sulfadoxine) and sulphones (e.g. dapsone) are often referred to, collectively, as a '**sulpha drugs**', first developed as antibacterial agents in the 1390s and also effective against *P. falciparum* [17]. They act by inhibiting plasmodial folate synthesis by competing for the enzyme dihydropteroate synthase (DHPS) [17]. **Sulfadoxine** is long acting drug which is rapidly and completely absorbed. The elimination half-life is about 5 days following oral or parenteral dosage in African children malaria [21]. **Dapsone** is rapidly absorbed and reaching peak plasma concentration in 3-6 hours. Dapsone has half-life time of 21 to 30 hours and eliminated quickly in comparison with sulfadoxine, with a mean half-life of about 26 hours [54]. Severe allergic reactions to sulpha drugs are uncommon, but severe and life-threatening (Steven-Johnson's Syndrome and granulocytosis) [16]. The sulpha drugs have generally been used in combination with pyrimethamine (sulfadoxine-pyrimethamine as Fansidar™) or dapsone with pyrimethamine (Maloprim™). More recently a novel combination has been under development: dapsone with chlorproguanil (LapDap™) [21].

Pyrimethamine was developed shortly after the Second World War as an antimalarial drug. Pyrimethamine is a dyhidrofolate reductase (DHPR) enzyme

inhibitor of the parasite. Pyrimethamine has a synergistic effect with sulfonamides because the two drugs interfere with different steps in the purine synthesis pathway [16]. Pyrimethamine is well absorbed after oral or parenteral administration, and is eliminated over several days (half-life/$T_{1/2}$ is 3 days) [55]. Temporary pancytopenia or granulocytosis is the adverse effects of pyrimethamine.

Proguanil is metabolized in vivo to cycloguanil (structurally similar to pyrimethamine), this active metabolite inhibits the parasite DHFR enzyme. Proguanil is contraindicated in individuals with liver disease and the dose should be reduced in people with kidney diseases [16]. Following ingestion, peak plasma concentrations of proguanil are reached in 2-4 hours. The elimination half-life is between 12 and 23 hours. Proguanil causes mild to moderate gastric intolerance and nausea. Mouth ulceration is common and may be severe [56].

1.3.2.2 Drug-combinations

Sulfadoxine-Pyrimethamine (e.g. Fansidar™) is a fixed-dose synergic combination. Pyrimethamine as competitive inhibitor of DHFR enzyme synergize with sulfadoxine (antifolate drug) as competitive inhibitor of DHFS enzyme in the purine synthesis pathway of all growing stages of the malaria parasite[16, 17]. SP is not a combination, in the 'resistance prevention' sense. They do not protect each other from resistance because the mechanism of action of the two drugs is linked [21]. Furthermore there different elimination half lifes (7 days for sulfadoxine and 3 days for pyrimithamine) result in longer exposure of parasites to the sulfa component, and this provides an opportunity for the parasite to emerge resistance. The toxicity of SP is almost entirely related to the sulfonamide-related [57, 58]. The sulfonamides may cause severe skin reactions (Stevens-Johnson syndrome), hepatitis, blood dyscrasias and other allergic reaction [21]. The recommended

treatment of SP is a single-dose treatment (25 mg/kg based on sulfadoxine and 1,25 mg/kg on pyrimethamine) for uncomplicated chloroquine-resistant falciparum malaria.

Proguanil -Atovaquone (e.g. Malarone™) is a new antimalarial marketed by The Glaxo Smith Kline (GSK). It has been marketed as both a prophylactic agent as well as the treatment of multidrug resistant strains of *P. falciparum*. Atovaquone, causing inhibition of nucleic acid and adenosine triphospate synthesis[59]. *In-vitro*, atovaquone affects the maturation of trophozoites and gametocytes of *P. falciparum.* Synergistic combination with proguanil is effective for infections with multidrug-resistant *P. falciparum* [21]. It is similar to halofantrine and lumefantrine in that oral absorption is augmented considerably by fats [60]. The elimination half-life is long (50-70 hours). Steady-state plasma concentrations are achieved in about 7 days [17, 60]. The combination is very well tolerated. The adverse effects are occasionally cause mouth ulcers, and at high doses abdominal discomfort [21]. The dosage of atovaquone-proguanil treatment in adults is four tablets (each containing 250mg atovaquone and l00mg proguanil) daily for 3 days, and one tablet daily as prophylaxis [14]. More over, for poor tropical countries the drug is far too expensive (US $42 for 12 tablets) [61].

1.3.3 Other antimalarial drugs

Piperaquine is a bisquinoline compound active against multi-drug resistant *P. falciparum* [62]. Piperaquine contains the 7-chloro-4-aminoquinoline structure found in all 4-aminoquinoline drugs, and for this reason some have suggested that piperaquine and aminoquinolines (e.g. chloroquine) are likely to share similar targets. Like chloroquine it probably acts by inhibition of the heme-digestion pathway in the parasite food vacuole is most convincing [63]. The absorption of piperaquine is slow,

with mean absorption halftimes of 9 hours. The mean terminal elimination half-life was long in both adults (23 days) and children (14 days), while the mean volume of distribution at steady state/bioavailability was very large in adults (574 L/kg) and children (614 L/kg) [64]. Common minor complaints have included mild headache, dizziness, nausea, abdominal pain and vomiting, although these symptoms are often difficult to distinguish from symptoms resulting from malaria itself [65-68].

Tetracyclines (e.g. doxycycline), the antimalarial activity of tetracyclines was first recognized in 1949. They are thought to affect protein synthesis in the plastid organelle (apicoplast) [17]. The absorption of ingested tetracyclines ranges from 30 to 80 percent and reduce by food, milk and antacids [17]. The half-life of doxycycline is 16 hours. Elimination is in the faeces and urine. Tetracyclines are contraindicated in individuals with hepatic dysfunction, in pregnant women, in children less than 8 years old because the risk of interference with the development of bone and teeth and discoloration of teeth. Common side-effects of tetracyclines are gastrointestinal symptoms, notably diarrhoea, *candida* vaginitis or stomatitis, light-sensitive rashes and itching of the skin [16].

1.3.4 Artemisinin derivatives

In 1971, Chinese scientists isolated an entirely novel compound from the leafy portions of the sweet wormwood plant *(Artemisia annua L).* In 1972, the active ingredient was purified and first named qinghaosu, and then later named artemisinin [69]. Qinghaosu (QHS) otherwise known as artemisinin is a sesquiterpene lactone which has a peroxide grouping and, unlike most other antimalarials, lacks a nitrogen-containing heterocyclic ring system [70]. Derivatives of artemisinin, such as dihydroartemisinin (DHA), artemether, and the water-soluble sodium artesunate, appear to be more potent than QHS itself [71]. They act rapidly, but are also quickly

eliminated. Their rapid disappearance may be a key reason why artemisinin resistance has been so slow to develop [69]. Many of the studies showed that they are the most rapidly acting of all antimalarial drugs, have broad stage specificity of antimalarial action and produce a faster clinical and parasitological response than other antimalarial drugs [71]. The rapid action of the artemisinin derivatives, even against multi-drug resistant strains of *P. falciparum* and their lack of toxicity have led to a considerable increase in their use, particularly in South East Asia, where multi-drug resistance is a major problem[52].

1.3.4.1 Structure and Activity

Artemisinin consists of a sesquiterpene lactone ring with a unique endoperoxide bridge [72]. The endoperoxide bridge is necessary for antimalarial activity, which was activated to hydroperoxide in the presence of intra-parasitic iron [70] into toxic free radicals[73], which then bind covalently to parasite proteins [74] which are particularly sensitive to this oxidative damage [17, 75, 76]. More recently a central role for SERCA (sarco- and endoplasmic reticulum calcium-ATPase) of *P. falciparum* (PfATP6) has been postulated to be central to the site of action of the artemisinins by inhibiting PfATP6 outside the food vacuole after activation by iron [77].

1.3.4.2 Formulation

Artesunate, the most widely used derivative is available in oral, parenteral and suppository. Artemether is administered as ampoules for intra-muscular injection or as capsules for oral giving medication[78]. Arteether, has recently been licensed and is available in an intra-muscular injection formulation. The active metabolite from

artemisinin derivatives is dihydroartemisinin (DHA), which is also available in oral preparation [52].

1.3.4.3 Pharmacokinetics

After oral medication, artesunate and artemether are rapidly, but incompletely absorbed [79], with considerable inter-individual variability in plasma concentration profiles [80]. There is extensive first pass metabolism and rapid biotransformation (demethylation of artemether and hydrolysis of artesunate) to DHA, the major active metabolite [81]. Artesunate is faster than artemether in the rate of biotransformation to DHA and occurs immediately, such that artesunate can be regarded as a prodrug [82, 83]. Following oral medication, peak of concentration are achieved rapidly: less than an hour for artesunate, 1 – 3 hours for artemisinin [84] and 3 hours for artemeter [85]. The drug is rapidly cleared from the body, elimination half-lives being 1.4 hours[79] for artesunate, 1.9 hours for DHA[85], 2-3 hours for artemisinin[86] and 4-5 hours for artemether[85]. The active metabolite, DHA seems to be eliminated more slowly than artemisinin derivatives and gives rise to nearly all antimalarial activity [85]. The peak bioactivity, that is the time when most of DHA or parent drug are present, appear almost similar in most study (1-2.6 hours) [80, 85].

1.3.3.2 Side effects

The artemisinin derivatives have been remarkably well tolerated in clinical studies. Although there are neurotoxic (spasticity and ataxia) effects of artemisinin derivatives at high doses in experimental animals [87], there have been no reliable neurological adverse effects in humans. In prospective study of over 3,500 patients in Thailand, the combined regimens of mefloquine plus an artemisinin derivative were associated with more side effects than those with an artemisinin derivative

alone. Moreover, there was no evidence for serious adverse effects [88]. The most common complaints were nausea, vomiting, anorexia, and dizziness, all of which are also characteristic of acute malaria. Artemisinin derivatives also appear to be safe for pregnant women [89], although animal studies have suggested some risk to the foetus in the first trimester.

1.4 Drug Resistance

The WHO defines resistance to antimalarials as the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a medicine given in doses usually recommended. Resistance to antimalarials arises because of the selection of parasites with genetic mutations or gene amplifications that confer reduced susceptibility [90].

In SEARO region, Thailand is the focus for the origin of multidrug-resistant malaria [8, 91, 92]. The spread of drug-resistant malaria parasites from SEARO to AFRO has provided an explanation for the rising mortality from malaria in this region since 1990 [93, 94]. As treatments lose their effectiveness, morbidity and mortality from malaria will inevitably rise and bringing the prospect of untreatable malaria ever closer [95].

1.4.1 Evolution of Drug Resistance

1.4.1.1 Globally

Chloroquine is widely available, cheap and effective, and for this reason has been the mainstay of antimalarial drug treatment for the past 40 years. However resistance is now widespread and only few countries are not affected [96]. Chloroquine resistance in high-level (so called R III) was first observed in Thailand in 1962 [97], then spread gradually throughout Southeast Asia; Malaysia [98],

Cambodia[99], Vietnam[100, 101], and Burma in 1969[102]. At about the same time, chloroquine resistance also appeared in South America [103]. Chloroquine resistance appeared later in Africa, being first observed in nonimmune travellers returning from East Africa [104] [105, 106]. More recently, *P. vivax* has also developed significant resistance to chloroquine in some parts of South East Asia and Oceania [107].

Despite the current high levels of chloroquine resistance in most malarious areas, the cost of less than $0.20 U.S. per treatment continues to apply sufficient financial incentive to maintain chloroquine as a first-line treatment in much of West and Central Africa [108].

Sulfadoxine-pyrimethamine (SP) was used in the early of the 1980s in areas with resistance to chloroquine, first in South East Asia and later Africa [109]. SP resistance was first noted on the Thai–Cambodian border in the 1960s, failures occurring in refugee camps in Thailand in the 1970s [110]. In sub-Saharan Africa resistance also rapidly emerged after SP was first deployed [111-114].

The decrease sensitivity to **quinine** has been noted for many years, although there is no well-documented high-grade resistance [107]. A recent efficacy study in Africa suggested that quinine resistance has emerged [115], and emphases the need to monitor the resistance

1.4.1.2 Indonesia

Since the first reports of chloroquine resistance in East Kalimantan and West Irian Jaya (Papua, Indonesia) in 1975 [116], chloroquine resistance has spread rapidly throughout the archipelago [117-119]. Many of studies for the emergence of antimalarial drug resistance are focused in Eastern Indonesia, particularly Papua. Previous studies have shown that Papua is the worst area affected by chloroquine

resistance *P. falciparum* in Indonesia [120-122]. The occurrence of chloroquine and other drug resistant strains of *P. falciparum* in Papua has been confirmed by several reports published since 1975[116, 123-125].

More recently, chloroquine resistance in *P. vivax* has also emerged, although this has been largely restricted to Papua (Indonesia)[126, 127] [9, 128-130]. With the coming of widespread resistance to chloroquine in this area, a combination of the antifolates sulfadoxine-pyrimethamine became more generally used as a second line therapy for uncomplicated malaria. However, since 1979 *P. falciparum* resistant to this drug was reported [119, 131]. Thereafter, several studies have been performed showing spread to at least 11 provinces where in vivo and in vitro resistance tests to this drug have been carried out [119, 121, 132-137]. Resistance of malaria parasites to the main antimalarial drugs; chloroquine and sulphadoxine-pyrimethamine, have also been confirmed by molecular analysis of *Plasmodium falciparum* isolates from different part of Indonesia [138]. Despite the rapid spread of resistance to these antimalarials in Indonesia, chloroquine and sulfadoxine-pyrimethamine are still being used as antimalarial drugs mainly for economic reasons.

In Indonesia, quinine resistance has been slow to emerge. This has also been the pattern elsewhere. Although not formally evaluated, quinine remains clinically effective [139]. Until recently, there has been no reported *in-vivo* quinine resistance by falciparum malaria. However, several isolates have shown *in-vitro* resistance [119, 140]. This makes malaria treatment in Indonesia less effective and underscores the need for new pharmacological initiatives to counter the increased incidence of malaria mortality and morbidity.

Amodiaquine is not available and, until recently, has never been used for malaria treatment in Indonesia. The *in-vitro* study for *P. falciparum* sensitivity has shown

resistance to amodiaquine [140]. Since 1985, several studies reported *P. falciparum* resistant to amodiaquine [121].

Mefloquine is also not available and has never been used for malaria treatment in Indonesia. *In-vitro* study of *P. falciparum* sensitivity to this drug found 4.8% isolates resistant to mefloquine [140]. *In-vivo* sensitivity testing of *P. falciparum* to mefloquine (15 mg/kgbw, single dose) was performed in 3 provinces and no resistant cases were found in these tests [141, 142]. In recent comparative study of mefloquine and chloroquine in Papua demonstrated the cumulative 28-day curative efficacies were 26% and 82% for chloroquine against *P. falciparum* malaria and *P. vivax* malaria, respectively. In contrast, mefloquine cure rates were far superior (96% against *P. falciparum* malaria and 99.6% against *P. vivax* malaria)[143].

1.4.2 Consequences of Drug Resistance

The emergence of drug resistant malaria is followed by a significant impact on health of a community. Initially, there is a delay in the therapeutic response [144] and the occurrence of recrudescent infections [145]. Treatment failure is also accompanied by a relative increase in the risk of carrying gametocytes[146] and thus an increase in the transmissibility of malaria. As resistance levels increase there is a greater incidence of anaemia [147]. In some areas of Africa an increasing prevalence of anaemia may be the first sign of worsening drug resistance [148] and results in a rise in the incidence of severe anaemia requiring hospitalization and the need for blood transfusions [149]. These factors combine to increase morbidity [150] and increase mortality (by 10-fold or more) of malaria [151], especially in the most vulnerable groups: children and pregnant women [145]. At the economic level, the cost to the patients and the society is increasing since patients need to be re-treated (some times with more expensive drugs) and also because of the cost

associated with the morbidity of recurrent episodes [152, 153], absenteeism from work or school, and mortality [145]. It is vital therefore to monitor the emergence of resistance and rationalize therapeutic approaches.

1.4.3 Methods of monitoring drug resistance

In general, three basic methods have been routinely used to study or measure antimalarial drug resistance: ***in vivo*** (clinical efficacy trials), ***in vitro***, and **molecular assays.**

1.4.3.1 Clinical efficacy trials

In 2003, WHO introduced the new guidelines for the assessment of therapeutic efficacy of antimalarial drugs for uncomplicated falciparum malaria in moderate and highly transmission areas. This standardized test is primarily designed to assess the *in-vivo* drug response in *Plasmodium falciparum.* Compared with the 1996 guidelines the most important change is the use of molecular techniques to distinguish recrudescent from reinfection [154]. Assessment of therapeutic efficacy includes assessments of both clinical and parasitological response. To assess the therapeutic efficacy of routine treatment regimens, the test should be carried out only in persons suffering with clinical manifestations and microscopically confirmed falciparum malaria, and ideally should include the most vulnerable population group, infants and young children. The therapeutic response is classified as Early Treatment Failures (ETF), Late Treatment Failures (LTF), and Adequate Clinical Response (ACR). **ETF** is defined as the development of one of the following considerations on the first three days of follow-up; i) there are danger signs or severe malaria on days 1, 2, 3 in the presence of parasitemia; ii) parasitemia on day 2 higher than on day 0; iii) parasitemia on day 3 (25% of the count on day 0, and iv) parasitemia on day 3 with

fever (axillary temperature ≥37.5°C). **LTF** is defined as the development of one of the following considerations from day 4 to day 28, without previously meeting any of the criteria of ETF, and divided in 2 sub-groups:

- Late Clinical (and Parasitological) Failures (LCF), (i) danger signs or severe malaria after day 3 in the presence of parasitaemia (same species/genotype as on day 0); and (ii) axillary temperature ≥37.5^{0}C in the presence of parasitaemia on any day between day 4 to day 28.
- Late Parasitological Failure (LPF): presence of parasitaemia on any of the schedule days of return (days 7, 14 or 28) with the same species/genotype as on day 0 and axillary temperature <37.5^{0}C without previously meeting any of the criteria of LCF.

ACR is defined as not acquiring any of the criteria of ETF or LTF, and parasite clearance confirmed through the 28-days follow-up [155]. Although this protocol is designed for uncomplicated *P. falciparum* infection, this test has been extended in this thesis to assess the therapeutic efficacy of antimalarial drugs for uncomplicated malaria infected by other human plasmodia. The results are aimed for providing information to policy makers for guiding antimalarial policy and for monitoring the efficacy of antimalarial drugs [156].

1.4.3.2 In vitro assessments

In vitro tests avoid many of the confounding factors which influence *in vivo* tests by removing parasites from the host response and placing them into a controlled experimental environment. In the most frequently used procedure, the micro-technique, parasites obtained from a venous blood sample are exposed in microtitre plates to precisely known quantities of drug and observed for inhibition of maturation into schizonts [157]. Antimalarial drug susceptibility can be tested by

measuring either the inhibition by different concentrations of drugs of parasite maturation to the schizont stage, or the degree of inhibition of radio labelled H-hypoxanthine uptake, or the synthesis of parasites specific enzymes (e.g. lactate dehydrogenase) [21].

A major problem with these assays is that although they maybe useful for monitoring temporal trends in parasite sensitivity their relation with the therapeutic response in an individual is often confounded by host factors such as the pharmacokinetics, immunity and stage of disease. Furthermore there are logistical difficulties of setting these assays up to process isolates fresh from the field.

1.4.3.3 Molecular assays

These tests are in the process of being developed and validated, but offer promising advantages to the methods described above. Molecular tests use polymerase chain reaction (PCR) to indicate the presence of mutations associated with biological resistance to antimalarial drugs [158].

1.4.4 Mechanisms of drug resistance

Malaria resistance appears through genetic mutations that confers reduced susceptibility to an antimalarial drug. When a parasite population containing a resistant mutant is exposed to a concentration of antimalarial drug sufficient to kill the susceptible but not the resistant parasites; in this case the mutants will be selected and preferentially transmitted [159]. The biochemical mechanism of resistance has been well described for chloroquine and the antifolates. Chloroquine resistance results from a reduced parasite accumulation of the drug, although the precise molecular mechanisms responsible have not been elucidated fully. The intra-erythrocytic malaria parasite consumes haemoglobin, detoxifying haem by

polymerization to haemozoin or malaria pigment. This process is inhibited by chloroquine and related arylamino alcohols [160]. Chloroquine resistance is associated with reduced concentrations of the drug in the parasites food vacuole. Both reduced entrance and increased efflux have been reported, although the current balance of evidence favours reduced accumulation [107]. Molecular analysis has linked chloroquine resistance with “point mutations” (also known as single nucleotide polymorphism - SNPs) in two major genes: *PfCRT* and *Pfmdr1* [161]. In contrast to the role of SNPs the drugs of the arylamino alcohol group (e.g. mefloquine, halofantrine and lumefantrine) lose their efficacy when the *Pfmdr1* gene (a gene encoding for a parasite homologue of the mammalian transporter protein) is amplified and expressed in larger copy numbers [162]. Amplification in *Pfmdr1* is the main cause of resistance to mefloquine in falciparum malaria [163].

No specific marker of artemisinin resistance has been identified, but isolates from South East Asia with higher copy number of *Pfmdrl* also demonstrate higher in vitro inhibitory concentrations [162]. Resistance to the antifolates is related to the point mutations in the genes encoding the inhibitors of dihydro-folate reductase (DHFR) and dihydro-pteroate synthetase (DHPS) enzymes. In DHFR, the target of action of pyrimethamine, resistance develops in an additive manner. A series of mutations is required to achieve complete resistance. Each mutation bringing the parasite a step closer to complete protection against this family of drugs[164].

Multidrug resistant *P falciparum* malaria is common in South East Asia, but difficult to identify and treat. Monitoring of *pfmdr1* copy number will be useful in epidemiological surveys of drug resistance in *P falciparum*, and potentially for predicting treatment failure in individual patients [163].

Widespread used of subtherapeutic doses antimalarial regimens are likely to play a major role in facilitating the emergence of drug resistance [159]. Sub therapeutic doses now result from under-dosing, fake or substandard antimalarials drug [165, 166] and patients failing to complete a full course of treatment (poor compliance). Poor compliance may arise because of the occurrence of adverse side effects, the cost of medication or because therapies are prolonged and complicated. Patients often stop therapy as soon as their acute symptoms have resolved; with drugs with short half lives this will lead inevitably to a failure of therapy since at least 7 days of treatment are needed to achieve a cure [167]. The use of presumptive treatment for clinical malaria has the potential for facilitating resistance by greatly increasing the number of people who are treated unnecessarily. This "selective pressure" can be particularly important in some areas where lack of proper diagnostic facilities in large numbers of patients being treated unnecessarily for malaria [168].

1.4.5 Strategies to prevent antimalarial resistance

Interventions aimed at preventing drug resistance, generally focus on reducing overall drug pressure through; more rational use of drugs by improving the way drugs are used with improving diagnosis, the use of drug with combinations, and develops of novel antimalarial compound.

In many parts of the world, malaria diagnosis without microscopy confirmation is common prior to treating malaria. This is relatively cheap but unreliable, resulting in treatments being wasted on suspected cases that do not in fact have malaria [169]. This emphasises the need for specific diagnostic techniques that is cheap and simple enough to be used by non-laboratory staff (e.g. rapid diagnostic test/RDT). Significant progress in RDT has been made with the development of dipstick

diagnostic kits. Hopefully this will achieve a major goal of improving access to good diagnosis.

Longer term strategies include development of new chemical agents to broaden therapeutic options. These programs need to be adequately funded and encouraged. Some progress has been achieved by initiatives such as Medicine for Malaria Venture (MMV). MMV is non profit foundation created to discover, develops and deliver affordable antimalarial drugs through effective public-private partnerships.

Compliance of patients to take adequate medication is very important. Therefore, drug development is needed to provide effective treatment for improving the way drugs are used (e.g. the short of course treatment). Artemisinin derivatives remain highly effective against most drug resistant isolates [159]. In combination with a slowly eliminated drug they ensure a rapid reduction in the biomass of parasites, thereby reducing the risk of recrudescence, and secondly the presence of the partner drug ensures that the parasites are never exposed to artemisinin derivative alone. Both of these factors may decrease the chances of drug resistance emerging [107]. There are other advantages of combinations containing an artemisinin derivative: firstly to improve the efficacy of a 'failing drug', with increasing cure rates, accelerating the early therapeutic response and prevent dangerous early high grade treatment failures. Secondly their activity against gametocyte carriage may slow the emergence of drug resistance and reduce the incidence of malaria in areas of low transmission [159]. Recently fixed dose artemisinin derivative combination therapies have been developed which will help increase the compliance of patients. The fixed dose combinations include artemether-lumefantrine (Coarthem®), amodiaquine-artesunate (Sanofi-Aventis®), dihydroartemisinin-piperaquine-trimetropim (Artecom®) and dihydroartemisinin-piperaquine (Artekin®).

1.5 Artemisinin Drugs

In uncomplicated malaria, the focus of therapy is on the relief of symptoms with rapid the eradication of parasites rapidly from the body, and the prevention of progression to severe disease and treatment failure. A secondary aim is to prevent transmission of resistance strain to other people. Pharmacodynamic measures, such as parasite and fever clearance and recrudescence rates are therefore useful parameters for estimating the efficacy of regimens in uncomplicated malaria [170]. Comparative studies have shown that artemisinin compounds act faster than any other antimalarial [171] with a mean time to fever clearance of 14.6 hours and a time to parasite clearance of 32 hours [52, 78].

1.5.1 Monotherapy

Despite the artemisinin derivatives producing faster clearance of parasites than other antimalarial drugs [71], failure rates in monotherapy are high and appear to be a function of the duration of therapy rather than size or frequency of the dose given. Although adequate cure rates have been reported following a five day course of an artemisinin derivative given alone [78, 172], failure rates with such a regimen rise to nearly 40% in patients presenting with high parasitaemias [173]. A higher initial dose of an artemisinin derivative (4 rather than 2 mg/kg) results in a faster early therapeutic response [52, 174], but subsequent doses should be kept to a minimum dose of 2 mg/kg for a further six days, to maintain adequate recrudescence rates [174]. Although higher efficacy can be obtained by 7 days regimens this is associated with reduced compliance in out-patients.

There are some theoretical advantages to using a liposoluble derivative (artemether and arteether); the biotransformation is slower than that for artesunate and hence the

effective antimalarial half-life in blood is longer. However, the lack of any demonstrable advantage for artemether over artesunate in large comparative studies [174-178] suggests that the pharmacokinetic and pharmacodynamic relationship for these antimalarial drugs may be complex. The choice of derivative should therefore be based upon availability, cost and quality of the preparation [52].

1.5.2 Combination therapy

1.5.2.1 The rationale

Although the artemisinin derivatives are extremely potent their short half life requires them to given for at least 7 days to ensure eradication of all parasites from the body. Such regimens are generally unpractical in an endemic setting. To shorten the duration of therapy artemisinin derivatives are recommended to be given in combination with a longer acting anti-malarial agent [179]. Combination therapy achieves its antimalarial effect through the rapid initial reduction in parasite biomass attributable to the short the acting but highly potent artemisinin, with the subsequent removal of the remaining parasites by the intrinsically less active but more slowly eliminated partner. For instance artesunate alone must be given daily for seven days, whereas in the presence of mefloquine, a 3-day regimen is sufficient [30, 180-182].

Other desirable properties of an antimalarial combination include the following: the two drugs would have unrelated modes of action on the parasite, at least one of the components must be fast acting, there must be no negative pharmacological interactions, the tolerability and toxicity profile must be good and the treatment must be active against all stages of the parasite (including gametocytes). Furthermore the drug therapy should be available in fixed combination so that neither drug component can be exposed to parasites alone. Finally cost is a major consideration.

Most patients with malaria come from poor societies and malaria endemic countries often do not have adequate resources to provide free therapy. However to ensure best access to good therapy the combination should be relatively inexpensive and affordable to all [183].

Antimalarial drug resistance involve mutations in the genes encoding target enzymes or transporters [184, 185]. Although these mutation arises spontaneously the probability that a mutant will arise depends on several external factors: i) the number of parasites exposed to the drug; ii) the concentrations of the drug to which these parasites are exposed; iii) the pharmacokinetic and pharmacodynamic properties of the antimalarial drug; iv) the degree of resistance that results from genetics changes; v) the level of host immunity; and vi) the simultaneous presence of other antimalarial drugs in the blood to which the parasite is not resistant[186]. The probability that a mutant will arise that is simultaneously resistant to two drugs, with different sites of action, is the product of the individual parasite mutation rates for each drug multiplied by the number of parasites in an infection that are exposed to the drugs. Example if one in 10^9 parasites are resistant to drug A and one in 10^{12} parasites are resistant to drug B, only one in 10^{21} will be simultaneously resistant to both A and B. Since patients present with a maximum of 10^{13} malaria parasites[186], one would predict a resistant parasite to be present approximately once every 10^8 infections treated with two drugs compared to a figure of 10% of infections treated with one drug[159, 186]. Therefore combinations of drug are less likely to incur resistance. The same rationale underlies antimicrobial policy in the treatment of tuberculosis and HIV.

Artemisinin drugs offer additional advantages over other combination regimens. Their potent action reduces parasites faster than the other antimalarials (by

approximately 10^4 per asexual cycle)[107] and therefore parasites are unlikely to be exposed to subtherapeutic concentrations for significant lengths of time[167] – this will also reduces the chances of a resistant mutant emerging during treatment[187]. The combinations will also serve to protect the artemisinin compounds since the latter will never be exposed to the parasites alone. Although this theory has not been proved formally in humans, experiments in animals support the hypothesis [188, 189]. Ideally such combinations should have well matched pharmacokinetic properties, be synergistic, manifest different modes of resistance and be amenable to good patient compliance [159].

The artemisinin combinations regimens reduce transmission potential in vivo through their gametocytocidal activity [190, 191]. This is most probably the result of the rapid reduction of the asexual parasite biomass and activity against the early stage sexual forms of the parasite [159]. Reduction of gametocyte carriage with ACT may therefore help prevent the spread of resistance. The inhibition of gametocytogenesis may therefore further help to slow the emergence of resistance [107]. In South East Asia, the most studied and used ACT is mefloquine plus artesunate. The combined actions of the two drugs translates into faster clinical and parasitological recovery [175] and higher cure rates. When this combination was deployed to a whole population, the higher cure rate and lower gametocyte carriage was associated with a 60% reduction in the incidence of malaria[150] and also halting of the progression of resistance to mefloquine [192].

1.5.2.2 Non-fix dosed combinations

1.5.2.2.1 Artesunate with mefloquine

Artesunate plus mefloquine has been extensively studied in areas of high multidrug resistant falciparum malaria in South East Asia [183] [52].

In Thailand, increasing resistance to chloroquine and SP led to the introduction of mefloquine in 1984[150]. Although a single dose of 15 mg of base/kg body weight initially gave cure rates in excess of 90%, the efficacy of this regimens declined rapidly after 1990, and the recommend dose was increase to 25mg/kg body weight [38]. By mid-1994, mefloquine monotherapy for uncomplicated falciparum malaria was discontinued, because the cure rates had fallen to nearly 50% and replaced by combination of 3 days of artesunate and mefloquine (25mg/kg) [178]. The regimen of mefloquine plus three days of artesunate is generally well tolerated and has a cure rates in excess of 95%[175, 178, 193, 194]. Although vomiting rates with mefloquine can be high particularly in children, the administration of artesunate before a split dose of mefloquine reduces this and heightens the absorption of mefloquine [36]. In Thailand, widespread deployment of combination artesunate with mefloquine has showed with a 60% reduction in the amount of malaria [150].

1.5.2.2.2 Artesunate with amodiaquine

Amodiaquine is generally effective against chloroquine resistant *P. falciparum* infections, but efficacy varies [195]. However, resistance to this drug follows the resistance to chloroquine and thus the useful therapeutic life of the combination is likely to be short [183]. WHO currently recommends the combination of artesunate-amodiaquine therapy in areas where the cure rate of amodiaquine monotherapy is greater than 80% [196]. A comparative study of artesunate-amodiaquine versus

amodiaquine alone, in uncomplicated falciparum malaria African children patients showed improved treatment efficacy with day 28 cure rates of 68% versus 41% in Kenya; 82% versus 79% in Senegal; and 85% versus71% in Gabon [197].

In a recent comparative study of artesunate-amodiaquine compared to monotherapy with chloroquine, amodiaquine or SP in Afghanistan, an area of high grade resistance to amodiaquine and CQ, showed improved treatment efficacy by the addition of artesunate to AQ or by use of SP (72% and 92% respectively). However, because SP remains effective it became the preferred option in combination with artesunate in this region [198]. In Sudan, a comparative study of artesunate-amodiaquine with artesunate-SP showed cure rates after 28 days of 91% for artesunate-SP and 92% for artesunate-amodiaquine [199].

In brief artesunate-amodiaquine is recommended artemisinin-based combinations by WHO, but in areas with high baseline amodiaquine resistance efficacy will be compromised [200].

1.5.2.2.3 Artesunate with Sulfadoxine-pyrimethamine

The combination of artesunate and SP has been shown to be effective in recent studies in Africa [201]. The partner drug (SP) is well tolerated and is given in a single dose. However, a major concern is the rapid emergence of parasites resistant to the DHFR and DHPS inhibitors and the continuous use of unprotected sulfadoxine-pyrimethamine that could compromise any attempt to use this combination [183]. There is also evidence that the use of sulfadoxine-pyrimethamine alone increases the production of gametocytes in the treated patients [202], a factor that could increase transmission and the spread of resistant mutants. The use of pyrimethamine-sulfadoxine combined with artesunate reduces this risk [203] and is particularly indicated in epidemics [204].

A comparative clinical study of artesunate-SP versus SP alone in falciparum malaria, in Irian Jaya (Papua, Indonesia) demonstrated excellent efficacy; day 28 cure rates of artesunate-SP being 95,6% and SP 84,8%. Combined artesunate plus SP resulted in more rapid fever and parasite clearance, was well tolerated, reduced risk of treatment failure, and lowered gametocyte carriage [205]. A recent comparative clinical study of three regimens; amodiaquine alone, combination of AQ-SP and artesunate-SP in uncomplicated falciparum malaria, in 379 Rwandan children patients demonstrated; cure rate at day 28, 77% following AQ, 83% following AQ-SP and 71% following ART-SP[206].

In conclusion, the combination of artesunate-SP is recommended artemisinin-based combinations by WHO, in areas where the cure rate of sulfadoxine-pyrimethamine is greater than 80%.

1.5.2.2.4 Other combinations

Artesunate plus chloroquine, in combination with artesunate is used in areas where *P. falciparum* is still sensitive to chloroquine. In several clinical trials conducted in African children have demonstrated to the good tolerability of oral artesunate when combined with standard antimalarial drugs. The cure rates of the different combinations were generally dependent on the degree of resistance to the partner drug. They were high for amodiaquine-artesunate, variable for sulfadoxine/pyrimethamine-artesunate, and poor for chloroquine-artesunate [207]. A comparative study of CQ alone and CQ combined with AS (CQ-AS) for treating uncomplicated *P. falciparum* malaria in 300 Burkina Faso children aged 6 to 59 months. By day 14, parasites were cleared in 81.6% CQ-AS treated children compared with 37.1% CQ treated children. Corresponding rates for day 28 were 49.0% vs. 19.0%. Despite the beneficial effects of adding AS, the high failure rate at

day 28 of CQ-AS precludes its use as the first-line regimen for treating CQ-resistant *P. falciparum* in Burkina Faso[208].

Overall in areas with high levels of chloroquine resistant, the combination of artesunate and chloroquine is not good enough.

Artesunate plus atovaquone-proguanil(Malarone™). Atovaquone-proguanil is a fixed combination regimen effective against multidrug resistant *P. falciparum* isolates [209, 210]. A study carried out in 1,596 Thailand patients with uncomplicated multidrug resistant falciparum malaria compared artesunate-atovaqune-proguanil, atovaqune-proguanil, and artesunate-mefloquine and showed that cure rates by day 42 with PCR corrected were all highly efficacy with > 95% in the 3 groups[211]. Artesunate-atovaquone-proguanil is a highly effective and well-tolerated treatment for multidrug-resistant falciparum malaria [211], its cost (>$40) prevents it wide spread use in endemic countries.

1.5.2.3 Fixed dose combinations

Fixed dosed combinations have significant advantages in improving compliance and ensuring that neither drug component can be exposed to parasites alone. Over the last decade there has been a lot of work towards developing such fixed dose ACT regimens. Although both “amodiaquine plus artesunate” and “mefloquine plus artesunate”, are now co-formulated, these regimens are not yet widely available. Only two ACTs are currently being marketed and widely available: artemether-lumefantrine (Coartemether) and dihydroartemisnin-piperaquine (Artekin or Duocotexcin).

1.5.2.3.1 Artemether with lumefantrine

Coartemether is a fixed tablet combination produced by Novartis which contains 20 mg artemether and 120 mg lumefantrine per tablet [212]. Artemether-lumefantrine (Coartemether) is produced to international Good Manufacturing Practise (GMP) and is now on the WHO essential drug list [213]. However, the combination is not registered for use in pregnant women, or children under 5 kg body weight [214]. Absorption lumefantrine, a lipophillic agent, varies with the level of fat ingested fat. Although it is recommended that coartemether be given with a fatty meal, most patients with acute malaria are initially anorexic and hence the absorption of the initial doses is often suboptimal. However, after clinical improvement and return to normal diet, the absorption of lumefantrine is increased [215].

Efficacy

The four dose regimen of artemether-lumefantrine has been shown to be effective, in terms of the 28-day cure rate, in regions where *P. falciparum* is not multidrug resistance and patients are partially immune[216]. However, in areas where the parasite is drug resistant, a six dose regimen is required to produce higher cure rates. In the early studies[217-220] of the four dose regimen of artemether-lumefantrine given at 0, 8, 24, and 48 hours resulted in a cure rate of less than 85%. In contrast, clinical trials have been conducted with artemether-lumefantrine show that the six dose regimen of artemether-lumefantrine has cure rates of more than 95%, while remaining equally safe and well tolerated[50, 51, 221, 222]. The six-dose regimen, therefore, fulfils the requirements of the WHO for effective and safe therapy of falciparum malaria[223].

There have been several efficacy studies comparing four-dose combination regimen of AL and monotherapy of several antimalarial drugs in uncomplicated falciparum

malaria patients. In two clinical trials, AL had fewer treatment failure rates compared to chloroquine [216, 218]. However, the failure rate of AL was significantly higher than after mefloquine plus artesunate[219]. These studies showed that the four-dose regimen of AL was more effective than chloroquine in areas of high chloroquine resistance, but tended to be less effective when tested against other drugs (the data did not reach statistical significance)[224].

In two studies of artemisinin combination therapy from Thailand, the six-dose AL was associated with more failures than mefloquine-artesunate [217, 221]. Failure rates were higher with AL (3% and 5%) than mefloquine-artesunate (0%), but this did not reach statistical significance. In comparative trial of two fixed combinations Dihydroartemisinin-Napthoquine-Trimethoprim (DNP) and AL in uncomplicated falciparum malaria in 130 Thailand patients, the cure rates at 28-day were showed 99% and 97% in DNP (twice a day for one day) and AL (six-dose regimen)[225]. In a Tanzanian study four dose unsupervised regimens was compared AQ, AQ with SP, AQ with artesunate and artemether with lumefantrine in 1,811 children with uncomplicated malaria. By day 28, the parasitological failure rates were 182 of 239 (76%) for amodiaquine, 282 of 476 (61%) for amodiaquine-sulfadoxine-pyrimethamine, 193 of 472 (40%) for amodiaquine-artesunate, and 103 of 485 (21%) for artemether-lumefantrine (six-dose regimen) [226]. In recent study of six-dose artemether-lumefantrine in 957 Mbarara patients was showed that artemether-lumefantrine has a high cure rate irrespective of whether given under supervision with food or under conditions of routine clinic practise[214].

In conclusion, the four dose regimen has now been dropped even in areas without drug resistance. The six dose regimen gives excellent efficacy in children and adults

even in areas with high levels of multidrug resistance. The WHO now recommends the use of the six dose regimen over 60 hours.

Effect on gametocyte carriage

Gametocyte carriage is significantly lower in patients treated with artemether-lumefantrine compared with patients treated with antimalarial therapy that does not include an artemisinin derivative. The effect on gametocytes in 260 Tanzanian children (aged 1-5 years) was also marked in comparison to chloroquine, as only 1.7% of those treated with artemether-lumefantrine were positive for gametocytes as compared to 9.1% of those children treated with chloroquine [218]. Patients treated with artemether-lumefantrine with gametocytaemia on day 7 were shown to have had a relative risk 14 times of subsequent treatment failure as compared to those patients that had not developed gametocytaemia [227].

Safety profile

The most common adverse effects reported following artemether-lumefantrine are nausea, vomiting and diarrhoea. However these complaints are also characteristic of acute malaria and it is difficult to assess how much of the symptoms are due to the drug and how much to the disease. Artemether-lumefantrine combination is better tolerated than other artemisinin combination, such as mefloquine plus artesunate, particularly with regard to nausea, vomiting, dizziness and neurological side-effects. A number of trials used for treatment falciparum malaria in young children and infants (over than 10 kg body weight), have also confirmed safety in this vulnerable age group [50].

Cardiotoxicity

The primary safety concern is related to the lumefantrine component and the possibility of cardiac adverse reactions, since other arylamino alcohols belonging to the class 2 blood schizontocidal compounds are known to prolong the QTc intervals. Extensive studies in more than 700 patients of the Phase III trials of coartemether have been conducted to investigate potential cardiotoxicity as an integral part of the clinical trial procedure. Although high dose intramuscular artemether prolonged the QT interval in dogs there has been no evidence of cardiotoxicity in humans, artemether-lumefantrine did not have significant cardiac effects at therapeutic doses [52]. There was no relationship between plasma concentrations of lumefantrine and QTc prolongation [51, 224].

Neurotoxicity

Animal studies in which high doses of lipid soluble artemisinin preparations were used have raised concerns about possible neurotoxicity [228]. However clinical trials described above have failed to show any serious neurotoxicity to artemether-lumefantrine [50, 216-219, 221, 229].

1.5.2.3.2 Dihydroartemisinin with piperaquine

Artekin is a fixed combination containing 40 mg dihydroartemisinin and 320 mg piperaquine per tablet. Dihydroartemisinin (DHA) is the active metabolite of artemisinin that is short acting, but has five to ten times more potent than the parent compound artemisinin [21]. The antimalarial properties of piperaquine a 4-aminoquinoline was first described in China in 1982 [62]. The first combination of artekin came out as China-Vietnam (CV4®: dihydroartemisinin (DHA), piperaquine, primaquine and trimethoprim), which was followed by CV8® (the same components

as CV4 but in increased quantities), Artecom® (in which primaquine was omitted) and Artekin™ or Duo-cotecxin™ (DHA and piperaquine phosphate only)[230].

Dihydroartemisinin consists of a sesquiterpene lactone ring with a unique endoperoxide bridge [52]. Piperaquine is a bisquinoline antimalarial and has received renewed interest in the last decade, with numerous studies showing good antimalarial activity against chloroquine-resistant Plasmodium strains [231]. The bulky bisquinoline structure may be important for activity against chloroquine-resistant strains, and may act by inhibiting the transporters that efflux chloroquine from the parasite food vacuole [232].

Pharmacokinetics

Dihydroartemisinin is rapidly cleared from the body, elimination half-lives being 1.9 h [85]. Antimalarial bioactivity is reaching a peak in 1- 2.6 h [15-17] before rapidly falling [79]. A study by Hung et al demonstrated that dihydroartemisinin (DHA) is rapidly absorbed. Piperaquine is slowly absorbed, with mean absorption halftimes (t1/2, abs) ranged from of 9.1 and 9.3 hours in adults and children [64]. The volume of distribution at steady state/bioavailability (Vss/F) was generally very large in adults (574 L kg-1) and children (614 L kg-1) with the mean of long half life (t1/2,z) was 23 days and a clearance (CL/F) that was markedly higher in children (1,85L h-1 kg-1) compared to adults (0,90L h-1 kg-1) [64].

Piperaquine is an antimalarial drug whose high lipid solubility suggests that its absorption can be increased by a high-fat meal [233], although studies specifically addressing this have not be carried out.

Efficacy

Several studies have confirmed the safety and efficacy of DP. A study in 106 Cambodian children and adults with uncomplicated falciparum malaria showed

excellent efficacy with a day 28 cure rate of 98.6% in children and 92.3% in adults[234]. This study used a four-dose regimen (mean total doses according to age were DHA 6.6–10.1 mg/kg and piperaquine phosphate 52.9–81.2 mg/kg) delivered over 32 hours. Studies of DP against multidrug-resistant falciparum malaria in Vietnam and Thailand also showed excellent efficacy with cure rates at day 56 of 98,7%[181] and at day 28 cure rates in the hospital-based study of 98.3% following DP[235]. A recent comparative clinical trials of DP versus artesunate-mefloquine in falciparum malaria, in Thailand and Laos demonstrated excellent efficacy; day 42 cure rates of DP being 95% and artesunate-mefloquine 96%[180].

Gametocyte carriage

DP, like other ACTs, has been associated with a low rate of gametocyte carriage. Of the Cambodian children and adults evaluated, all the patients that had gametocytes at follow-up had low gametocytaemia (<70parasites / μl)[234]. From Thailand only 3 patients in the DP group developed patent gametocytaemia [235].

Toxicity and tolerability

In general, studies have shown piperaquine monotherapy to be well tolerated with few patients reporting adverse events [65-68]. The situation is similar in studies of piperaquine administered as part of ACT [234, 236]. The most common minor adverse effects have concerned gastrointestinal effects particularly diarrhoea, nausea, abdominal pain and vomiting, although in acute malaria these symptoms are often difficult to distinguish from symptoms resulting from malaria itself[64-68]. In several studies, no haematological, biochemical, cardiac or hepatic abnormalities were described, but it is unclear whether they were specifically evaluated [65-68]. However, a detailed safety and tolerability evaluation performed by Karunajeewa et al.[237] in 62 Cambodian patients (including 32 adults and 30 children) with

uncomplicated malaria showed no significant cardiotoxicity or electrocardiographic changes (including the corrected QT interval), changes in plasma glucose or postural hypotension following treatment with DP at the manufacturer's recommended doses. In addition, there are no published data relating to the safety of piperaquine in pregnancy, lactation or children younger than 2 years.

1.5.2.3.3 Other fixed dose combinations

A fixed-dose combination of **artesunate with pyronaridine** with a 3 days regimen is currently in phase 3 clinical trials. Pyronaridine tetraphosphate is a blood schizonticide antimalarial therapy synthesized in China in 1970. Pyronaridine has good schizontocidal activity against *P. falciparum* and *P. vivax*. Available data indicate that pyronaridine is effective in cases of chloroquine resistance both in vivo and in vitro and seems to be satisfactorily tolerated. However, more studies are required to determine the pharmacokinetic and dynamic properties as well as the efficacy and toxicity of pyronaridine, following the limited data from China and few other trials in other countries[238, 239]. Originally pyronaridine was deployed as an enteric-coated formulation for monotherapy (which had poor oral bioavailability), and was given as a three or five day course. A study conducted to determine the clinical effective dose, safety, efficacy of orally administered artesunate-pyronaridine in South East Asia and Africa, is still ongoing.

Two new fixed dose combinations ACTs, nonpatented malaria treatments are expected to become available in the second half of 2006. There were **artesunate-amodiaquine (AS-AQ, Sanofi-Aventis) and artesunate-mefloquine (AS-MQ, Far-Manguinhos)**. These will help to simplify the dosing regimen therefore, ensuring better patient compliance. AS-AQ and AS-MQ will also be available in a

low-dose paediatric formulation: one smaller tablet a day for three days. In addition, the tablets are water-soluble to facilitate absorption for younger children. In a recent study in Thailand the cure rate at day 63 were 94.1% for the fixed-dose combination and 92% for the separate tablet formulation. Findings also indicated that AS-MQ and the existing formulation have similar safety profiles, with low and comparable rates of minor adverse events such as dizziness, lack of appetite, headaches and sleep disturbance. The most commonly reported adverse event for mefloquine is early vomiting, and it was lower in the AS-MQ regimen (3%) than in the separate tablet formulation (8.4%). (source; www.dndi.org, the Drugs for Neglected Diseases initiative (DNDi)).

1.5.3 Use of ACT in special groups

There are few data on ACT use in pregnancy [240], although case reports of up to 500 exposed women haven't identified any foetal toxicity [241]. WHO currently has advises against the use of artemisinin drugs in the first trimester, unless in a lifesaving situations where no other drugs are suitable. In later pregnancy however alternative therapies should be used if available and efficacious. ACT has been used safely in children as young as 1 month [242] and paediatric formulations (such as dihydroartemisinin-piperaquine granules) are available in some cases [230].

1.6 Conclusion

Malaria is a major health problem particularly in eastern Indonesia. There were around 15 million malaria cases annually and approximately 1.2% (23,483 deaths) of all deaths cause by malaria.

The national malaria control program aims to decrease the mortality and morbidity of malaria by focusing on early diagnosis, optimizing treatment protocol,

surveillance and vector control. The optimizing treatment protocol is the most sufficient and effective tools for decrease the morbidity and mortality malaria. Up until 2004, chloroquine remained the first line therapy, but more recently Indonesia has adopted the combination of artesunate with amodiaquine as the first line therapy for uncomplicated malaria.

Nowadays, the emergence and widespread of drug resistance particularly to chloroquine and SP has contributed to an increase of malaria burden in Indonesia. The consequences of drug resistance are recurrent infections, more severe disease / deaths, more malaria (anaemia, low birth weight, lost productivity) and greater financial cost.

One of the strategies to prevent antimalarial resistance is the use of combination artemisinin derivative, and favour as a fixed dose combination related with compliance to the patient. Only two ACTs are currently being marketed and widely available: artemether-lumefantrine (Coartemether) and dihydroartemisinin (Artekin).

1.7 Aims of this Thesis

In view of the significant burden of chloroquine resistance documented elsewhere in Papua province we undertook a series of chemotherapeutic studies in the Timika region to address the follow aims:

Aim 1: Determine the efficacy of local antimalarial protocols for uncomplicated malaria to all four species.

Hypothesis 1.1 Chloroquine $\pm$ SP as a first line antimalarial drugs are no longer effective for the treatment of uncomplicated falciparum malaria and vivax malaria in Timika.

Hypothesis 1.2 Chloroquine is still effective for treatment of ovale malaria and malariae malaria in Timika.

Aim 2: Compare the safety and efficacy of two fixed dose artemisinin combination therapies in order to inform local policy makers and health service providers of the most suitable alternative therapies.

Hypothesis 2.1 Both combination therapies are highly effective for the treatment of uncomplicated malaria.

Hypothesis 2.2 Dihydroartemisinin-piperaquine for the treatment of uncomplicated falciparum and vivax malaria is more effective than artemether-lumefantrine in preventing relapse of *P. vivax* and reinfections with *P. falciparum*.

The therapeutic studies carried out in this work represent an ongoing collaboration between the National Institute of Health Research and Development in Jakarta and the Menzies School of Health Research, in Darwin, Australia. The field work was carried out in partnership with the local Dinas Kesehatan Kabupatan in Timika and the Public Health and Malaria Control Programme PT Freeport Indonesia.

2 Methodology

2.1 Study sites

The clinical studies included in this these were conducted in Timika on the southern coast of Papua, Indonesia. Two rural clinics were chosen located to the west of Timika, 20 km west of the city of Timika (75 minutes from Timika town by car). The total population of the areas is about 130,000 [243]. Due to economic migration the ethnic origin of the local population is diverse, with highland Papuans, lowland Papuans and non-Papuans all resident in the region. There is an established malaria control program (MALCON- PH) – but with massive clearing of forest areas for housing & gardens, high rainfall and poor drainage vector breeding remains an ongoing challenge. *Anopheles faraunti, Anopheles koliensis* and *Anopheles punctulatus* [244] [245] breed throughout this region and have been shown to be the main vectors. This lowland area has mesoendemic malaria transmission with annual incidence of 938 per 1000 per year, divided 57:43 between *P. falciparum* and *P. vivax* infections. There are currently more than 800 slide positive cases of malaria per week and over 150 cases of severe malaria admitted into the local hospital each year (unpublished data). In view of the high number of infections in non-immune patients, local protocols recommend that all patients with patent parasitaemia are given antimalarial therapy.

2.2 Study overview

An initial pilot study was conducted to determine the efficacy of antimalarial drugs currently deployed in the region. This was an open study based on the 2003 World Health Organization (WHO) *in vivo* antimalarial drug sensitivity protocol [154], modified to include mixed infections and any level of parasitaemia. Patients were followed for 42 days in the falciparum malaria and 28 days for other species of infection. Recruitment of *P. falciparum* and *P. vivax* took place between April 2004 and July 2004, whereas the enrolment of *P. malariae* and *P. ovale* took much longer and was complete in August 2005. The comparative study was undertaken to compare the safety and efficacy of two fixed dosed artemisinin combination therapies for uncomplicated *falciparum* and *vivax* in children and adults with uncomplicated symptomatic malaria. Patients were followed for 42 days using a standardized drug efficacy record form. The pilot study was undertaken at one clinic (SP9), whereas the comparative study was undertaken from at two clinics 5 Km apart: SP9 and SP12.

2.3 Patients

Patients who presented to two established outpatient clinics in Timika (referred to SP9 and SP12), with fever and symptoms of acute uncomplicated infection with *P. falciparum, P. vivax* or both species, *P. ovale*, *P. malariae* were, after laboratory confirmation of the diagnosis and who agree to fulfil the requirements of this study. Patients were recruited if slide-positive with fever (axillary temperature ≥37.5°C) or history of fever in the last 48 hours. No parasite threshold was used and so anyone with fever +/- history of fever and any *P. falciparum, P. vivax, P. ovale* or *P. malariae* parasites visible of the blood film were enrolled. Pregnant or lactating

women, children under 10 kg, patients with signs of severity [246], a parasitaemia >4%, or concomitant disease requiring hospital admission, known hypersensitivity or allergy to chloroquine or sulfadoxine-pyrimethamine or artemisinin derivatives, serious underlying disease (cardiac, renal or hepatic) and inability to tolerate oral treatment were all excluded. Informed consent was signed by an adult patient or by a parent/guardian for children. If the subject was illiterate, consent was obtained in the presence of a literate witness. All patients were given an information sheet in their own language.

2.4 Treatment allocation

In the comparative studies patients enrolled with either *P. falciparum* or *P. vivax* were randomized to receive one of two treatments. An independent statistician used computer-generated randomization to generate blocks of patient numbers for each treatment. Randomization was carried out in groups of 20, and each code given a sealed opaque envelope containing that patient treatment group and which was only opened when a patient had been allocated a code number.

2.5 Trial procedures

2.5.1 Clinical examination

Clinical history, symptoms and signs were recorded for all patients using a standardized data form daily during the first week until they became afebrile and aparasitaemic. The nurse took a history, a full clinical examination and measured the axillary temperature using a digital electronic thermometer, then asked presenting and non presenting complaints using the standardized form under supervised the study physician.

2.5.2 Laboratory examination

Blood was taken for blood film, haematocrit, and white cell count. Parasite counts were determined on Giemsa-stained thick films as the number of parasites per 200 white blood cells (WBC). All slides were read by a certified microscopist with 10 years experience. A thick smear was considered negative on initial review if no parasites were seen in 100 high power fields. A thin smear was also examined to confirm parasite species and used for quantification if parasitaemia was greater than 200 per 200 WBC. All slides were cross-checked by another experienced microscopist. Upon cross checking the whole field was checked before a slide was considered negative. Blood specimens were collected from a finger prick for thin and thick smears on days 0, 1, 2, 7, 14, 28, 35, 42 and on any other day when the patient developed fever or became unwell; for genotyping PCR 3x50µl blood spots on filter paper (Whatman 3MM chromatography paper) on days 0 and on any other day when parasitemia recurrence; for checked haemoglobin level using a battery operated portable photometer (HemoCue™ Hb201+) on day 0 to confirm were not severe anaemia and at any time after day 2 if they came to follow up; for patients with *P. vivax* or mixed *falciparum*/*vivax* infection or *P. ovale* were checked with G6PD test to determine G6PD level on day 1 in clinic (see table)**.**

Heparinized venous blood samples were taken from patients that agreed to venesection on day 0, 7, 28 or day of recrudescence. Plasma drug levels of chloroquine (CQ) and its major metabolite desethylchloroquine (DCQ) were assayed by high performance liquid chromatography to ascertain the compliance with therapy and the relationship between drug levels at these times and treatment failure.

Table of study procedures

Day	0	1	2	3	4	5	7	14	21	28	35*	42*
Medical history, previous medication, physical examination	X											
Trial medication	XXX	XXX	XXX							#		#
Vital signs	X	X	X	(X)	(X)	(X)	X	X	X	X	X	X
Finger prick for parasite microspcopy	X	X	X	(X)	(X)	(X)	X	X	X	X	X	X
Finger Prick PCR Spot	X						R	R	R	R	R	R
Axillary temperature	X	X	X	(X)	(X)	(X)	X	X	X	X	X	X
Blood Sample **	X						X	R	R	X	R	R*
Concomitant medication	X	X	X	(X)			X	X	X	X	X	X
Adverse experience(s)	X	X	X	(X)			X	X	X	X	X	X

(x) = patients without clearance; x = once daily, xx = twice daily

#14 days of unsupervised primaquine given day 28 (*vivax* malaria) or day 42 *falciparum* malaria

** In those agreeing to be venesected: for FBC, and drug levels

R if recrudescence

*for *falciparum* malaria

2.5.3 Procedure descriptions

2.5.3.1 Parasite counts

Thin films were performed if the parasitaemia was greater than 1000 per 200 WCC and for confirmation of species other than *P. falciparum.* A total of 200 thick film fields were examined (tally counter) before a slide was pronounced negative. If asexual forms of *P. falciparum* or *P. vivax* were present, a parasite count was determined from the thick or thin smear and the associated white cell count or haematocrit if available. If the latter was not available an average value was taken: WCC 7500 per ul and Hct 33%. If gametocytes were found, a separate gametocyte count was performed.

2.5.3.2 Blood sampling

On admission, if the smear was positive for *P. falciparum* or *P. vivax* then a venous sample was requested from all patients; 15 ml of whole blood (l ml/kg up to

10ml in children) was taken from those agreeing to be venesected and collected into sterile vacutainer tubes containing potassium EDTA (Becton Dickinson). This sample was processed at the laboratory in Rumah Sakit Mitra Masyarakat Hospital (RSMM) for the following: enrolment thick and thin blood film, 3x50ul blood spots on filter paper (for PCR) and coulter counter (for WBC, Hb). The sample was spun down and the pellet processed and stored for *in vitro* culture and parasite genotyping at a later date.

At each subsequent visit a finger prick was be performed for blood smear and (at the weekly follow ups) for haematocrit. In those agreeing to venesected during recovery, an additional 10 ml (l ml/kg up to 5ml in children) whole blood sample was be requested **on day** 7 **and day** 28. These were processed for drug levels to ascertain the compliance with therapy and the relationship of drug levels at these times and treatment failure.

If recrudescence is noted during follow-up, then a repeat EDTA blood sample was requested and processed as on admission. In those patients refusing to be venesected, then a finger prick was performed and from this the following was performed: a repeat thick and thin blood film processed in RSMM, 3x50jal blood spots on filter paper (for PCR) and capillary for assessment of haematocrit. Filter spots were taken on admission and at the time of parasite "recrudescence". In the case where falciparum recrudescence occurs during follow up, these samples were genotyped by PCR (Merozoite Surface Proteins-1 (MSP-1), Merozoite Surface Proteins-2 (MSP-2) and Glutamaterich Protein (GLURP)) [247] to assess whether the reappearance of parasitaemia was due to reinfection or true recrudescence.

2.6 Management of patients failing therapy

Early Treatment Failure (ETF) was be defined as the development of one of the following conditions during the first three days of follow-up; patients occurred of the danger signs or severe malaria on days 1, 2, 3 in the presence of parasitaemia; parasitaemia on day 2 higher than on day 0; parasitaemia on day 3 (25% of the count on day 0; and parasitaemia on day 3 with axillary temperature >37.5°C.

Patients were referred for medic review. Current therapy was terminated and the patient given quinine orally or intravenously at the discretion of the attending physician.

Late Treatment Failure (LTF) was be defined as the development of one of the following conditions from day 4 to day 42, without previously meeting any of the criteria of ETF, and divided in 2 sub-groups; firstly **Late Clinical Failure (LCF):** patients with danger signs or severe malaria after day 3 in the presence of parasitaemia (same species / genotype as on day 0); and patients with axillary temperature >37.5°C in the presence of parasitaemia on any day between day 4 to day 42. Secondly **Late Parasitological Failure (LPF):** patients with presence of parasitaemia on any of the schedule days of return (days 7 to 42) with the same species/genotype as on day 0 and axillary temperature <37.5°C without previously meeting any of the criteria of LCF.

Retreatment of late failures varied between studies and is described separately for each chapter.

2.7 Removal of patients from trial or analysis

The following events were considered sufficient reason for a patient to discontinue the trial: whenever the patient decided that it is in his/her best interest; whenever the investigator considered it advisable or in the patient's best interest; intolerable adverse experiences; lack of therapeutic response resulting in intolerable symptoms; major violation of the clinical trial protocol; non-compliance of the patient (e.g. missing more than two follow up visits) and development of any exclusion criteria.

2.8 Quality assurance

Site monitoring visits were scheduled on a regular basis by the study supervisors. During these visits, information recorded on the case report forms was verified against source documents (e.g. laboratory records and clinic registers). After the case report forms were collated at the end of the trial, they were reviewed for completeness and accuracy. The data are entered into a database, where specially designed computer checks were used to identify selected protocol violations and data errors.

All blood films on admission were read at the clinic as well as by a senior microscopist at the RSMM hospital laboratory. A random selection of 10% of all blood films analysed at the clinic will also be collected and reread by an independent expert microscopist at NIHRD in Jakarta.

Data were double entered and validated using Epidata software version 3.0 (Epidata Association, Odense Denmark).

2.9 Analytical Methods

Data were double entered and validated using EpiData 3.02 software (EpiData Association, Odense, Denmark) and analysis performed using SPSS for Windows (SPSS Inc, Chicago, Illinois, USA). Sample size calculations for the prospective randomised studies (chapters 3, 6 and 7) were estimated using Statcalc (a component of Epilnfo 6). For categorical variables percentages and corresponding 95% confidence intervals were calculated Wilson's method. Proportions were compared by calculating the % with Yates' correction or by Fisher's exact test where appropriate. Normally distributed continuous data were compared by Student's t test and analysis of variance. Data not conforming to a normal distribution were compared by the Mann-Whitney U test or Kruskal-Wallis analysis of variance. The association 2 continuous variables were assessed using Spearman's rank correlation coefficient.

2.9.1 Analysis of Efficacy

For the comparative analysis of efficacy the primary end point was the overall reappearance of any parasitaemia during a 42 day follow up using a Modified Intention-To-Treat (ITT) Population. All patients randomized were included in the analysis and anyone withdrawn from the study for either parasitological or non-parasitological reasons (listed below with the exception of lost to follow up and withdrawal of consent) were evaluated as failures.

The Per-Protocol (PP) Population was defined as all randomized patients who completed a full course of treatment and did not violate any of the inclusion / exclusion criteria listed in the protocol. Patients who did not have a reappearance of

parasitaemia and who were withdrawn from the study prior to complete follow up (i.e. non-parasitological) were included up until their last day of follow-up. If they were followed for longer than 28 or 42 days they were included in the binary endpoints as successfully treated. Those patients with recurrent vomiting or adverse drug effect which required early termination and the administration of rescue therapy were regarded as treatment failures.

Reasons for Withdrawal: Parasitological: patients with Early Treatment Failure / develop warning signs, Late Treatment Failure (LTF) and **Non Parasitological:** patients with enrollment Protocol Violation (e.g. Pregnancy), failure to complete treatment (compliance), recurrent vomiting, intolerable adverse experiences (early), withdrawal of consent to further follow up, use of antimalarial drugs or antibiotics (not for rescue), concomitant disease during follow up and lost to follow up.

The parasite species at enrollment was be categorised into three groups: pure *P. falciparum,* pure *P. vivax* and mixed *P. falciparum* and *P. vivax*. There were similar categories for the species at the time of treatment failure.

The following **secondary endpoints** were assessed: time to reappearance of *P. falciparum,* time to reappearance of *P. vivax,* time to true recrudescent *P. falciparum* (PCR corrected) and time to reinfection with *P. falciparum* (PCR corrected). In each case the results were assessed overall as well as after stratifying by the initial infecting parasite species. If follow up was terminated before completion of the study the last day seen was recorded and the status at that point recorded depending upon whether the recurrence was reappearance of *P. falciparum* (overall, recrudescence or reinfection) or *P. vivax.* Comparative efficacy was assessed as

summary proportions of patients failing therapy at day 28 and day 42. To compare efficacy rates with other study sites a subgroup analysis conforming to standard WHO protocols was also performed.

When computing the PCR-corrected cure rates patients for whom the PCR was unavailable or uninterpretable were included up until there last negative smear and regarded on balance as reinfections.

Parasite, fever and symptom clearance times were assessed as proportion of patients still parasitaemic or febrile days 1 to 3.

Gametocytes carriage was assessed at weekly follow-up on days 7 and 14, and expressed as the proportion of patients with patent gametocytaemia (Gametocyte Positivity Rate: GPR). Because of the transient nature of gametocytaemia patients missing either of the follow-up appointments had to be excluded from the calculation of the cumulative GPR. In order to include all patients followed Person Gametocyte Week rates (PGW) were also calculated as a measure of transmission potential. These were defined as the number of weeks in which blood slides were positive for gametocytes during a 6 week follow up divided by the total number of weeks followed-up, and were expressed per 1000 person weeks.

2.9.2 Analysis of Safety and Tolerability

Adverse experiences were recorded during the trial (together with their incidence, duration, severity and relationship to trial drug). Physical examination, vital signs and haematological parameters were also assessed.

Safety and tolerability were evaluated in detail on the basis of NIH/NCI Common Toxicity Criteria grades and deviations from laboratory normal ranges. Adverse

experiences were evaluated on the basis of their incidence, duration, severity and relationship to trial drug.

Safety and tolerability measurements were summarised for the ITT patient population. The incidence of adverse experiences were tabulated by severity and trial period in which the AEs started (e.g. present at baseline (Day 0), started after baseline). A separate summary for AEs which were felt to be related to trial drug by the investigator was also produced.

2.10 Ethics

This study was approved by the Ethics Committee of National Institute of Health Research, Ministry of Health, Jakarta, Indonesia (Approval: KS.02.01.2.1.4042), the Ethics Committee of Menzies School of Health, Darwin, Australia (Approval: 03/64 and 05/16), and the Oxfordshire Tropical Research Ethics Committee, Oxford, UK (Approval:).

All studies were also registered at the clinical trials website: http://www.clinicaltrials.gov/ct: Pilot recorded as NCT 00157859, phase 1 comparative NCT 00157833 and phase 2 comparative NCT 00157885.

3 Efficacy of existing antimalarial drugs for uncomplicated malaria in Timika, Papua, Indonesia

3.1 Introduction

Since the 1950s, chloroquine has been the most extensively used antimalarial for uncomplicated malaria. It has the advantage of being widely available, cheap, well tolerated and safe [139]. Drug resistant strains of *P. falciparum* first emerged in Cambodia in 1959 [97, 248] and subsequently spread throughout Asia and Africa. Resistance of *P. falciparum* to chloroquine therapy was first noted in Indonesia almost 30 years ago [249], and has now been documented through out the country [117, 137, 250] with the highest levels reported from northern Papua [251]. Declining efficacy has also been noted to the second therapy of sulfadoxine-pyrimethamine (SP) [130, 252].

The emergence of drug resistant strains of *P. vivax* was only described in 1989 in northern Papua province (formerly Irian Jaya) [126]. Other studies of *P. vivax* have subsequently confirmed a high prevalence of chloroquine resistance in Sumatra, and Northern Papua [130, 251, 252] and elsewhere in Indonesia [253]. Resistance to SP has also been noted [135].

P. ovale and *P. malariae* account a minor number of cases of malaria, although in some regions their prevalence in symptomatic patients can reach as high as 10%

[250]. Few studies have addressed the efficacy of chloroquine to these strains. A recent study in South Sumatra did however indicate the resistant strains of *P. malariae* may be emerging [254].

In Mimika district on the Southern part of Papua, malaria transmission is unstable and causes an appreciable burden of disease despite an extensive malaria control programme. A small unpublished study in 2001 reported that the day 28 cure rate for *P. falciparum* had fallen to 21% following sulfadoxine-pyrimethamine alone and 25% for chloroquine plus sulphadoxine-pyrimethamine [255]. Failure rates of chloroquine monotherapy for vivax malaria in Timika are unknown, however in the same study chloroquine three days plus a single dose of sulfadoxine-pyrimethamine and primaquine on day 0 resulted in a day 28 cure rate of 25 %.

As part of a series of studies to rationalize antimalarial protocols in this region we undertook a series of chemotherapeutic trials to determine the efficacy of prevailing protocols for uncomplicated malaria.

3.2 Methods

3.2.1 Study Procedures

Methods were as described in section 2. This was a prospective open label drug efficacy study of existing recommended therapy (chloroquine and sulphadoxine-pyrimethamine for falciparum malaria and chloroquine alone for vivax malaria, malariae malaria and ovale malaria in children and adults with uncomplicated symptomatic malaria. The study was based on the 2003 World Health Organization (WHO) in vivo antimalarial drug sensitivity protocol.

Treatment courses of chloroquine (CQ) with sulfadoxine-pyrimethamine (SP) for *P. falciparum* (alone or mixed with *P. vivax*), and monotheraphy CQ for pure *P. vivax*,

P. ovale or P. malariae infections were administered. The CQ+SP regimen consisted of CQ (CQ: P.T Bayer, Jakarta, Indonesia-150 mg base /tablet, 25 mg/kg over 3 days) and SP (Suldox; PT Dumex-Alpharma Indonesia, Jakarta; 25 mg Pyrimethamin + 500mg Sulfadoxin/tablet) was given as a single dose on day 0 (25 and 1.25 mg/kg respectively). All drug administrations were supervised and participants observed for 30-60 minutes to exclude adverse reactions and to ensure the medication was not vomited. If vomiting occurred within 60 minutes the whole dose was repeated once. If vomiting occurred again within 60 minutes the patient was withdrawn from the study. If the axillary temperature was ≥38°C, paracetamol was given.

Treatment failures were retreated with quinine (10mg of salt /kg body weight/dosage orally 3 times a day for 7 days) plus doxycycline (100mg bd for 7 days) if ≥8 years age and not pregnant. From May 2004 those patients with reappearance of *P. vivax* within 28 days were given a 3 day course of supervised amodiaquine if they consented (Flavoquine™: Aventis -153 mg base /tablet, 30 mg/kg over 3 days). Primaquine (0.5 mg of base/kg of body weight for 14 days) was administered to those individuals with *P. vivax* or *P. ovale* infection or mixed infection on day 28 of their participation in the study.

Endpoints were as described in section 2. Since chloroquine concentrations persist at levels above the minimum effective concentration (MEC>15ng/ml) for *P. vivax* beyond 28 days [129], any recurrence within this time were considered to be therapeutic failures.

3.3 Results

3.3.1 Baseline Characteristics

In total 207 patients were enrolled in the study between April 2004 and August 2005. Patients with *P. falciparum* (n=88), *P. vivax* (n=40) and mixed infections (n=15) were enrolled between April and September 2004. Patients with *P. malariae* (n=50) and *P. ovale* (n=14) were enrolled between April 2004 and August 2005. Patients with *P. falciparum* or mixed infections were treated with chloroquine plus sulfadoxine-pyrimethamine (Cq+SP) and the remainder were treated with chloroquine monotherapy.

3.3.2 *P. falciparum* and Mixed Infections

The 103 patients infected with either pure *P. falciparum* infection or *P. falciparum* mixed with *P. vivax* were equally distribution among adults and children, but there was predominance of males 61% (63/103) and patients of Papuan origin 57% (59/103). Other baseline characteristics are given in table3.3.3a**.**

Seven patients (6.8%), all of whom had pure *P. falciparum*, had recurrent vomiting and required rescue therapy. A further 10 patients (all with *P. falciparum*) failed to come to the clinic for supervised therapy. **(figure1: flowchart).** Of those patients who received a full treatment course, the mean dose of chloroquine administered was 27.5 mg/kg [SD=2.9].

3.3.3 *P. vivax*

In SP9 Clinic there were 40 patients with *vivax* malaria patients enrolled in the study (table3.3.3a). Patients with *P. vivax* were significantly younger with 28% (11/40) being under 5 years of age compared to 9% (8/88) of those with *P. falciparum*

(Relative Risk RR: 3.0 [95%CI: 1.3-6.9]; p=0.014**.** The geometric mean parasitaemia on admission was 578 (340-992), significantly lower than that in patients with *P. falciparum* (p=0.006). There was a slight predominance of males (55%) and Papuan patients (65%) (table3.3.3a). Of these 40 patients 2 (5%) patients had recurrent vomiting and required rescue therapy and a further 1 patient failed to come to the clinic for supervised therapy (figure1: flowchart). Of those patients who received a full treatment course for *P. vivax* infections, the mean dose of chloroquine administered was 26.3 mg/kg [SD=2.6].

3.3.4 *P. malariae*

There were 50 patients enrolled with *P. malariae* between September 2004 and August 2005 (table3.3.3a). Patients enrolled were equally distributed between children and adults, but there was a predominance of males (62%) and Papuan patients (76%). The geometric mean parasitaemia on admission was 646 (493-847), significantly lower than that in patients with *P. falciparum* (p=0.01). Of these 50 patients one (2%) patient had repeated vomiting and required rescue therapy and a further four patients failed to come to the clinic for supervised therapy (figure1: flowchart). In the 45 patients who received a full course of treatment, the mean dose of chloroquine administered was 26.4 mg/kg [SD=2.7]**.**

3.3.5 *P. ovale*

Between October 2004 and June 2005 14 patients infected with *P. ovale* arm were enrolled and treated with chloroquine monotherapy (table3.3.3a). The geometric mean parasitaemia on admission was 791 (95%CI: 354-1170), but numbers recruited were low and did not differ significantly from patients with *P. falciparum*. One patient 1 (7%) had repeated vomiting and requiring rescue therapy (figure1: flow

chart). In the 13 patients who received a full course of treatment, the mean dose of chloroquine administered was 26.3 mg/kg [SD=2.7]**.** Of those patients who received a full treatment course for *P. ovale* infections, the mean dose of chloroquine administered was 27.5mg/kg [SD=4.8].

Table3.3.3a: **Baseline characteristics of uncomplicated malaria study patients**

Species	Falciparum Arm		Vivax Arm	Malariae Arm	*Ovale* Arm
	PF	MIX	PV	PM	PO
No. of Subjects Enrolled	88	15	40	50	14
Males N (%)	64%(56)	47%(7)	55%(22)	62%(31)	64%(9)
Papuan	56%(49)	67%(10)	65%(26)	76%(38)	79%(11)
Age Median (range) in years	17.5(1-60)	10(4-60)	15(1.8-60)	15.5(2-49)	29(3-53)
Age <5 N (%)	9%(8)	28%(4)	28%(11)	10%(5)	7%(1)
Age 5-14 N (%)	36%(32)	27%(4)	23%(9)	38%(19)	14%(2)
Age >14 N (%)	55%(48)	47%(7)	50%(20)	52%(26)	79%(11)
Temperature (°C) >37.5°C N (%)	35% (31)	33%(5)	15%(6)	14%(7)	14%(2)
Haemoglobin (g/dl) Mean (SD)	10.2±1.9	9.8±2.2	10.5±2.1	9.5±2.1	12,1±3.0
HB <10 N (%)	44%(39)	47%(7)	38%(15)	68%(34)	36%(5)
Geometric Mean (95% CI) parasite count per µl blood	1651(1071-2547)	3618 (1206-10,885)	578 (340-992)	646 (493-847)	791(354-1770)
Gametocyte carriage (%)	8% (7)	40% (6)	13%(5)	28%(14)	43%(6)
Spleenomegaly	37%(31/85)	27%(10)	39%(15/39)	38%(19)	36%(5)

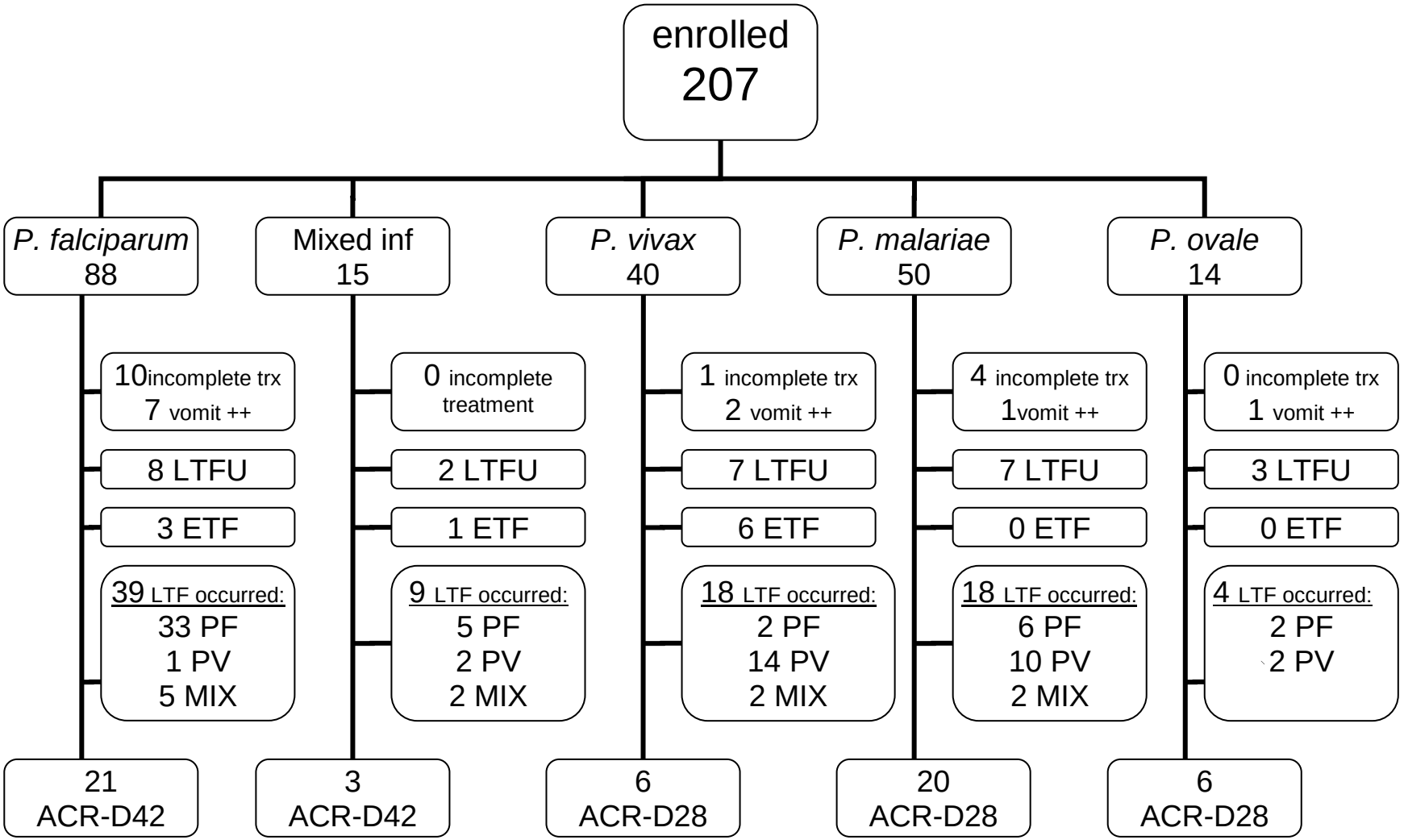


Figure1: The flowchart of patients enrolled in existing therapies study

3.3.6 Drug Tolerability

Overall vomiting of the first dose of chloroquine within an hour of administration occurred in 6.8% (14/207) of patients and did not differ between species of infection or co-administration of SP. Vomiting any dose of chloroquine occurred in 16% of children (15/95) compared to 1.8% (2/112) in adults (RR=8.8 [95%CI: 2.1-38], p<0.001).

3.3.7 Early Therapeutic Response

Early parasitological failure was observed in 10 patients: 3 with *P. falciparum*, 6 with *P. vivax* and one mixed infection. Four patients developed warning signs or markers of severity and required rescue with intravenous quinine. One adult with *P. falciparum* developed convulsions and coma and a child with falciparum malaria developed respiratory distress on day one. An adult and a child, both with *vivax* malaria, were developed severe vomiting and diarrhoea on day one. The remaining six patients received oral quinine and all made a complete recovery.

Table 3.3.7a: Early therapeutic response of standard therapies

	P. falciparum	**Mixed Infections**	***P. vivax***	***P. malariae***	***P. ovale***
Early Treatment Failure % (N)	**4.2%** (3/88)	**6.7%** (1/15)	**16%** (6/40)	**2%**(1/50)	**7.1%**(1/14)

Table3.3.7b: Proportion of patients still parasitaemic during follow up

STILL PARASITAEMIC	Pf	Mix	Pv	Pm	Po
Day 1	**88%** (66/75)	**100%** (14/14)	**82%** (31/38)	**57%** (24/42)	**55%** (6/11)
Day2	**36.4%** (24/66)	**60%** (9/15)	**53%** (17/32)	**18%** (8/43)	**0%** (0/12)
Day3	**6.9%** (4/58)	**38%** (5/13)	**28%** (8/28)	**3%** (1/39)	**0%** (0/13)

Parasite clearance was delayed following treatment of *P. vivax* (either alone or mixed with *P. falciparum*) with 32% (13/41) still parasitaemic by day 3. This was significantly longer compared to only 7% (4/58) in those with pure *falciparum* infection (RR=4.6 [95%CI: 1.6-13], p=0.003) and 1/52 (2%) of those with either *P. malariae* or *P. ovale* (RR=16.5 [95%CI:2.3-120.9], p=0.0002).

Although all patients presented with a history of fever, only 25% (51/207) actually had a recorded fever on admission. Patients with *P. falciparum* (alone or mixed) were at significantly greater risk of fever compared to non *falciparum* malaria (**table3.3.3a**, RR=2.4 [95%CI 1.4-4.2], p=0.001). Of those patients presenting with a fever 94% (33/35) had defervesced by day 2.

3.3.8 Late Therapeutic Response

Recurrence of infection during recovery was observed in 88 patients: 48 of whom had *P. falciparum*, 29 had *P. vivax* and 11 had mixed infections.

Table3.3.8a: The species at recurrence of malaria following standard therapies

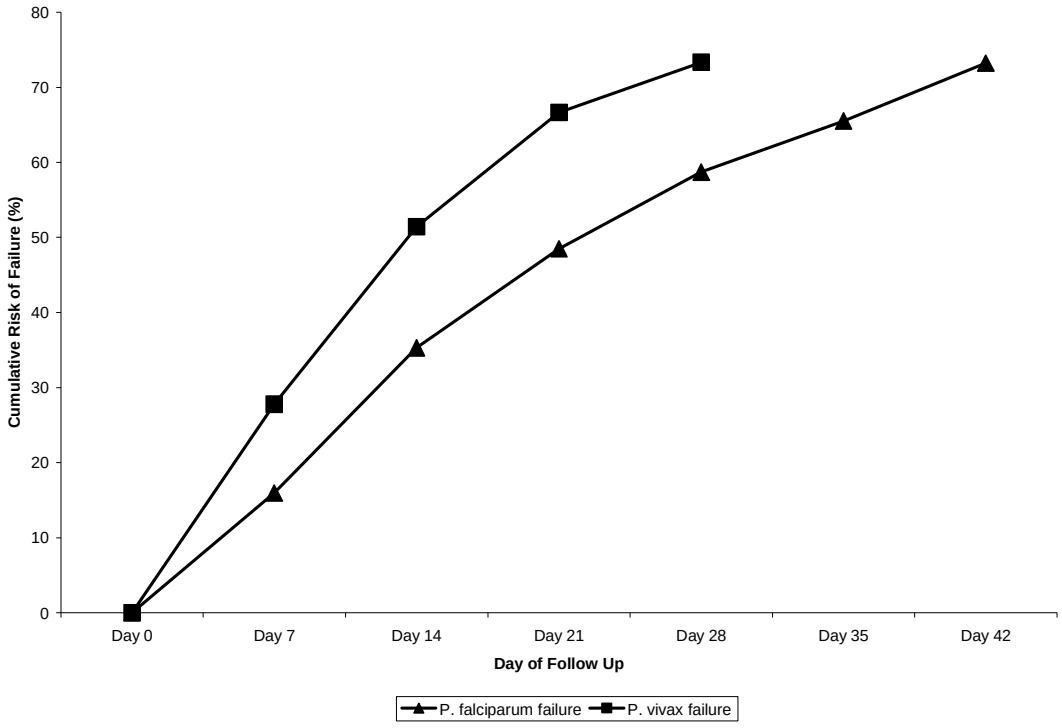
SPECIES AT TIME OF LATE TREATMENT FAILURE					
	***P.falcp* †**	**Mixed Infections †**	***P. vivax* ‡**	***P. malariae*‡**	***P. ovale*‡**
Mixed Infection	5	2	2	2	0
P. falciparum	33	5	2	6	2
P. vivax	1	2	14	10	2

† Up to day 45; ‡ up to day 31

Overall failure rates were defined as either early treatment failure or the recurrence of the same species after treatment (either alone or mixed).

Figure 2: Cumulative incidence of Therapeutic Failure of patients following treatment of *P. falciparum*

with CQ+SP and *P. vivax* with CQ alone



Failure rates after *P. falciparum* and *P. vivax* infections were high (table3.3.8b). By day 42 the failure rate of pure *P. falciparum* and mixed infection was 73% (41/56) and 77% (10/13) respectively. *P. vivax* infections were followed up for 28 days prior to administration of primaquine. By this time 73% (22/30) had failed therapy. Although recurrence of *P. falciparum* and *P. vivax* occurred in 34% (22/64) with *P. malariae* or *P. ovale* infections, there was no occurrence of treatment failure with the same species.

Table3.3.8b: Late therapeutic efficacy of standard therapies

	P.FALCP †	**MIXED INFECTIONS †**	***P. VIVAX* ‡**	***P. MALARIAE*‡**	***P. OVALE* ‡**
Failure rate day 7	**16%** (11/69)	**13%** (2/15)	**28%** (10/36)	**0%** (0/42)	**0%** (0/13)
Failure rate day 28	**59%**(37/63) *	**77%**(10/13) ^	**73%** (22/30)#	**0%** (0/27)**	**0%** (0/8)^^
Failure rate day 42	**73%**(41/56) *	**77%**(10/13) ^	-	-	-
Time to Recrudescence Days Median (range)	**16** (7-24)	**15** (7-24)	**12** (1-22)		

*Reappearance of *falciparum* or mixed infections. ^ Reappearance of either *falciparum* or *vivax* malaria. # Reappearance of *vivax* or mixed infections. ** Reappearance of *malariae* malaria. ^^ Reappearance of *ovale* malaria.

PCR Correction

Parasite genotyping was successful and informative in 78% (35/45) of the late *P. falciparum* or mixed isolates and identified 18 true recrudescence and 10 new infections, with indeterminate results in 7 cases. Therefore the rate of *P. falciparum* recrudescence by day 42 corrected by PCR was 52% (29/56) [95%CI: 39-64].

3.3.9 Chloroquine levels

On admission 61% (63/103) patients with *P. falciparum* and 65% (26/40) patients with *P. vivax* had chloroquine detected in their blood. In 29% (18/63) cases these plasma drug levels were above the recognized minimal effective concentration (MEC) (30ng chloroquine plus desethylchloroquine (CQ+DSC)/ml plasma)[129]. In total 27% (7/26) of patients presenting with vivax malaria had detectable chloroquine levels on admission and all exceeded the recognized MEC for *P. vivax* (15ng CQ+DCQ/ml plasma) [129, 256].

Table 3.3.9a: Chloroquine levels in blood of falciparum malaria and *vivax* malaria

	MEC >30ng/ml of *falciparum* malaria		**MEC >15ng/ml of *vivax* malaria**
	P. falciparum	**Mixed infection**	***P. vivax***
Day 0 **Mean(95%CI)**	**29% (17/58)** **100(33 - 215)**	**20%(1/5)** **48(48 - 48)**	**27% (7/26)** **104(27 - 208)**
Day 7 **Mean(95%CI)**	**96% (30/31)** **213(62 - 561)**	**75%(3/4)** **422(129 - 578)**	**91% (10/11)** **214(74 - 426)**

MEAN and 95%CI with three columns for Species

Chloroquine levels were determined in 67% (14/21) patients who recrudesced with *P. falciparum* or mixed infections and 17% (6/34) of these patients had plasma levels above the MEC [Range 25-108ng/ml]. Chloroquine levels were determined in 17% (6/34) patients with *P. vivax* during follow up. The median CQ+DCQ level on this day was 44 [range 25-108], with 100% (7/7) of patients having levels in excess of the MEC.

3.3.10 Gametocyte Carriage and Anaemia

On admission gametocytes were present in 26% (31/119) compared to only 8% (7/88) of patients with pure falciparum malaria (p=0.001). During follow up only 1 patient with infected with non falciparum malaria had patent gametocytaemia, compared to 46% (32/70) after falciparum malaria on day 7 and 45% (24/53) on day 14.

On day of admission anaemia (HB under 10 g/dl) were present in 48% (100/207) of patients. Those with *P. malariae* were at higher risk of anaemia compared to with other species (p=0.001). During follow up patients who failed therapy were 2.2 fold [95%CI 1.1-4.3] more likely to be anaemic on day 7 (p=0.03).

3.3.11 Re-treatments

Overall 52 patients had *P. falciparum* or mixed infections detected during follow up requiring re-treatment , of whom 24 (46%) adults and 28 (54%) children (less than 14) agreed to be reenrolled for further follow up. Adult received an unsupervised 7 day course of quinine plus doxycyclin, however by day 28 57% (8/14) had had a further recurrence of *P. falciparum*. Children were retreated with 7 days unsupervised quinine and by day 28, 76% (19/25) had failed therapy again.

Of the 20 patients with *P. vivax* infection during follow up, 12 patients were treated with supervised amodiaquine and 6 patients with unsupervised quinine. Only 8 were successfully followed to day 28 after treatment: 3 following amodiaquine and 5 following quinine therapy.

3.4 Discussion

Since 2004, the combination of artesunate-amodiaquine has become the drug of choice for uncomplicated malaria in Indonesia. However, prior to policy change the

majority of uncomplicated infections were still treated with chloroquine (CQ) in Timika, Papua. We therefore evaluated the therapeutic efficacy of the existing antimalarial drugs in symptomatic patients with uncomplicated falciparum and vivax malaria in Timika using the 1997 WHO guidelines. Patients were treated with chloroquine and sulfadoxine-pyrimethamine (SP) for uncomplicated malaria falciparum and followed for 42 days. Patients with uncomplicated malaria due to other species (*P. vivax, P. ovale, or P. malariae)* were given chloroquine monotherapy and followed for 28 days before receiving a 14 day course of unsupervised primaquine (*P. vivax* or *P. ovale*). Patients failing therapy received unsupervised oral quinine +/- doxycycline for 7 days.

During the execution of this study, we were also able to evaluate the efficacy of amodiaquine in a relatively small number of patients with reappearance of uncomplicated vivax malaria.

Baseline features of uncomplicated malaria patients

In SP9 clinic, uncomplicated falciparum malaria, vivax malaria, malariae malaria and ovale malaria were observed predominantly in males (60%) patients and in Papuans (65%) patients. This may reflect that adult Papuans males are likely to have greater exposure to areas of malaria transmission. Whereas non-Papuan people in this region have a much greater awareness and greater use of bed nets (Personal observations).

The rate of anaemia (haemoglobin <10g/dl) was higher in those patients with *P. malariae* (68% 34/50) compared to that with other species (42% 66/157), (p=0.001). During follow up, (42% 40/95) of patients had anaemia on day 7 with no difference between species of infection (p=0.9). By day 28 no patients were anaemic. During follow up patients who failed therapy were 2.2 fold [95%CI 1.1-4.3] more likely to

be anaemic on day 7 (p=0.03). This is consistent with previous observations showing that drug resistant is associated with a greater risk of anaemia [147, 257]. Gametocytes carriage on admission was higher in patients with non-falciparum malaria occurring in 26% patients vs 8% of patients with pure *P. falciparum* infection (p=0.001). Conversely during follow up only 1 patient non falciparum malaria had patent gametocytaemia, compared to 46% on day 7 and 45% on day 14 following falciparum malaria. These results confirm the relationship between high levels of drug resistance of *P. falciparum* and increased gametocytes carriage [162, 258], and provide an explanation for increased transmission driving the spread of resistance.

Therapeutic efficacy

The cumulative day 28 failure rate for *P. falciparum* was 59% [95% CI:46-70] compared to and 73% [95% CI:56-86] for pure *vivax* infections. After PCR correction for reinfection the day 42 failure rate for *P. falciparum* was 73% [95% CI: 60-83]. These high rates of late treatment failure were associated with an extremely poor early therapeutic response with 5% (4/86) of patient with falciparum malaria and 16% (6/37) of those with *vivax* malaria required rescue therapy. These results confirm the poor efficacy of chloroquine plus sulfadoxine-pyrimethamine for falciparum and chloroquine alone for vivax malaria seen in the northern part of the province [121, 135, 251].

Retreatment with unsupervised courses of quinine +/- doxycycline was associated with an equally poor outcome. By day 28 57% (8/14) of patients with recurrence parasitaemia retreated with quinine plus doxycycline failed therapy again and this figure rose to 76% (19/25) in children treated with quinine alone. Quinine treatment was unsupervised and although the poor efficacy could be attributable to quinine

resistance a more likely explanation is poor adherence to a difficult regimen (three times daily for 7 days) which is frequently associated with unpleasant side effect (cinchonism, nausea and dizziness). Although a study of supervised therapy would help to resolve this, in practice supervision is not an option and hence the usefulness of this regimen appears limited in uncomplicated malaria.

In total 11 patients with recrudescent *P. vivax* were retreated with amodiaquine monotherapy. Early parasite clearance was rapid in all cases with no early treatment failures. Although only three patients were followed for 28 days none failed therapy during this time. This is one of the first reports of the efficacy of amodiaquine against chloroquine-resistant strains. Although it suggests that its efficacy may be superior to chloroquine, further studies are needed to confirm this.

In contrast to the poor efficacy of *P. falciparum* and *P. vivax* there were no early treatment failures when chloroquine was used alone for *P. malariae* malaria or *P. ovale* infections. Although late treatment failures did occur (18 following *P. malariae* and 6 following *P. ovale*), none of these were with the same species, suggesting either reinfection or recrudescence from pre-patent parasitaemias in the initial infection. Hence the success rate of either of these species to chloroquine remains at 100%.

3.5 Conclusions

There is a high prevalence of multidrug resistance to *P. falciparum* and a high grade chloroquine resistant *P. vivax*. However chloroquine retains adequate efficacy against *P. ovale* and *P. malariae* in Papua. Moreover unsupervised treatment with quinine +/- doxcycline maybe a useful life saving treatment, but is not a suitable alternative for uncomplicated malaria in the community.

Alternative treatment regimens in this region are clearly needed. The World Health Organisation (WHO) recommends the use of artemisinin combination therapy (ACT) in regions where antimalarial drug resistance is emerging. Artemisinin derivatives have been shown to be safe and effective against both drug resistant falciparum and vivax malaria. Current national recommendations advocate the use of a combination of artesunate and amodiaquine, although alternative regimens, such as artemether-lumefantrine and dihydroartemisinin-piperaquine are also available. In the next study we set out to compare to suitable alternative therapies for falciparum and vivax malaria.

4 Artemether-lumefantrine versus dihydroartemisinin-piperaquine for multi-drug resistant *P. falciparum* and *P. vivax* in Papua, Indonesia

4.1 Introduction

Resistance to antimalarial drugs is emerging through out the tropical world and poses a significant challenge to malaria control programmes. To ensure high cure rates and to combat this threat the World Health Organization (WHO) has recommended the use of artemisinin combination therapy (ACT), although debate still continues as to the most suitable combination and how ACTs should be deployed and funded. The rationale underlying ACT is to improve antimalarial efficacy, facilitate adherence to a full treatment course, and minimize the selection of drug resistant parasites[52, 107]. If possible preparations should be formulated in a single tablet, to improve adherence and ensure that neither parasites are exposed to either component alone. Until recently the only ACT available fulfilling this requirement was artemether-lumefantrine (AL). The 6 dose regimen of AL is now one of the preferred options for uncomplicated malaria in most countries where antimalarial policy is changing to an ACT.

Although the antimalarial activity of piperaquine has been recognised since the 1960s, until recently its use in clinical practice has been restricted to the People's Republic of China where it replaced chloroquine as the antimalarial recommended by the national control programme in 1978. In the 1990s piperaquine was been

combined with dihydroartemisinin (DHA), the active metabolite of the artemisinin derivatives. The co-formulation has been marketed under a variety of names including artekin or duocotecxin [75], which contain 40mg/tablet DHA and 320mg/tablet of piperaquine. Comparative drugs studies have shown that DP (DP) is well tolerated and has excellent efficacy against multidrug resistant strains of *P. falciparum* [181, 234, 235, 259]. In 2005 it became the first line antimalarial in the Vietnamese national malaria programme.

In Timika on the southern coast of Papua Province, Indonesia we have recently reported, that the cure rate of chloroquine plus sulfadoxine + pyrimethamine for *P. falciparum* has fallen to 52% and that of chloroquine monotherapy for *P. vivax* to 35%, with 28% of patients failing therapy within the first week (submitted to Indonesia Medical Journal). These figures are similar to those reported in the northern part of the province [251] and emphasize the urgent need to develop alternative treatment strategies. In line with global strategy the Indonesian Ministry of Health has committed to implementations of ACT which in Eastern Indonesia is required to be effective against both multidrug resistant *P. falciparum* and *P. vivax.*

Most antimalarial drug trials are designed to determine drug efficacy for the treatment of pure infections of *P. falciparum*; indeed World Health Organisation protocols specifically encourage the exclusion of mixed infections to decrease extraneous factors that may influence the therapeutic response [154]. In South East Asia *P. vivax* exerts a huge burden with as many as 270 million cases per year [8]. Although chloroquine resistant strains of *P. vivax* are mainly limited to Indonesia and Papua New Guinea [53], sporadic reports of tolerance have been reported elsewhere in India and South America and resistance is highly likely to spread across Asia. The relevance of these co-infections is usually underappreciated and rarely

reported [260]. Since microscopic diagnosis of malaria is often difficult with misidentification of parasite species and underreporting of mixed infections [261], it is likely that drug trials which deliberately ignore *P. vivax* either initially or during subsequent during follow up, may not necessarily reflect the true potential of antimalarials in practice.

We report a pragmatic randomized comparative study to determine the safety and efficacy of DP and AL for the treatment of patients with uncomplicated malaria presenting to rural clinics with infection with *P. falciparum*, *P. vivax* or a mixture of both species.

4.2 Methods

4.2.1 Study Procedures

Methods were as describe in section 2 with some minor variations.

This was an open randomized, parallel group, single centre 6 week trial to compare the safety and efficacy of a six dose AL regimen with DP for the treatment of acute, uncomplicated falciparum and vivax malaria in adults and children (>l0 kg).

Treatment, AL (Coartem®: Novartis, Basel, Switzerland, containing 20mg of artemether and 120mg of lumefantrine) was dispensed in blister packs with each dose determined according to weight. Patients weighing 10-15kg received 1 tablet per dose, 15-25kg received 2 tablets, 25-35kg received 3 tablets and greater than 35kg received 4 tablets. In total six doses were administered over 3 days: on admission, then at 8, 24, 36, 48, 60 hours. Although patients were instructed to take each dose with a biscuit or milk, only the morning dose was supervised and the patient then given the evening dose to self administer.

DP (Artekin®: Holley Pharmaceutical Co., PRC; containing 40 mg dihydroartemisinin and 320 mg piperaquine) was administered as a weight per dose regimen of 2.25 and 18 mg/kg per dose of dihydroartemisinin and piperaquine, respectively, rounded up to the nearest half tablet. Three supervised doses were given; on admission and after 24 and 48 hours.

When drug administration was observed after AL or DP and vomiting occurred in less than 30 minutes, administration of the full dose was repeated. If vomiting occurred between 30 and 60 minutes half the dose was repeated. If the axillary temperature was ≥38°C, paracetamol was given.

Those patients failing therapy with recurrence of *P. falciparum* were retreated with quinine (10mg of salt /kg body weight/dosage orally 3 times a day for 7 days) plus doxycycline (100mg bd for 7 days) if ≥8 years age and not pregnant. Patients with reappearance of *P. vivax* were given a 3 day course of supervised amodiaquine (Flavoquine™: Aventis - 153 mg base /tablet, 30 mg/kg over 3 days). Primaquine (0.5 mg of base/kg of body weight for 14 days) was administered unsupervised to those individuals with *P. vivax* infection or mixed infection on day 28 of their participation in the study.

Statistical analyses were as described in section 2. A pilot study suggested that 60% of patients presenting to the clinic with uncomplicated malaria have *P. falciparum*, 25% *P. vivax* and 15% mixed infections. Assuming an efficacy of 96% for both AL and DP when drug administration was fully supervised, a total sample size of 750 patients would determine with 95% confidence the cure rate for each regimen within ±3% and that of *P. vivax* within ±3.5% of the true cure rate (allowing for 30% loss to follow up). In addition the study had 80% and 90% confidence to detect a 10% reduction in the overall efficacy which may have resulted from a policy of only

supervising administration once daily (a practical constraint of widespread deployment). Those patients with recurrent vomiting or adverse drug effects which required early termination of treatment and the administration of rescue therapy were regarded as therapeutic failures. Preliminary analysis demonstrated that 87% (26/30) of treatment failures were reinfections. Hence in the 13 (30%) cases when the PCR result was unavailable or uninterpretable the reappearance of *P. falciparum* was regarded as a reinfection.

Gametocyte carriage was assessed by calculating Person Gametocyte Week rates (PGW) as a measure of transmission potential. These were defined as the number of weeks in which blood slides were positive for gametocytes during a 6 week follow up divided by the total number of weeks followed-up, and were expressed per 1000 person weeks [190].

4.3 Results

Seven hundred and seventy four patients with uncomplicated malaria were enrolled between July 2004 and June 2005. The DP and artemether-lumefantrine groups included 387 patients each. Patients were of all ages from 1 to 60 years old with slightly higher male than female. There were 446 (61%) patients were infected with *P. falciparum* alone, 175 (24%) with pure *P. vivax*, 111 (15%) with a mixture of both species. Forty two patients were excluded from the per protocol analysis: 5% (19/387) patients had protocol violations (8 DP and 11 AL) and 6% (23/387) patients had incomplete treatment (14 DP and 9 AL), see figure 3.

In total, 732 patients (367 patients AL and 365 DP) were included in the final evaluation per protocol. 160 (21%) patients did not complete follow up to the day

of treatment failure or day 42 (71 in the AL group and 89 in the DP). Of these, 147 (92%) patients were lost to follow up (64 in the AL group and 83 in the DP), 7 (4%) patients took other antimalarials outside of the study protocol (3 following AL and 4 following DP), 6 (4%) patients developed concomitant disease deemed unrelated to study drug which required hospital admission during 42 days follow-up (4 following AL and 2 following DP).

Therefore of those enrolled a total of 560 (72%) patients could be assessed for treatment efficacy at day 42. The trial profile is given in figure 3.

4.3.1 Baseline Characteristics

There was no significant difference in baseline characteristics between treatment groups (see Table4.3.1a). However patients at SP12 were more likely to be adults, severely anaemia and Papuan (see Table4.3.1b).

Overall 714 of patients received a full course of treatment with AL and DP. Of those 356 of patients who received a full course of AL, the mean dose of artemether and lumefantrine were 10.1 mg/kg [SD 2.1 mg] and 60.5 mg/kg [SD 12.3 mg]. The remains 358 of patients who received a full course of treatment with DP, the mean dose of DHA and piperaquine were 6.7 mg/kg [SD 0.9 mg] and 53.6 mg/kg [SD 6.7 mg].

Table4.3.1a: Baseline Characteristics

	Artemether-Lumefantrine	**Dihydroartemisinin-Piperaquine**
Number of patients enrolled	387	387
No. in Per Protocol Population	367	365
Species at Enrollment: *P. falciparum* *P. vivax* Mixed infections Other*	 238 (61%) 85 (22%) 57 (15%) 7 (2%)	 236 (61%) 91 (24%) 55 (14%) 5 (1%)
Males N (%)	231 (60%)	224 (58%)
Median Weight kg (Range)	46 [10-104]	48 [10-103]
Age (years) : Median [Range] in years <5 N (%) 5-14 N (%) >14 N (%)	 18 [1-60] 51 (13%) 112 (29%) 224 (58%)	 17 [1-60] 48 (12%) 121 (31%) 218 (57%)
Temperature (^{o}C): >37.5; N (%)	130 (34%)	121 (31%)
Haemoglobin (g/dl) Mean (SD) <7 g/dl N (%)	 9.8 (2.5) 53 (14%)	 9.8 (2.4) 51 (13%)
Geometric mean (95% CI) Parasite count per µl blood	3.241 (2.709-3.877)	3.173 (2.660-3.785)
Gametocyte carriage N (%)	126 (33%)	149 (39%)
Ethnicity : Papuan Non Papuan	 268 (69%) 119 (31%)	 273 (70%) 114 (30%)
Splenomegaly N (%)	270 (70%)	253 (66%)
Hepatomegaly N (%)	76 (20%)	82 (21%)
Total Dose /kg DHA / Artemether Median mg/kg [Range]	9.8 [4.6-16]	6.6 [4.6-9.2]
Total Dose Lumefantrine or PiperaquineMedian mg/kg [Range]	58.8 [27.7-96]	52.5 [36.9-73.9]

**P. ovale, P. malariae* and negative smears were all regarded as protocol violation.

Table4.3.1b: Differences between sites of enrollment clinic

	SP9 (n=363)	**SP12**(n=411)	
Adults	51% (186/363)	62% (256/411)	P=0.002
Severe Anaemia	11% (38/359)	16% (66/409)	P=0.03
Papuan	56% (204/363)	82% (337/411)	P<0.001

Patients infected with *P. falciparum* or mixed infections were at greater risk of being febrile on admission, having severe anaemia, a palpable spleen or carrying gametocytes, whereas those with pure *vivax* malaria were more likely to be infants (see table4.3.1c)

Table4.3.1c: Baseline differences in patients on admission with differing species of infection

	P. falciparum	***Mixed***	***P. vivax***	
Infants	10% (49/473)	13% (15/114)	19% (33/175)	P=0.02
Severe Anaemia	13% (59/473)	24% (26/110)	11% (19/175)	P=0.004
Fever	35% (167/474)	43% (48/112)	19% (33/176)	P<0.001
Spleen	71% (335/474)	70% (78/112)	57%(101/176)	P=0.005
Gametocytes	22% (105/471)	63% (69/110)	58%(100/174)	P<0.001

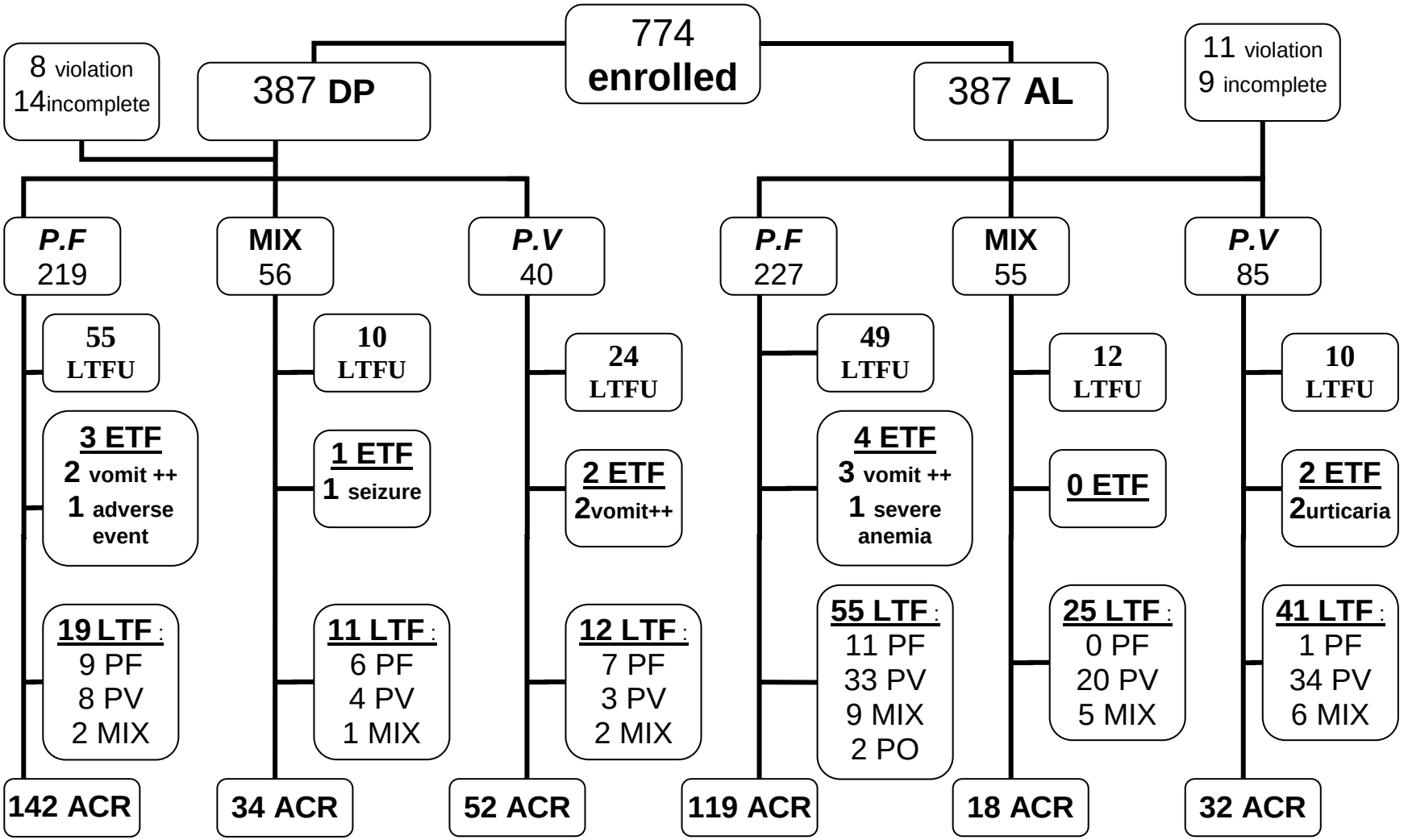


Figure3: The flowchart of patients enrolled in FC study

4.3.2 Drug Tolerability

Both regimens were well tolerated. There was no difference between vomiting within 1 hour rates in AL and DP. On admission 21 patients were vomited medication, 76% (16/21) patients were occurred following the first dose. The rate of vomiting within one hour of any dose was higher in children: 11% (11/99) in infants compared to 3% (7/233) in older children and 0.7% (3/442) in adults ($p<0.001$). Vomiting was also significantly higher in patients on non Papuan ethnic origin 6% (13/233) compared to Papuans (1.5% 8/541, $p=0.003$); this was still apparent after stratifying by age and treatment.

4.3.3 Early Therapeutic Response

The initial responses to the two treatment groups were similar; twelve patients (6 in each group) required early termination of therapy and administration of rescue therapy and were therefore classified as early treatment failures: Seven had repeated vomiting (4 after DP and 3 after AL) and 3 children developed urticarial rashes (2 after AL and 1 after DP). A 5 year old girl presenting with mixed infection and high fever developed a complex partial seizures 30 minutes after administration of DP. She made a rapid recovery but was transferred to hospital for intravenous therapy. She made a full recovery. A 3 year old boy presenting with falciparum malaria was treated with AL, but returned to the clinic the following day having developed warning signs (tachypneoa of 60 per minute, unable to eat with profuse diarrhoea). He had not taken his second dose. He was transferred to hospital and given intravenous fluids and quinine. He made a full recovery.

Following treatment with AL 7% (15/217) of patients with pure falciparum malaria were still parasitaemic on day 2 compared with 1% (2/209) of patients treated with

DP (RR=7.2 [95%CI: 1.7-32], p=0.004). Fever clearance was also slower after AL. After 24 hours 15% (17/111) of those patients who were febrile on admission had yet to resolve their fever compare to 6% (6/101) after DP (RR=2.6 [95%CI: 1.1-6.4], p=0.05). By day 3 all patients in both groups were aparasitaemic and afebrile (see figure 4). Figure 5 shows the percentage of patients with positive slide in both groups. By day two, 18 (5.5%) of 328 patients in the AL recipients and 4 (1.2%) of 335 DP recipients still had a positive blood film (RR=4.6[95%CI: 157-13.44], p=0.004).

Figure4: The percentage of patients with fever in both groups.

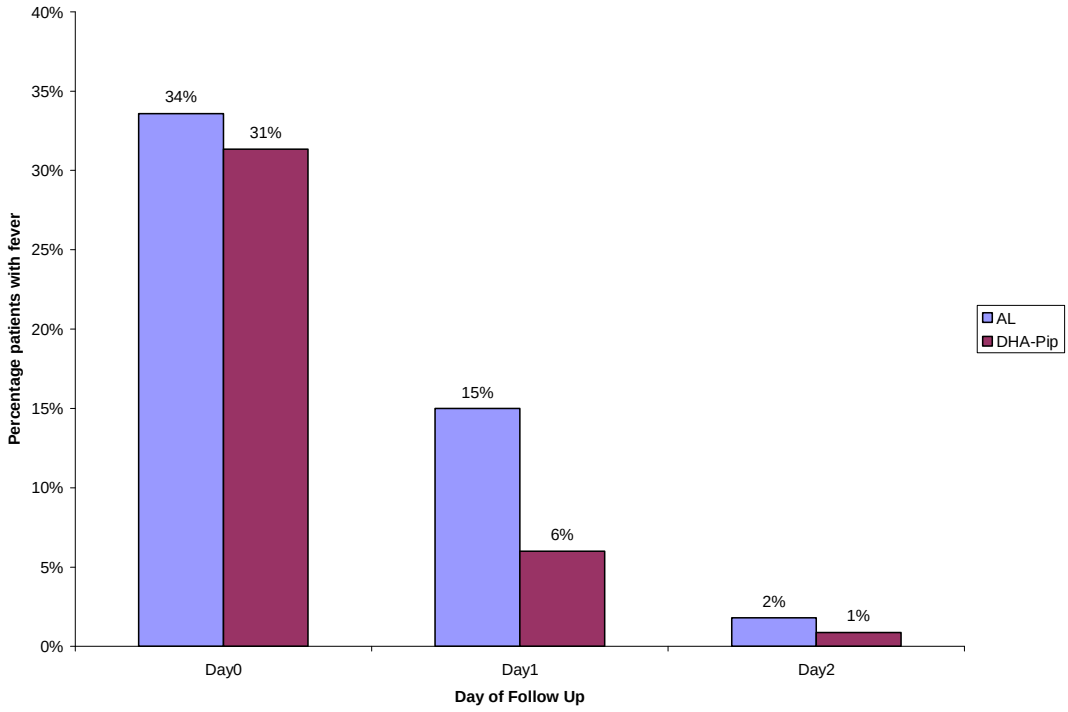
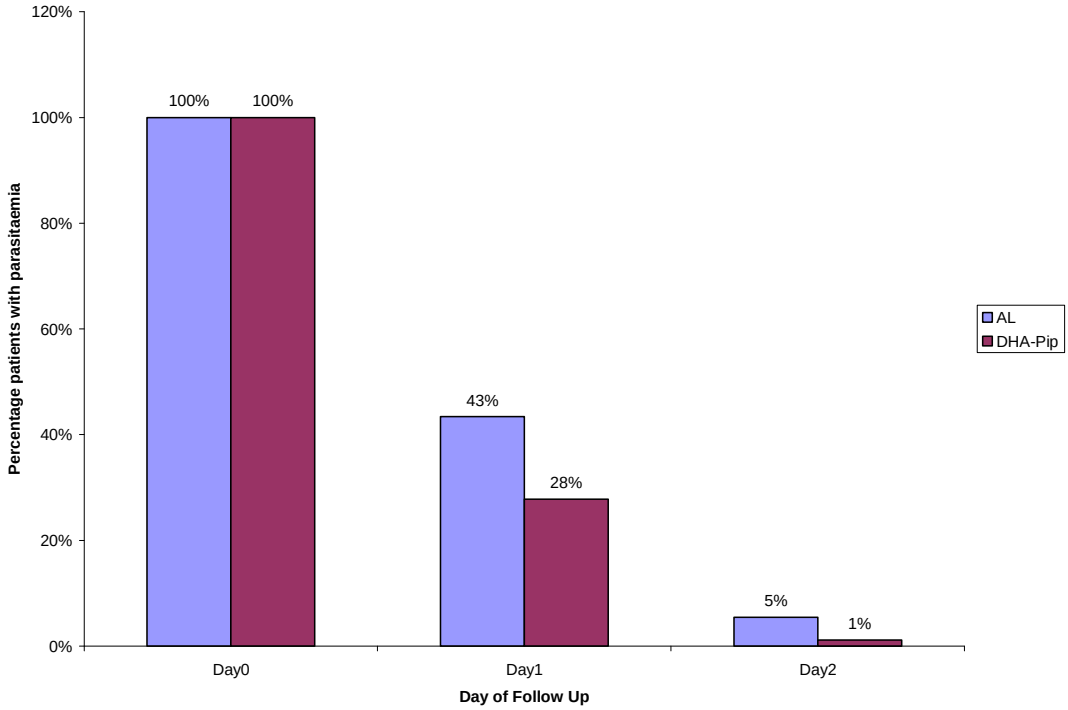


Figure5: The percentage of patients with positive slide in both groups.



4.3.4 Per protocol population

In the late response of per protocol population, *P. falciparum* was noted in 59 patients during follow up (34 monoinfections and 25 mixed with *P. vivax).* In total 64% (37/58) of these infections were symptomatic, 33% (18/54) were anaemic and 25% (15/59) were associated with gametocyte carriage. Moreover, late treatment failures with *P. vivax* were observed in 127 patients during follow up (102 monoinfections and 25 mixed with *P. falciparum).* In total 45% (55/122) of these infections were symptomatic, 35% (33/95) were anaemic and 32% (40/127) were associated with gametocyte carriage.

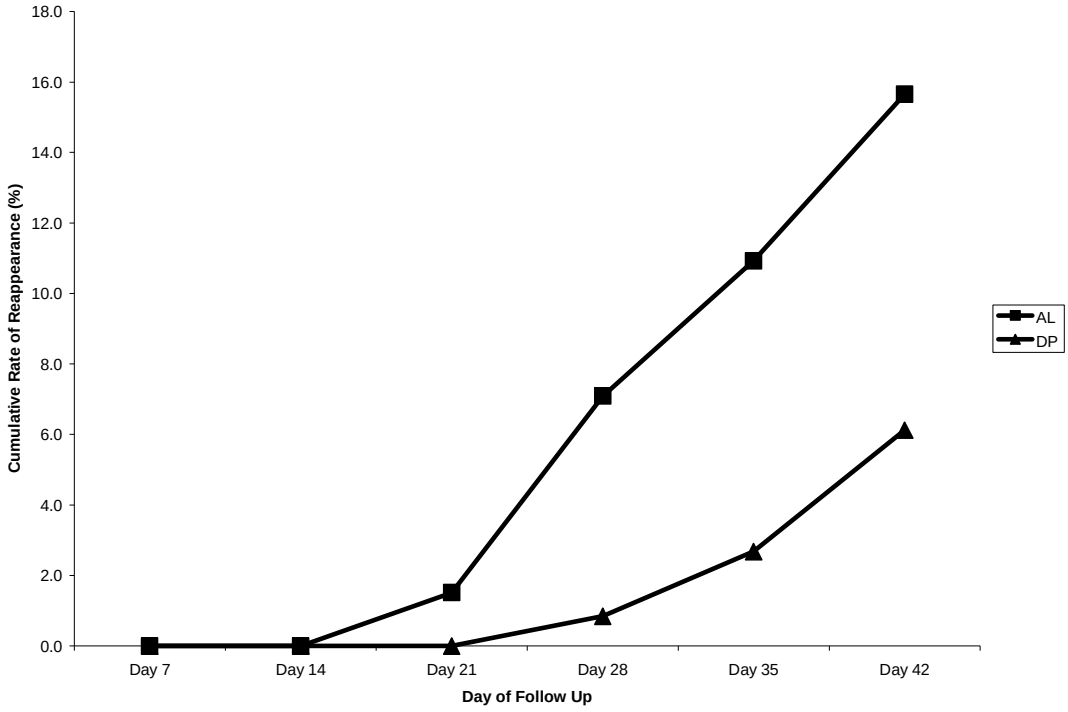
4.3.4.1 Appearance of *P. falciparum*

Overall during the 42-day follow-up period, 14.5% (32/220) reappeared with *P. falciparum* or both mixed with *P. vivax* infections after AL and 10.2% (27/264) after

DP recipients (p=0.19). The median time to failure with *P. falciparum* was significantly longer after DP(median 38 days [Range 21-45]) compared to 34 days [19-43] after AL; p=0.004.

In those patients initially with *P. falciparum* or mixed infections, 16.1% (29/180) reappeared with *P. falciparum* or mixed infections after AL and 10.7% (22/205) after DP (p=0.16). After stratifying by enrollment site there was no difference in the reinfection rate at the Sp9 clinic, but at the SP12 clinic the use of DP was associated with a significantly fewer reinfections than AL (RR=2.56[95%CI:1.06-6.19]), (p=0.02) (figure 6).

Figure 6: Recurrence rate in SP12



In those patients with a recurrence of *P. falciparum*, PCR could be performed on both the pre and post isolate in 67% (33/48) cases (29 new infections and 4 recrudescence infections). The PCR corrected cure rates at day 42 for *P. falciparum* (including early treatment failures) were similar: 96% (144/150) following AL and 97% (182/188) with DP.

Table4.3.4.1a: Day 42 failure rates with initial species *P. falciparum* or mixed infection

	AL	DP	
Day 28	4.7%(11/232)	1.7%(4/233)	P=0.11
Day 42	14.2(25/176)	9.0%(18/201)	P=0.15
Day 42 PCR corrected	1.4%(2/144)	1.1%(2/183)	P=0.7

4.3.4.2 Appearance of *P. vivax*

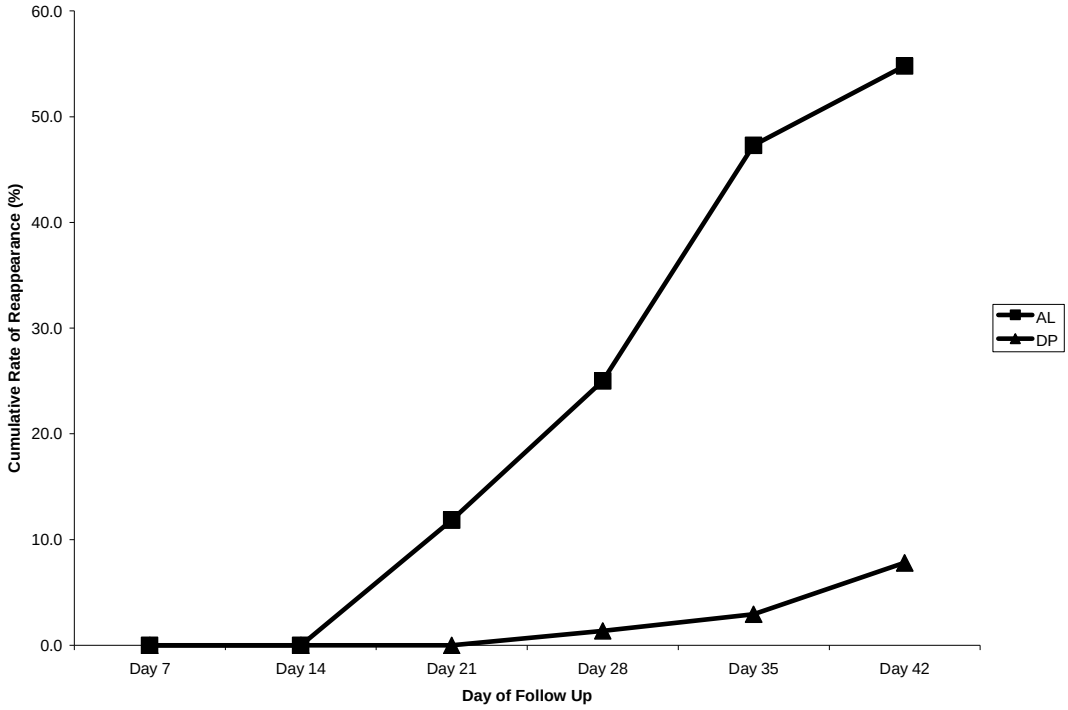
During the 42-day follow-up period, 37.9% (107/282) *P. vivax* infection occurred after AL and 7.8% (20/257) after DP recipients; (RR= 4.88 [95%CI 3.12-7.62], p<0.001). Although recurrence of vivax was significantly more likely to occur if the initial infection was *P. vivax* or mixed 34.7% (75/216), it also occurred in 40.9% (52/323) of pure *P. falciparum* infections. Failure rates were significantly lower following DP irrespective of the initial species of infection (see table 4.3.4.2a). In all cases the difference between treatments was apparent by day 28.

Table4.3.4.2a: Day 42 failure rates of *P. vivax* (pure or mixed)

Initial Infection	Artemether-lumefantrine	DP	Relative Risk	
Pure *P. falciparum*	25%(42/167)	6%(10/156)	3.9[95%CI:2.0-7.6]	P<0.001
Pure *P. vivax*	56%(40/72)	8%(5/60)	5[95%CI:2.4-10.4]	P<0.001
Mixed Infection	58%(25/43)	12%(5/41)	4[95%CI:1.9-8.9]	P<0.001

The median time [range] to reappearance of *P. vivax* was significantly longer following DP therapy (35 days [14-44]) compared to 40 days [28-43] after AL; p<0.001. In total 45% (55/122) of these infections were symptomatic, 35% (33/95) anaemic and 31.5% (40/127) carried gametocytes.

Figure 7: Reappearance *P. vivax* after treatment of pure vivax



4.3.4.3 **WHO Protocol**

To compare the results with other sites elsewhere in Indonesia we did a subgroup analysis applying the standard WHO inclusion criteria for pure *vivax* (parasitaemia greater than 250ul) and pure *falciparum* infections (parasitaemia greater than1000ul). Following treatment of DP was associated with a fewer failures than AL, although numbers in the subset of patients less than 5 years of age were low and did not reach significance (see Table4.3.4.3a).

Table4.3.4.3a: Failure Rates according to WHO Criteria

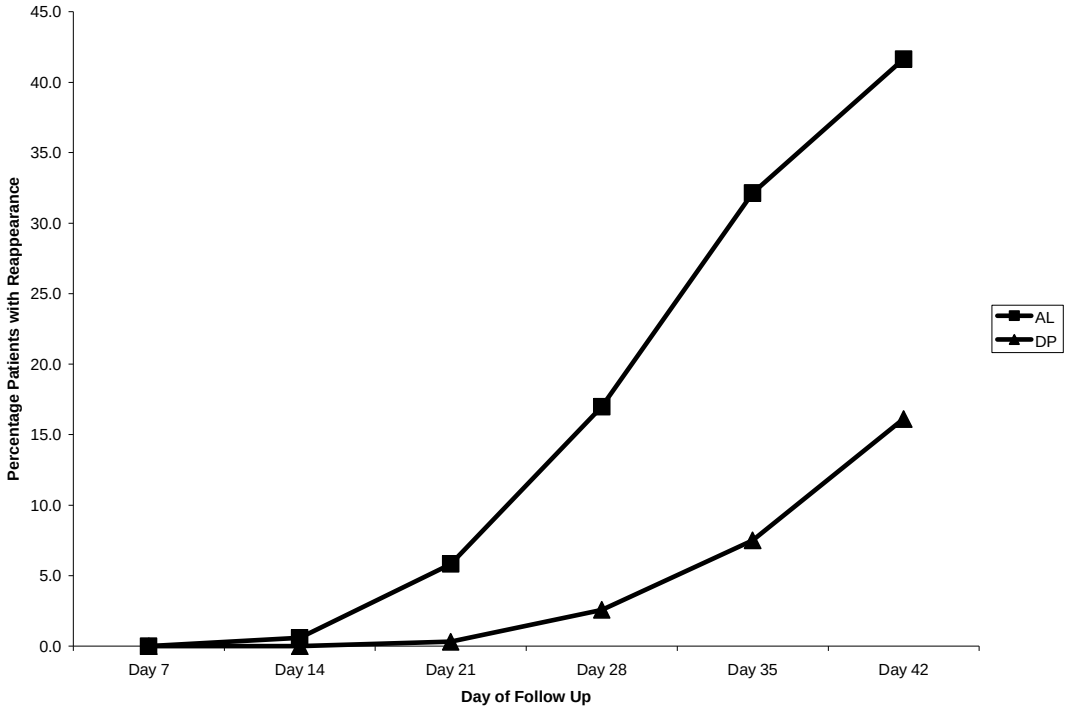
	Artemether-lumefantrine	**Dihydroartemisinin--Piperaquine**	
All Ages			
Initial infection with *Pure Pf*			
Day 28 failure Rate	5% (8/150)	3% (5/153)	NS
Day42 failure Rate*	5% (5/91)	4% (5/122)	NS
Initial infection with *Pure Pv*			
Day 28 failure rate	30% (20/67)	5% (3/63)	P<001
Age < 5yrs			
Initial infection with *Pure Pf*			
Day 28 failure Rate	23% (3/13)	11% (2/18)	NS
Day42 failure Rate*	50% (5/10)	14% (2/14)	NS
Initial infection with *Pure Pv*			
Day28 failure Rate	27% (3/11)	20% (3/15)	NS

*PCR Corrected

4.3.4.4 Modified Intention to Treat (ITT) population

In the modified ITT, by day 42 overall treatment failure was observed in 41.6% (122/293) of patients treated with AL compared to 16.1% (44/273) of those treated with DP (RR=2.58 [95%CI 1.91-3.50], p<0.001).

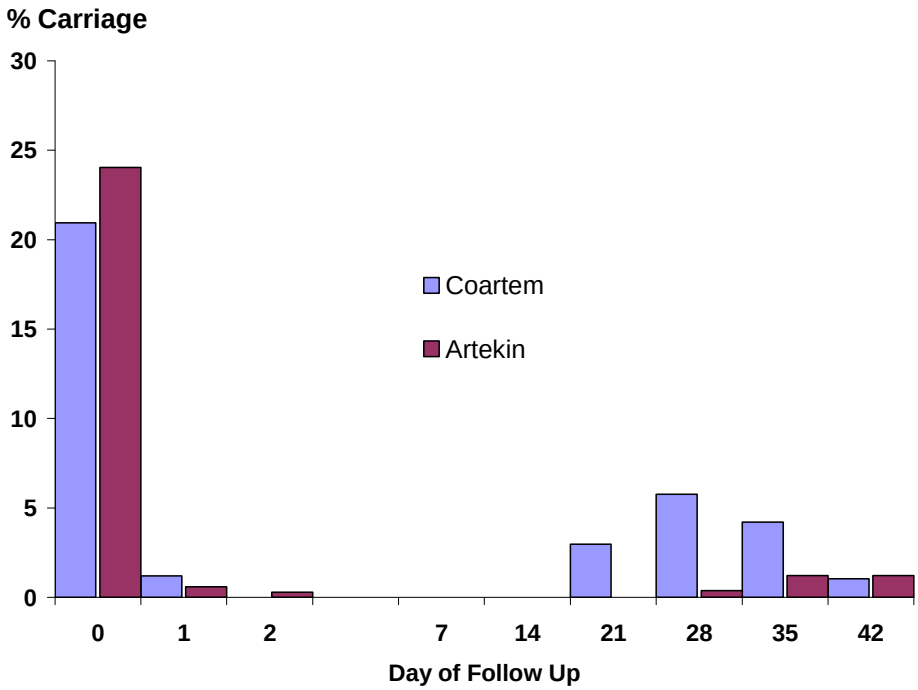
Figure 8: Reappearance of *P. falciparum, P. vivax* and mixed infections after treatment of falciparum and vivax malaria



4.3.5 Gametocyte Carriage Rates

On admission falciparum gametocytes were present in 21% (122/574) of patients with *P. falciparum* / mixed infections compared to 1.7% (3/173) of patients with pure *P. vivax* ($p<0.001$). In those patients without gametocytes on presentation the falciparum gametocyte carriage during follow up was 5.7 per 1000-person weeks with no difference between treatment arms. Vivax gametocytes on admission were present in 56% (160/284) of patients with *P. vivax* infections (alone or mixed) compared to 1% (5/463) of patients with pure *P. falciparum* ($p<0.001$). After day 7, vivax gametocytaemia was always associated with recurrence of *P. vivax* asexual stages, carriage occurring at a rate of 3.7 per 1000-patient weeks after DP compared to 24.6 after AL (RR=6.6 [2.8-16], $p<0.001$).

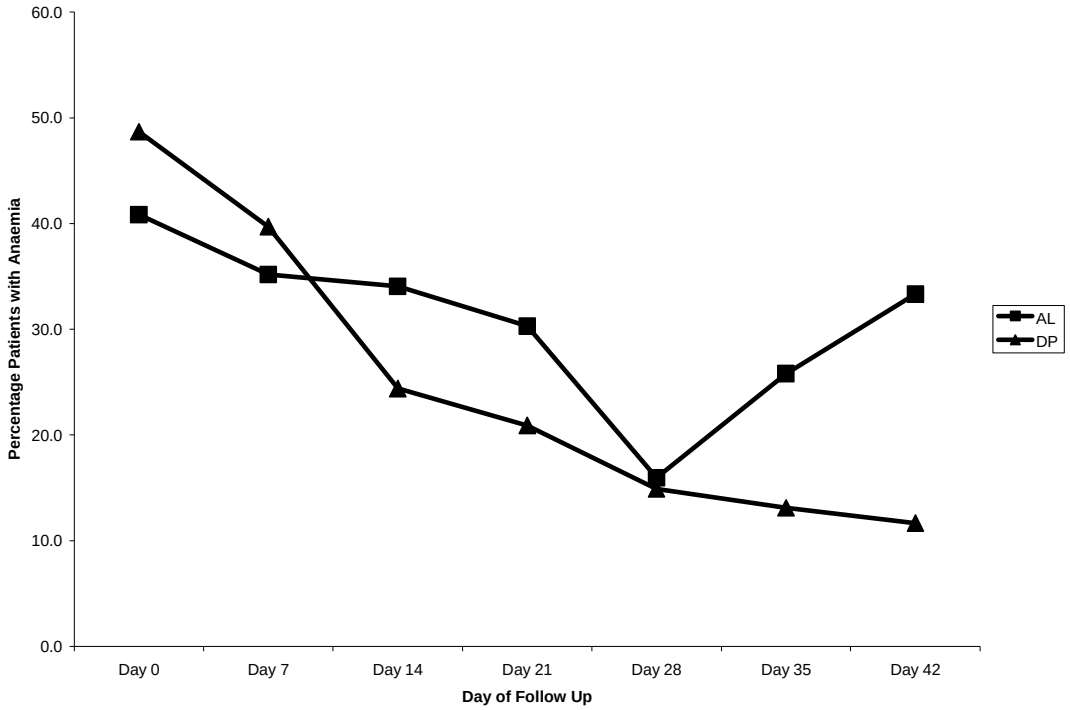
Figure 9: *P. vivax* Gametocyte Carriage Following Treatment



4.3.6 Haematological Recovery

The mean haemoglobin on admission was 9.8 [95%CI: 9.6-10.0], with 51% (374/728) of patients having a haemoglobin less than 10g/dl and in 14% (100/728) of patients less than 7g/dl. Anaemia on admission was equally distributed 50% (182/366) of those with AL and 53% (192/362) in those with DP. After stratifying by the species of the initial infection, there were no significant differences between treatment groups at day0, day 7 or day 28. By day 42 33% (6/18) of patients with adequate clinical response treated with AL were anaemic, compared to 11.6% (7/60) of those who received DP (RR=2.9 [95%CI 1.1-7.4], p=0.03).

Figure 10: Proportion of Patients with Anaemia in those of Adequate Clinical Response



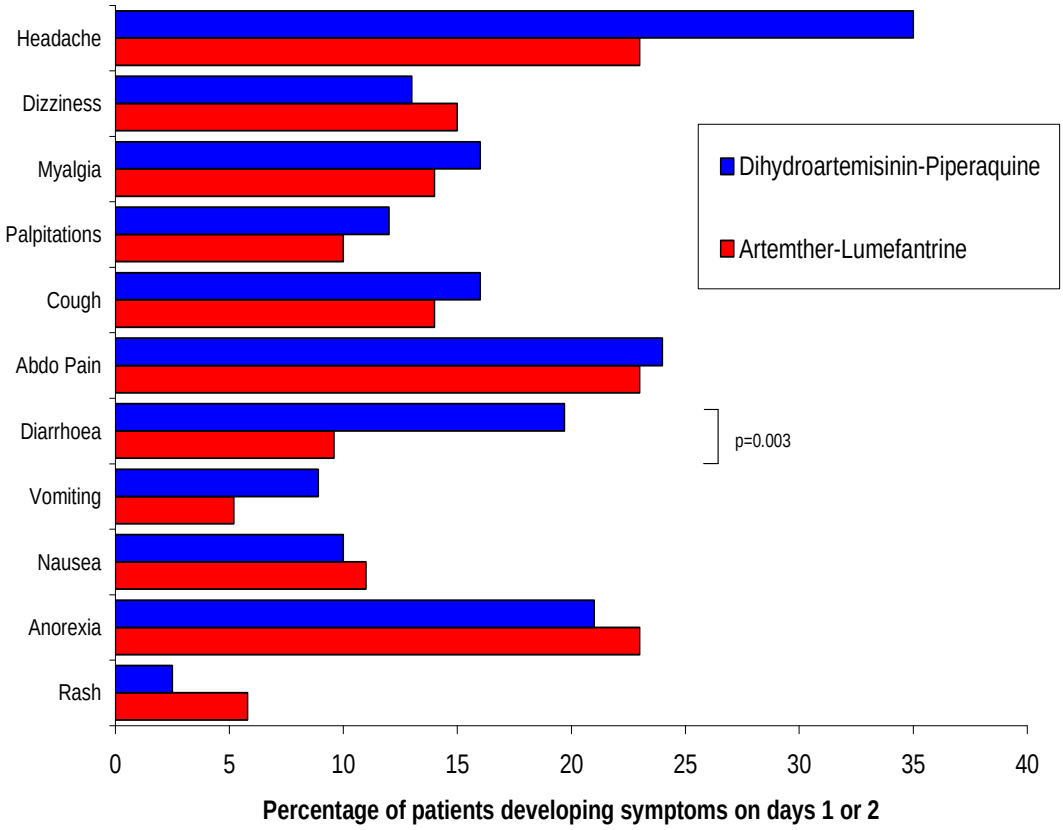
4.3.7 Adverse Side effects

Adverse events possibly related to treatment were defined as symptoms or signs that were not present on admission and that developed after the start of treatment. All adverse events, including those probably related to malaria were recording and compared among treatment groups. On admission patients with falciparum malaria were more likely to be febrile (RR=2.0 [95%CI 1.5-2.8], p<0.00l). Otherwise initial species had no effect on symptomatology on admission or thereafter.

The rates of symptoms on day 1 or 2 which were not present on admission are highlighted in figure 11. The only significant difference between the regimens was in the prevalence of diarrhoea which developed in 21% (50/244) of patients treated with DP and 10% (22/22) after AL (RR=2.1 [1.3-3.4], p=0.001). By day 7 the prevalence of diarrhoea had fallen to 5% (20/395) with no difference between

treatment groups. Although 35% (12/34) of patients developed a headache on days 1-2 after DP compared to 22% (9/40) patients following AL, numbers were small as headache was a common presenting symptom, and the difference did not reach significance. There was no significant difference between regimens in the rates of any of the symptoms questioned in the first week or thereafter.

Figure 11: Development of symptoms on day 1 or 2 in those with out the symptoms on admission



Three patients developed urticaria after AL therapy. In two cases, a 20 year old and a 2 year old, both female with vivax malaria, the reaction occurred after 24 hours and required cessation of the study drug and rescue therapy with quinine. They were regarded as treatment failures. Both had a rapid and complete recovery. The third case was in a 28 year old man with vivax malaria, who developed urticaria on day 2

at the end of therapy. He made a rapid response to antihistamines and was followed for 36 days with no failure recorded.

Adverse event reports, considered possibly or probably drug related were completed on a further three patients. A twenty seven year old man developed severe gastritis and tachypnoea 24 hours after starting DP. He required hospitalization and was given intravenous quinine rescue therapy and made a rapid and full recovery. A three year old boy developed arthritis in his knee 48 hours after starting AL. He was transferred to hospital given antibiotics and made a full recovery. A three year old boy treated with DP developed a new cough on day 1 was treated with amoxyl. The next day he developed a rash. Although it was felt that this was most likely attributable to amoxyl, the study drug was stopped and he was changed to quinine rescue therapy.

4.3.8 Re-treatments

In total, 163 patients had a reappearance of a parasitaemia during the follow-up period. Of the 59 patients representing with *P. falciparum* (alone or mixed), 6 did not come back for retreatment. One three year old boy with pure *P. falciparum* infection represented with danger signs, was transferred to the hospital and treated with intravenous quinine; he made a full recovery. Four patients were reenrolled and successfully treated with AL (2) and DP (2). In total 48 patients were treated with an unsupervised course of quinine ± doxycycline recurrence of a parasitaemia by day 28 was observed in 31 (12 with *P. falciparum*, 11 with *P. vivax* and 8 with mixed infections) giving an overall day 28 cure rate of 35% [95%CI 23-50].

Of the 102 patients with a recurrence of pure *P. vivax*, 9 did not come back for re-treatment. A 6 year old boy previously treated for *P. falciparum* infection represented on day 28 with pure vivax infection and coma (GCS=5). Repeat

microscopy (three times) revealed only *P. vivax* and HRP2 dipstick was negative. No other cause for his coma was found. He was given intravenous quinine and made a full recovery. One patient retreated with AL failed therapy again at day 21. Twenty six patients were retreated with an unsupervised course of quinine ± doxycycline plus primaquine of whom 18 had a recurrent parasitaemia by day 28 observed (1 with *P. falciparum*, 9 with *P. vivax* and 8 with mixed infections) giving an overall day 28 cure rate of 31% [95%CI 17-50]. Of the 65 patients retreated with a supervised course of amodiaquine plus primaquine, 2 patients had recurrent vomiting, 4 patients failed to complete therapy and 18 had a recurrent parasitaemia by day 28 observed (1 with *P. falciparum*, 9 with *P. vivax* and 8 with mixed infections). By day 28 the overall cure rate (any recurrent parasitaemia) was 70% [95%CI 56-84] with an efficacy for *P. vivax* of 74% [95%CI: 60-88].

4.4 Discussion

This is the first study to compare the efficacy of two fixed dose artemisinin combination therapies. Artemether-lumefantrine and DP were both well tolerated, safe and highly effective in treating the multidrug resistant strains of *P. falciparum* prevalent in Papua, Indonesia.

Therapeutic efficacy of Artemeter-Lumefantrine versus DHA-Piperaquine

The patients recruited into the study were mainly adult Papuan males, consistent with these a high risk group from an area meso-endemic for malaria transmission. Although the initial parasite clearance was significantly faster following DP, within 48 hours 97% of patients were aparasitaemic and 98% afebrile. By day 42 PCR corrected cure rates for *P. falciparum* exceeded 98% in both groups. These findings are similar to those reported previously for AL [50, 214] and DP [181, 234, 235].

However when vivax recurrences and falciparum reinfections were included, the overall parasitological failure rate by day 42, the a priori primary endpoint of this study, was significantly higher after AL 41.6% (122/293) compared to that after DP 16.1% (44/273); RR=2.58 [95%CI 1.91-3.50] p<0.001. Determining the actual cure rate of *P. vivax* is challenging since recurrence of malaria can be due to recrudescence from the same isolate, reinfection with a new isolate or relapse from hypnozoite stages [262]. The relapsing isolate can be from the same strain or a different strain, making the interpretation of genotypic data difficult. In this area the incidence of *P. vivax* infection suggests that the reinfection rate would be approximately 0.8% per week. We did not genotype *P. vivax* isolates, but if one assumes that vivax cure rates were as high as those for P. falciparum, then by day 42 one would expect approximately 2% recrudescences, less than 5% reinfections with the remainder accounted for by relapses.

The discrepancy in failure rates between DP and AL is likely to be a consequence of the wide difference between the terminal *elimination* half life of lumefantrine (~4 days) [49] and that of piperaquine (28-35 days) [64, 263]. Similar differences in the overall failure rates have been reported previously in comparative studies of AL and mefloquine plus artesunate (a combination with a similarly long terminal elimination half life as DP) [50].

Artemisinin combination therapies achieve their antimalarial effect through an initial rapid reduction in parasite biomass attributable to the short-acting but highly potent artemisinin derivative (artemether in AL and dihydroartemisinin in DP), with the subsequent removal of the remaining parasites by the intrinsically less active but more slowly eliminated lumefantrine (AL) or piperaquine (DP). Overall cure rates depend upon there being sufficient partner drug to remove the residual parasite

biomass left by the artemisinin derivative. After eradication of the asexual stages of the parasite from the peripheral blood, patients who remain in an endemic area are at risk of reinfection. In areas where *P. vivax* is endemic, patients are also at risk of relapse from the liver stage hypnozoites, even if they leave the transmission area. In Papua and equatorial regions *P vivax* relapses in up to 60% of patients, the first relapse occurring at approximately 21 days [264]. Slowly eliminated antimalarial drugs will exert a greater post treatment prophylactic effect than those more rapidly eliminated and this becomes apparent in both the rates of reinfection and relapse. The greater the risk in reinfection or relapse the more apparent this prophylactic effect is likely to become.

In the present study the first vivax recurrence occurred after 17 days after AL and by day 42 37% of patients had relapsed. In contrast the first relapse after DP didn't occur until day 29. The delay in *P. falciparum* reinfection was also significant but more modest: a median delay of 4 days. We were unable to follow patients any longer than 6 weeks, but if we had done so it is likely that the survival curves would have eventually merged as the plasma concentrations of piperaquine fell. What therefore is the benefit of a transient post treatment prophylaxis?

Malaria infections are associated with haemolysis and suppression of haemopoeisis recovery from which takes 4-6 weeks. Patients failing therapy take significantly longer for their haemoglobin to return to normal [257]. In the present study there was a steady recovery in the mean haemoglobin, although as the reinfections and relapses began to occur in the AL group, a difference emerged between the treatment groups so that by the end of the study those treated with DP had significantly higher haemoglobin levels and were 2.1 fold less likely to be anaemic compared to those treated with AL.

Carriage of *P. falciparum* gametocytes in both groups was low with no difference in *P. falciparum* gametocyte carriage between ACTs. In contrast the gametocyte carriage of *P. vivax* differed considerably. Sexual stages of *P. vivax* occur at or before the time of the onset of clinical symptoms, unlike those of *P. falciparum* which more commonly occur later. In our study 56% of patients with pure *P. vivax* infections had gametocytes on presentation. Unlike *P. falciparum*, *P. vivax* gametocytes retain sensitivity to most schizonticidal agents and by day 4 no patients had a patent gametocytaemia. Reappearance of *P. vivax* gametocytes during recovery was only present in those patients with a recurrence of asexual stages of *P. vivax*. After 42 days the lower rate of vivax recurrence following DP was associated with a 6.6 fold lower rate of gametocyte carriage.

Hence the delay in relapse and reinfections conferred by DP not only gave people a longer period free from symptomatic malaria it also allowed greater time for haematological recovery and, in the case of *P. vivax*, significantly reduced the transmission potential to the mosquito vector. Although the public health implications of these observations need to be confirmed in studies with longer follow up and with repeated exposure, our observations suggest that further benefits are likely to accrue with repeated treatments and that the benefit will be more apparent in patients at greatest risk of reinfection and vivax relapse.

The major concern with deploying long half life antimalarial drugs and combinations with pharmacokinetic mismatching is that there will be a greater risk of selecting drug resistant isolates [265]. Indeed the long subtherapeutic tail of the antifolate sulfadoxine-pyrimethamine has been directly implicated in the rapid spread of resistance to this agent throughout Asia and Africa [266]. The emergence and spread of resistant parasites is determined by two major components: selection de novo and

selective transmission. The former has been argued to be a function of biomass and therefore more likely to occur in the initial infection rather than the reinfecting strains [92]. In vivo the rapid reduction of an infecting biomass with an artemisinin derivative reduces the number of asexual parasites exposed to the second drug and in doing so will reduce the chances of a resistant mutant emerging during treatment [92].

If DP is deployed in an area prior to the emergence of piperaquine resistance and if the artemisinin component can delay significantly the emergence of de novo resistance then the real benefits accrued by the long acting combination argue in favor of DP. Although piperaquine has not been previously used in Papua and in vitro studies have failed to demonstrate any prior evidence of tolerant parasites (unpublished), resistance to piperaquine has been observed after 20 years of wide spread use as monotherapy in China [267]. Once resistance strains emerge in an area, selective transmission in the prolonged sub-therapeutic tail of DP is likely to fuel the spread of resistance and herald the demise of the regimen. Careful monitoring of in vivo and in vitro antimalarial efficacy must remain a priority therefore. Reassuringly despite the considerable mismatching of mefloquine and artesunate the widespread deployment of this combination in Thailand has been associated with more than 15 years of excellent efficacy [150].

We only supervised the first daily dose of AL and it is possible that some of the difference in efficacy may be related to a lack of patient adherence to a complete course of therapy. However patients on the whole confirmed that they had completed therapy, and this is supported by the very low recrudescent rates observed for *P. falciparum*. These findings support the observations that when administered

with clear instructions unsupervised AL can still retain good cure rates [214] and suggests that adherence in our study population was good.

Neither of AL or DP have any efficacy on the hypnozoite stages of *P. vivax*. The only registered antimalarial agent with activity against hypnozoites is the 8-aminoquinoline primaquine. It is possible that early administration of primaquine would have prevented the high rates of vivax relapse that we observed. However radical cure of *P. vivax* in this area requires 30mg primaquine daily over 14 days [268, 269] and in practice such long courses of unsupervised therapy are adhered to rarely. In a subsequent clinical study in which early administration of unsupervised primaquine immediately after DP failed to decrease the recurrence rate of vivax (unpublished data). Hence in this area although the post treatment prophylaxis afforded by DP will not prevent subsequent relapses it will provide the only practical means currently available of delaying the timing of these relapses. Alternative strategies to deal with the hypnozoite stage are needed as a research priority but as yet are not forthcoming.

Although the emergence of chloroquine resistant strains of *P. vivax* have been well documented, few studies have identified suitable treatment regimens for endemic communities [143] and only study has determined the efficacy of an ACT [130]. We describe one the first accounts of the use of amodiaquine for chloroquine resistant *P. vivax* recurring from patients after AL or DP treatment. There were no high grade failures in any of the 65 patients treated and the cure rate at day 28 was 74% [95%CI: 60-88], significantly better than the 25% [95%CI: 9-53] observed previously following treatment with amodiaquine monotherapy (chapter3). However in the current study patients were detected actively and generally had low levels of

peripheral parasitaemia; hence these figures are likely to represent the upper limit of efficacy. Therefore the use of monotherapy amodiaquine can not be advocated.

Unsupervised courses of quinine with or without doxycycline were associated with recurrence rates in excess of 42% (20/48) following *P. falciparum* and 17% (19/48) for *P. vivax* infections. These high failure rates are likely to arise from poor adherence to a complicated regimen and side effects profile, rather than resistance. In practice this highlights that quinine based regimens alone or in combination are unlikely to provide a useful long term strategy for uncomplicated malaria.

4.5 Conclusions

DP and AL are safe and highly effective for the treatment of multidrug resistant uncomplicated malaria. However DP was demonstrated to be a well tolerated, safe and effective treatment for multidrug resistant *P. falciparum* and *P. vivax.* In this region alternative treatment strategies are limited, but DP's simple three day regimen, low price (~ $1.4 per adult treatment), faster clinical response and post treatment prophylactic effect offer significant benefits over artemether-lumefantrine, the only other fixed dosed ACT currently available. Although priority should be given to ensuring low recrudescence rates the clinical relevance of reinfections and relapses should not be ignored. Further longitudinal studies are needed to quantify more precisely the clinical implications of repeated exposure, but in the meantime DP has become the treatment of choice in southern Papua, Indonesia.

5 Discussion

In Timika (Papua, Indonesia), malaria poses a significant public health risk. There are currently more than 1000 cases of uncomplicated malaria per week (divided 60:40 between *P. falciparum* and *P. vivax*) despite an extensive vector control program. Current local protocols advocate the use of combination chloroquine plus SP for falciparum malaria and chloroquine alone for vivax malaria, ovale malaria and malariae malaria. There is a need to assess the therapeutic efficacy of existing antimalarial drugs based on parasitological and clinical responses[156] to act as a baseline data for comparison with future studies. The first clinical study in this thesis evaluated the efficacy of chloroquine ± sulfadoxine-pyrimethamine for all four malaria species causing uncomplicated malaria in Timika. The second study was aimed at identifying the most suitable combination therapy for the district

In the first study we have demonstrated that the level of antimalarial drug resistance for *P. falciparum* and *P. vivax* has reached unacceptable levels in southern Papua, Indonesia. The day 42 failure rate of CQ+SP after correcting for reinfections for falciparum malaria was 73% [95% CI: 60-83] compared to a day 28 cure rate for *P. vivax* of 73% [95% CI: 56-86]. In both cases the recurrent infection occurred in the presence of plasma chloroquine concentrations above the predicted minimum effective concentrations, suggesting the occurrence of true parasite resistance rather than poor drug absorption. Retreatment with unsupervised quinine ± doxycycline resulted in further recurrence of malaria in 48% [95%CI: 31-65] of *P. falciparum* infections and 70% [95%CI: 37-100] of *P. vivax* infections. These findings are similar to those previously reported of chloroquine or SP studies for falciparum malaria [116, 245, 264].

The consequences of such high levels of drug resistance are likely to be appreciable. As the results are more malaria, hospital cases, lots of anaemia, economic cost and decrease the productivity of people. Although we haven't presented these epidemiological studies in this thesis other associated work are addressing the burden of disease.

In view of the poor efficacy of existing therapies we then undertook a large comparative study to determine suitable alternative strategies. Since 2004, the standard of treatment guideline in Indonesia used the combination of artesunate with amodiaquine for uncomplicated malaria. Even though, this is the cheapest ACT, but it is not a fixed combination regimen and this can decrease the adherence of patients taking the medication. In addition, the possibility of cross resistance of this combination in areas with high resistance of chloroquine may compromise this regimen and lead to the rapid emergence of resistance to the regimen. In the northern part of the province a recent study suggested that amodiaquine efficacy against *P. falciparum* was already compromised such that of the addition of artesunate may add little to improve cure rates [270]. To investigate alternative ACTs we investigated two fixed combination artemisinin combinations to determine the safety and efficacy of artemether-lumefantrine (AL) and dihydroartemisinin-piperaquin (DP). Both regimens were safe and effective treatment for drug resistance *P. falciparum* with a cure rate greater than 95% after PCR corrected.

The partner drug (lumefantrine in AL and piperaquine in DP) is act to remove the residual parasite biomass left by the artemisinin derivative. Piperaquine is a slower eliminated antimalarial drug than lumefantrine. In the present study the first *P. vivax* recurrence occurred earlier following AL. In contrast the first relapse following DP did not occur until day 29. The delay in *P. falciparum* reinfection was also

significant, with a median delay of 4 days. Later, as a result of the difference emerged between the treatment-groups were DP 2.1 fold less likely to be anaemic to those treated with AL. It is associated with that patients failing therapy take significantly longer for recovery their haemoglobin [257]. More over, after 42 days the lower rate of vivax recurrence after DP was associated with 6.6 fold lower rate of gametocyte carriage. DP had more significantly diarrhoea with 2.0 fold an increased risk on day 1 and 2, however this is transient and by day 7 the rate of diarrhoea was 5% in both treatment arms. This suggests that further benefits are likely to accrue with repeated treatments and that benefit will be more apparent in patients at greatest risk of reinfection and vivax relapse.

Recently the standard treatment guideline in Indonesia has changed to recommend the combination of artesunate with amodiaquine for uncomplicated malaria. Between 2003 and 2005, several efficacy studies of combination artesunate-amodiaquine in uncomplicated falciparum malaria showed the cure rate by day 28 ranging from 80% to 90%[11]. A recent comparative study of 3 days artesunate-amodiaquine versus 2 days DP, in uncomplicated malaria in Bangka Island, Indonesia showed similar PCR corrected day 28 cure rates: 94% and 97% respectively (p=0.488) (unpublished). However a comparative study of 3 days artesunate-amodiaquine versus 3 days DP in uncomplicated malaria in Timika showed that the day 42 cure rate was significantly better after DP than Artesunate –amodiaquine : 95% and 80% respectively ($p<0.001$) (unpublished). Other ACTs are also being tested. A phase 2 study multicentre conducted to determine the clinical effective dose, safety and efficacy of orally administered fixed dose artesunate-pyronaridine in north Sulawesi is in process.

In summary, the existing standard antimalarial drugs should be replaced by artemisinin combination therapy for uncomplicated malaria in Timika. Of the two

fixed combination artemisinin derivatives, artekin may be used for replacing the standard regimen for uncomplicated malaria in Timika.

Since 1st March 2006 DP has become the treatment of choice for uncomplicated malaria in southern Papua, Indonesia. As a guideline to the health care practice in Timika, NIHRD made a “handbook”; to optimize the use of combination artemisinin derivative (DP). The change of policy to DP in Timika will be observed carefully to quantify more precisely the clinical impact and the cost effectiveness of widespread deployment of DP. It is hoped that the results of this study will provide information that will help to inform policy change elsewhere in Indonesia.

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Appendix 1 ***Publications***

ABSTRACTS

H Siswantoro, A Ratcliff , E Kanangalem, P Ebsworth, Jeanne Rini, M Edstein, N Anstey, E Tjitra, RN Price. **High Prevalence of multidrug resistant *P. falciparum* in Papua, Indonesia.** Marseille meeting 11-15 September 2005, France.

A Ratcliff, **H Siswantoro**, E Kanangalem, R Rumaseuw , EP Ebsworth, MD Edstein, N Anstey, E Titra, RN Price . **High grade chloroquine resistant *Plasmodium vivax* in Papua, Indonesia: potential for amodiaquine as salvage therapy.** Marseille meeting 11-15 September 2005, France.

A Ratcliff, **H Siswantoro**, R Rumaseuw, E Kenangalem, EP Ebsworth, NM Anstey, E Tjitra, RN Price. **Coartemether versus artekin for drug resistant *P. falciparum* and *P. vivax* in Papua, Indonesia.** Marseille meeting 11-15 September 2005, France.

PAPERS

A Ratcliff, **H Siswantoro**, E Kenangalem, M Wuwung, R Rumaseuw, A Brockman, M Edstein, F Laihad, P Ebsworth, NM Anstey, E Tjitra, RN Price. **Multi-drug resistant *P. falciparum* and *P. vivax* in southern Papua, Indonesia**. Transactions of the Royal Society of *Tropical Medicine and Hygiene – in press 2006*.

H Siswantoro, A Ratcliff, E Kenangalem, M Wuwung, A Brockman, R Rumaseuw, M Edstein, P Ebsworth, NM Anstey, RN Price, E Tjitra. **Efficacy of existing antimalarial drugs for uncomplicated malaria in Timika, Papua, Indonesia**. *Medical Journal of Indonesia*: Submitted.

H Siswantoro, A Ratcliff, E Kenangalem, M Wuwung, R Rumaseuw, A Brockman, F Laihad, P Ebsworth, NM Anstey, E Tjitra, RN Price. **Artemether-lumefantrine versus dihydroartemisinin-piperaquine for multi-drug resistant *P. falciparum* and *P. vivax* in Papua, Indonesia**: *Lancet*: Submitted

Appendix 2 ***Signs or symptoms indicative of severe malaria***

Signs or Symptoms Indicative of Severe Malaria

- Impaired Conscious Level (Blantyre Coma Score <5, GCS<15)
- Severe Anaemia (Hct<15%)
- Bleeding Disorder (Epistaxis, bleeding gums, frank haematuria…)
- Respiratory Distress (Deep Breathing RR>30)
- Jaundice
- Shock – circulatory collapse with BPs<80mmHg

Warning Symptoms

- Not able To Drink/Eat
- Vomiting Excessively
- Recent History Of Convulsions
- Altered Mental State
- Unable To Sit/Stand Up

Appendix 3 ***Information Sheet***

A randomized trial to determine the efficacy and safety of coartemether and artekin for the treatment of acute falciparum and vivax malaria in Timika, Papua.

Malaria can be a serious disease if we don't treat it quickly and effectively. In previous studies in Timika we found that chloroquine and Fansidar sometimes work but are no longer very good treatments. Studies in other countries have shown that there are several new drugs are very effective even against the worse strains of malaria. They work very fast and have few known side effects. We are conducting a study in this Puskesmas to show whether these new drugs work well against malaria in Papua, so that we can introduce better treatments for the Timika people.

In order to show that these new drugs are as good or even better than the present drugs we will give you/your child either the old treatment (chloroquine + Fansidar) or one of two new treatments (coartemether or artekin). To find out which is the best treatment we will ask you questions about your symptoms and examine you. We will also see you every week for 6 weeks, to see whether your malaria returns.

If you agree to be in the study the following procedures will happen:

1. You will be asked a questionnaire about your symptoms and have an examination by a doctor.

2. You will then be given antimalarial tablets and asked to wait one hour to make sure you don't vomit your medication.

3. You will then be asked to return to the clinic each day until you are feeling better. At each visit we will ask questions to see whether you are getting better, we will also prick your finger to see whether the malaria parasites in your blood are decreasing.

4. A nurse will give your medication each day to be taken in front of her/him. Some patients (but not all) will have tablets to be taken at home 12 hours later. It is important that you/your child take all the tablets prescribed.

5. After the first week you will be asked to return to the clinic every week for 42 days to see whether your treatment has been successful.

6. If your symptoms or fever returns you should come as soon as possible to the Puskesmas for a blood test. If your/your child's malaria returns we will treat you/your child with another drug which is standard treatment at this Puskesmas.

7. If you/your child agrees we would also like to take a blood sample from your arm now and then again in 7 days time and 28 days time. This will be used to test the sensitivity of the malaria parasite infecting you, test your immunity to infection and look at the amount of drug in your body.

The potential benefit of being part of this study is to give you / your child a good treatment against malaria and help to introduce the best treatment for everybody in the Timika region. You will not be charged any money for your malaria treatment or to be seen on the follow up visits. You will be reimbursed for costs of transportation back to the clinic.

If you do not wish to participate in this study it will NOT affect your right to receive standard health care administered at this clinic.

Any time you can withdraw you/your child from the study and still receive the treatment as you would normally.

If you have any questions about this study, you may contact Dr Emiliana Tjitra or the study doctors in this clinic.

In case of an emergency you should return to this clinic or if it is after hours present yourself to the Rumah Sakit Mitra Masyarakat Hospital Emergency Room and inform the doctor that you have been a participant in this study.

If you have a complaint about the study then these should be addressed to Dr Liliana Kurniawan, the Chairperson of the Ministry of Health Ethics Committee, Jakarta. Telephone Number: 021 – 425 9860.

Appendix 4 ***Consent Form for Adults***

A randomized trial to determine the efficacy and safety of coartemether and artekin for the treatment of acute falciparum and vivax malaria in Timika, Papua.

I …… agree to participate in the above study.

I DO / DO NOT * agree to having a venous blood sample taken at enrollment, day7 and day 28 .

(* Delete as applicable)

I am aware that I can withdraw consent at any time of my own choosing.

Signature of Patient ……

Date

……

Witness Name

……

Signature

……

Appendix 5 ***Consent Form for Children***

A randomized trial to determine the efficacy and safety of coartemether and artekin for the treatment of acute falciparum and vivax malaria in Timika, Papua.

I agree as the PARENT / LEGAL GUARDIAN of ……..……. that he / she can be enrolled in the above study.

I DO / DO NOT * agree to him / her having a venous blood sample taken at enrollment, day7 and day 28 (* Delete as applicable).

I am aware that I can withdraw our consent at any time of my own choosing.

Signature of Parent ……

Date

……..……

Witness Name

……..……

Signature

……..……

Appendix 6 ***Data sheet used in the clinical study***

CATATAN MEDIK PENDERITA MALARIA

Puskesmas: _____ **Tanggal:** _ _ / _ _ / _ _
(Health Center) (Date: Day / Month/ Year)
Nama {**pertama**}: **_____** **Nama** {**keluarga**}:**_____**
(First name) (Family name)
Nama {**ortu/wali**}: **_____**
(Parent/Guardian's name)

Tanggal kelahiran: _ _ / _ _ / _ _ **Umur:** _ _ th _ _ bl
(Date of birth: Day / Month/ Year) (Age) Year Month
Seks : L / P **BB (kg)**::**_____**
(Sex M/F) (Weight, kg)
Suku:_____
(Origin)

Alamat:_____
(Address)
BAGIAN PENGOBATAN: ***Falciparum / Vivax***
(Treatment Arm)

KELOMPOK PENGOBATAN:
(Treatment Group)

Primary / Failure

Chloroquine	**Quinine**
SP	**Doxycycline**
Primaquine	**Amodiaquine**

Termasuk didalam kriteria (Inclusion Criteria)
Berat Badan > 10kg ……**Ya / Tidak**
(Weight >10kg)
Umur > 1 years …… **Ya / Tidak**
(Age > 1years)
Panas atau riwayat panas dalam 24 jam terakhir… **Ya / Tidak**
(Fever / Hx of Fever)
Parasitemia *Pf* atau *Pv* …….. ……. **Ya / Tidak**
(Any Pf or Pv)
Dapat memenuhi protokol…… **Ya / Tidak**
(Able to comply)
Menandatangani surat persetujuan…… **Ya / Tidak**
(Sign informed Consent)

Yang Tidak termasuk (dalam criteria) (Exclusion Criteria)
Hamil atau menyusui…… **Ya / Tidak**
(Pregnancy or Lactation)

Tak dapat minum obat…… **Ya / Tidak**
(Unable to take Oral)

Tanda-tanda malaria berat ……**Ya / Tidak**
(Signs of severity/ danger)

Alergi terhadap obat……**Ya / Tidak**
(Drug Allergy)

Penyakit utama lainnya (jantung, ginjal, hati)…… **Ya / Tidak**
(Underlying disease)

Parasitemia >4% ……**Ya / Tidak**
(Parasitaemia > 4%)

Pernah berpergian ke kecamatan lain { kecamatan} : **Ya / Tidak**
Visited any other subdistrict within last 14 days

Bila Ya, ke:_____
(If yes, to:)

Bila Ya, ke:_____
(If yes, to:)

Bila Ya, ke:_____
(If yes, to:)
Gejala sakit malaria terakhir:
Recent Episodes of malaria

Berapa kali dalam 1 bulan terakhir: _____
(How many in last month)

Gejala terakhir (berapa hari yang lalu):_____
(Last episode ; days ago)

Riwayat minum obat antimalaria dalam 4 mg terakhir: Ya / Tidak
(History of taking antimalarial drugs in the last 4 weeks?

Bila Ya,: 1=Klorokuin / 2=Primakuin / 3=Sulfadoksin-Pirimetamin / 4=Kina

Dimana Saudara biasanya dirawat? _____
Where were you treated?

Penelitian terdahulu / Kode: _____
Previous Study / Code

Pemeriksaan Fisik
(Examination on Admission)

Suhu aksila (^{0}C): _ _. _ ℃
(Axillary temperature, ^{0}C)

Nadi/menit: _____ **Pernafasan/menit:** _____
(Pulse/min) (Respiratory rate/min)

Hepatomegali (cm) _____
(Hepatomegaly, cm)

Splenomegali (H):_____
(Splenomegaly, Hackett)

Tanda bahaya: _____
Danger signs:
0= no danger sign/ 1=Not able To Drink/Eat/2=Vomiting Excessively/ 3=Recent History Of Convulsions/ 4=Lethargic/Unconscious State / 5=Unable To Sit/Stand Up

Catatan:
(Comments:)

TANGGAPAN TERHADAP PENGOBATAN AWAL

(Early Therapeutic Response)

	H0 (Day0)	H1 (Day 1)	H2 (Day 2)	H3 (Day 3)	H4 (Day 4)	H5 (Day 5)
DATE						
Suhu aksila (^{0}C) (Axillary temperature ^{0}C)						
Hepatomegali (cm) (Hepatomegaly)						
Splenomegali (cm) (Spenomegali cm)						
Riwayat panas dlm 24 j akhir (History of fever in the last 24 hours)						
Tidak sehat (Unwell)						
Tanda bahaya * (Danger signs):						
Sakit kepala (Headache)						
Pegal linu / nyeri sendi (Myalgia / Arthralgia)						
Mual (Nausea)						
Muntah (Vomiting)						
Sakit perut (Abdominal pain)						
Anoreksia (Anorexia)						
Mencret (Diarrrhoea)						
Pusing (Dizzy)						
Gangguan tidur (Sleep disturbance)						
Palpitasi/berdebar (Palpitations)						
Kelainan perilaku/aneh (Strange Behaviour)						

Tanda-tanda bahaya:

0= no danger sign / 1=Not able To Drink/Eat / 2=Vomiting Excessively

3=Recent History Of Convulsions / 4=Lethargic/Unconscious State / 5=Unable To Sit/Stand Up

TANGGAPAN TERHADAP PENGOBATAN LAMBAT

(Delayed Therapeutic Response)

	H7 (Day 7)	H14 (Day 14)	H21 (Day 21)	H28 (Day 28)	H35 (Day 35)	H42 (Day 42)
DATE						
Suhu aksila (^{0}C) (Axillary temperature ^{0}C)						
Hepatomegali (cm) (Hepatomegaly)						
Splenomegali (cm) (Spenomegali cm)						
Riwayat panas dlm 24 j akhir (History of fever in the last 24 hours)						
Tidak sehat (Unwell)						
Sakit kepala (Headache)						
Pegal linu / nyeri sendi (Myalgia / Arthralgia)						
Mual (Nausea)						
Muntah (Vomiting)						
Sakit {perut} (Abdominal pain)						
Anoreksia (Anorexia)						
Mencret (Diarrrhoea)						
Pusing (Dizzy)						
Gangguan tidur (Sleep disturbance)						
Palpitasi/berdebar (Palpitations)						
Gangguan perilaku/aneh (Strange Behaviour)						

Efek – efek yang lainnya: Termasuk hari terjadinya: Selama berapa lama: Seberapa berat:

(Other adverse effects: Include Date / Duration / Severity)

KELOMPOK PENGOBATAN:
(Treatment Group)

CHLOROQUINE (VP Study)

Dosis Dose	**Tgl/Jam** Date/Time	**Obat** Drug	**Dosis** Dose	**Tab/ml** Tab / ml	**Supervisi** **Supervised**	**Muntah1*** Vomit1	**Muntah2*** Vomit2
1		**Chloroquine**			**Ya / Tidak**		
		Other					
2		**Chloroquine**			**Ya / Tidak**		
		Other					
3		**Chloroquine**			**Ya / Tidak**		
		Other					

* Dosis setelah muntah (Catat waktu pemberian obat setelah kejadian muntah)
Vomited Dose (State time after administration vomiting occurred)

Minum Obat Antimalaria atau Antibiotik Selama Penelitian:
(Antimalarial / Antiobiotic)

Ya/ Tidak

Bila Ya: **Obat apa? (Which?)** **Kapan? (When?)**

1. _____ _____

2. _____ _____

KELOMPOK PENGOBATAN:
(Treatment Group)

CHLOROQUINE & SP
***(*FP Study)**

Dosis Dose	**Tgl/Jam** Date/Time	**Obat** Drug	**Dosis** Dose	**Tab/ml** Tab / ml	**Supervisi** **Supervised**	**Muntah1*** Vomit1	**Muntah2*** Vomit2
1		**Chloroquine**			**Ya / Tidak**		
		SP					
2		**Chloroquine**			**Ya / Tidak**		
		Other					
3		**Chloroquine**			**Ya / Tidak**		
		Other					

* Dosis setelah muntah (Catat waktu pemberian obat setelah kejadian muntah)
Vomited Dose (State time after administration vomiting occurred)

Minum Obat Antimalaria atau Antibiotik Selama Penelitian:
(Antimalarial / Antiobiotic)

Ya/ Tidak

Bila Ya: **Obat apa? (Which?)** **Kapan? (When?)**

1. _____ _____

2. _____ _____

KELOMPOK PENGOBATAN:
(Treatment Group)

Amodiaquine

Dosis Dose	**Tgl/Jam** Date/Time	**Obat** Drug	**Dosis** Dose	**Tab/ml** Tab / ml	**Supervisi** **Supervised**	**Muntah 1*** Vomit1	**Muntah 2*** Vomit2
1		**Amodiaquine**			**Ya / Tidak**		
		other					
2		**Amodiaquine**			**Ya / Tidak**		
		other					
3		**Amodiaquine**			**Ya / Tidak**		
		other					

* Dosis setelah muntah (Catat waktu pemberian obat setelah kejadian muntah)
Vomited Dose (State time after administration vomiting occurred)

Minum Obat Antimalaria atau Antibiotik Selama Penelitian:
(Antimalarial / Antiobiotic)

Ya / Tidak

Bila Ya: Obat apa? (Which?) Kapan? (When?)

1. _____ _____

2. _____ _____

KELOMPOK PENGOBATAN: (Treatment Group) Quinine +/- Doxycycline

Dosis Dose	**Tgl** Date	**Obat** Drug	**Dosis** Dose	**Tab/ml** Tab/ml	**Supervisi** **Supervised**	**Muntah1*** Vomit1	**Muntah2*** Vomit2
1		**Quinine / Doxy**			**Ya / Tidak**		
2		**Quinine**					
3		**Quinine**					
4		**Quinine / Doxy**			**Ya / Tidak**		
5		**Quinine**					
6		**Quinine**					
7		**Quinine / Doxy**			**Ya / Tidak**		
8		**Quinine**					
9		**Quinine**					
10		**Quinine / Doxy**			**Ya / Tidak**		
11		**Quinine**					
12		**Quinine**					
13		**Quinine / Doxy**			**Ya / Tidak**		
14		**Quinine**					
15		**Quinine**					
16		**Quinine / Doxy**			**Ya / Tidak**		
17		**Quinine**					
18		**Quinine**					
19		**Quinine / Doxy**			**Ya / Tidak**		
20		**Quinine**					
21		**Quinine**					

COMMENTS:

Date	Day	Time	Kepadatan parasit (Asexual Count) per 200 WBC	Kepadatan parasit (Asexual Count) per 1000 RBC	Species (Species) Pf / Pv / Mix	Kepadatan Gametosit (GametoCount) per 200 WBC	Hct	Hb (g%)	Leukosit (µl) N / L / M / E (WBC µl)	Plt
	D0.1									
	D0.2									
	D1									
	D2									
	D3									
	D4									
	D5									
	D6									
	D7									
	D14									
	D21									
	D28									
	D35									
	D42									

Pada saat masuk penelitian :(On Admission)
Spot PCR (PCR Spot) Ya / Tidak
Spot PK (PCR Spot) Ya / Tidak
Sampel darah WB (WB Sample) Ya / Tidak

Tgl Gagal Pengobatan: (Day of Failure)
Spot PCR (PCR Spot) Ya / Tidak
Spot PK (PCR Spot) Ya / Tidak
Sampel darah WB (WB Sample) Ya / Tidak

Tanggal akhir pemeriksaan tindak lanjut: _ _ / _ _ / _ _
(Date of last follow up)

Hari pemeriksaan tindak lanjut: _____
(Days followed in study)

Terminasi dini: Ya / Tidak
(Early termination)

Bila Ya : 1 = Early Treatment Failure / Develop Warning Signs
2 = Late Treatment Failure
3 = Lost to follow up
4 = Concomitant Disease during followup
5 = Withdrawal of consent
6 = Protocol Violation
7 = Intolerable adverse experiences

Pemberian primakuin untuk eradikasi pada terminasi:

(Primaquine) Vivax / MixOnly

Ya /Tidak/ Menolak

Parasit Rekrudesen / Reinfeksi: Ya / Tidak
(Parasite Recrudescence)

Kepadatan parasit per 200 WBC: _____
(Asexual Count)

Species Pf / Pv / Mix: _____
(Species)

Kepadatan Gametosit per 200 WBC: _____
(Gameto Count)

Pengobatan Gagal: _____
(Failure Treatment)

Penelitian Gagal / Kode: _____
Failure Study / Code

Appendix 7 ***Ethical Approval letter of "Clinical trial to determine the efficacy and safety of coartemeter and artokin for the treatment, of acute falciparum and vivax in Timika, Papua" study***

This study was approved by:

The Committee on Health Research Ethics, National Institute of Health Research and Development, Ministry of Health, Jakarta, Indonesia.

Approval was signed by Chairwoman, dr. Liliana Kurniawan, on 18 November 2003 for "**A randomized trial to determine the efficacy and safety of coartemeter and artokin for the treatment, of acute falciparum and vivax in Timika, Papua**"

Submitted on 10 October 2003, by: **Dr. Emiliana Tjitra, Ph.D**

The clinical studies included in this Thesis followed the principles of the Declaration of Helsinki and were approved by two ethics committees: the Human research Ethics committee of Northern Territory Dept of Health & Community services and Menzies School of Health Research (Approval: 03/64 and 05/16) and the Ethics committee of the National Institute of Health research and Development, the Indonesian Ministry of Health (Jakarta, Indonesia) (Approval: KS.02.01.2.1.4042),.

All three studies were also registered at the clinical trials website:

http://www.clinicaltrials.gov/ct:

Pilot study was recorded as NCT 00157859,

Comparative study was recorded as NCT 00157833

Ethical clearance for the use of human subject

MINISTRY OF HEALTH
NATIONAL INSTITUTE
OF HEALTH RESEARCH AND DEVELOPMENT

Jl. Percetakan Negara No. 29
Telp.4261088-4244693-4243314
Jakarta 10560
P.O. BOX. 1226 Jakarta 10012Fax. (021)424393?

No.:KS.02.01.2.1.4042

TO WHOM IT MAY CONCERN ETHICAL CLEARANCE FOR THE USE OFHUMAN SUBJECT

The Committee on Health Research Ethics, after conducting review on the research protocol with the following title:

"A randomized trial to determine the efficacy and safety of coartemeter and artokin for the treatment, of acute falciparum and vivax in Timika, Papua"

submitted on 10 October 2003, by: dr. Emiliana Tjitra, Ph.D

has hereby declared that the above protocol whereby human subjects will be used has been approved for implementation.

Please notify that all the ethical aspects in the study should be considered very carefully for protecting the human right of the respondents. The progress and final summary report for this research should be submitted to NIHRD ethics committee

Jakarta,18 November 2003
Committee Health Research Ethics
Chairperson,

dr. Liliana Kurniawan M.Sc, DTMH

BADAN PENGAWAS
OBAT DAN MAKANAN
REPUBLIK
INDONESIA

Persetujuan Pelaksanaan Uji Klinik
Nomor: PO.01.01.3.3317

Sesuai dengan Keputusan Kepala Badan Pengawas Obatdan Makanan R.I.NO.02002/SK/KBPCM tanggal 28 Pebruari 2002, dengan ini diberikan Persetujuan Pelaksanaan Uji Klinik:

Judul Protokol:

"A Randomized Trial to Determine The Efficacy And Safety of Coartemether and Artekin for Treatment of Acute Falciparum and Vivax Malaria in Timika, Papua "

Peneliti Utama:

Dr. Emiliana Tjitra - NIHRD, Jakarta

Persetujuan Komisi Etik :

Nomor KS.02.01.2.1.4042 tertanggal 18 November 2003 dari Komite Etik Penelitian Kesehatan, Badan Penelitian dan Pengembangan Kesehatan Depkes Rl.

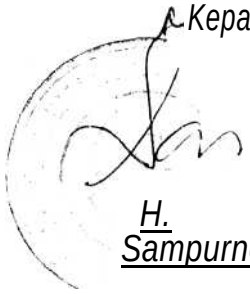
Dengan ketentuan:

1) *Teiah dilakukan kaji ilmiah terhadap Uji Klinik oleh Komisi Etik.*
2) *Uji klinik dilakukan sesuai dengan prinsip-prinsip Cara Uji Klinik yang Baik.*
3) *Persetujuan Pelaksaksanaan Uji Klinik No. PO.01.01.3.1705 tertanggal 15 april 2004 tidak berlaku !agi*
4) *Persetujuan ini berlaku 2 (dua) tahun sejak tanggal ditetapkan.*

Jakarta, 27 Juli 2004

Badan Pengawas Obat dan Makanan

Kepala, fa



H. Sampurno

Appendix 8 ***Information Sheet in Bahasa***

Penjelasan Penelitian Malaria

Malaria merupakan penyakit yang biasa terjadi di Indonesia dan dapat menjadi berat. Dalam lembaran ini akan dijelaskan mengenai penelitian bagaimana orang dapat dilindungi dari malaria dan reaksi terhadap pengobatan.

Penelitian 1: (Penelitian Pendahuluan)

"Sejauh mana keberhasilan pengobatan malaria di daerah Timika?"

Malaria dapat menjadi berat apabila tidak segera ditangani secara efektif. Ada kemungkinan Klorokuin dan Fansidar tidak efektif lagi di daerah Timika; hal ini disebabkan karena meningkatnya daya tahan parasit malaria terhadap obat-obatan tersebut. Beberapa orang masih ada reaksi terhadap pengobatan tersebut karena mereka mempunyai daya tahan yang kuat.

Kami mengadakan penelitian rintisan ini di Puskesmas untuk meneliti sejauh mana keberhasilan obat anti malaria yang biasa digunakan di klinik. Jika obat-obatan tersebut ternyata tidak baik, maka kami akan mengadakan penelitian yang lebih besar untuk mencari alternative pengobatan.

Kami akan memberikan anda dan anak anda obat malaria yang biasa dipakai (Chloroquine +/- Fansidar). Kami akan menanyakan beberapa hal mengenai gejala-gejala penyakit anda dan akan melakukan pemeriksaan fisik anda. Kami akan mengunjungi anda setiap minggu selama 4-6 minggu, untuk melihat apakah penyakit malaria yang diderita kambuh lagi.

Jika anda menyetujui untuk berpartisipasi dalam penelitian ini, maka akan dilakukan beberapa prosedur berikut:

1. Anda akan ditanyakan mengenai gejala-gejala yang dirasakan dan akan diperiksa oleh dokter.
2. Anda akan diberikan tablet anti malaria dan diminta untuk menunggu selama 1 jam untuk memastikan anda tidak muntah setelah minum obat.
3. .Kemudian anda akan diminta kembali ke klinik setiap hari sampai anda merasa sehat. Dalam setiap kunjungan akan ditanyakan apakah anda merasa lebih baik, kami juga akan mengambil sedikit darah dari ujung jari untuk memastikan apakah jumlah parasit malaria di dalam darah sudah berkurang.
4. Perawat akan memberikan obat setiap hari dan obat tersebut harus diminum didepan mereka. Penting sekali anda atau anak anda untuk minum obat yang telah diberikan.
5. Setelah minggu pertama, anda akan diminta kembali ke klinik setiap minggu selama 28 atau 42 hari untuk melihat apakah pengobatan telah berhasil.

6. Jika gejala atau panas timbul lagi maka anda harus ke klinik segera untuk dilakukan pemeriksaan darah. Jika malaria anda kambuh, anda atau anak anda akan di berikan pengobatan lain yang sudah terbukti efektif didaerah lain di Indonesia.

7. Jika anda/ anak anda setuju, kami juga akan mengambil contoh darah dari lengan (20 ml atau 4 sendok teh, 10 ml atau 2 sendok teh untuk anak) dan akan diulang pada hari ke 7 (2 sendok teh pada dewasa dan 1 sendok teh pada anak). Contoh darah ini akan digunakan untuk menilai sensitivitas parasit malaria, menilai respons kekebalan tubuh terhadap infeksi dan melihat kadar obat yang ada dalam tubuh. Contoh darah yang sama akan digunakan untuk penelitian lain yang akan dijelaskan dibagian belakang lembaran ini: kedua penelitian ini akan menggunakan contoh darah yang sama.

PENELITIAN 2: "Bagaimana orang dapat terlindungi dari malaria ?"

Jika seseorang terinfeksi malaria, tubuh akan berusaha untuk melawan infeksi tersebut melalui berbagai cara. Penelitian ini berusaha mencari apakah orang yang terlindungi dari malaria lebih mampu membuat zat seperti Nitrit Oksida (NO) dalam tubuh dibandingkan orang yang terkene serangan malaria berat. Jika hal ini benar, maka pengobatan malaria baru dapat diusulkan pada masa yang akan datang.

Penelitian ini akan menggunakan darah yang sama diambil dari penelitian 1. Partisipasi anda pada penelitian ini tidak akan membebani anda dan keluarga anda dengan pengeluaran uang ekstra. Dokter anda mungkin akan merekomendasikan tes dan prosedur lain untuk mendapatkan diagnosis yang lebih baik dan mengobati infeksi anda.

Beberapa ml darah yang telah anda berikan akan disimpan sebagai zat yang disebut DNA. Ini akan digunakan untuk mengetes bagaimana tubuh anda dan anak anda dapat melawan infeksi malaria. DNA ini akan dilabel dengan nomor dan akan disimpan secara berhati-hati, sehingga tidak akan ada seorangpun yang dapat mengatakan bahwa DNA ini kepunyaan siapa. Di masa yang akan datang, peneliti tidak akan dapat mengidentifikasi hasil anda/anak anda dengan nama. Oleh sebab itu kami tidak dapat memberitahukan hasil pemeriksaan ini kepada anda. Setelah kami melihat hasil dari semua pasien kami akan membuat laporan penemuan kami. Laporan akan diberikan ke RSMM.

Keuntungan dari penelitian ini: Menjadi bagian dari penelitian ini membuat anda /anak anda mendapatkan pengobatan terbaik tehadap malaria dan membantu memperkenalkan pengobatan ini kepada masyarakat di Timika. Anda tidak akan di kenakan biaya untuk mendapatkan pengobatan ini ataupun selama kunjungan petugas kerumah anda. Uang transportasi ke klinik akan diganti.

Jika anda menolak untuk ikut dalam penelitian ini, penolakan anda tidak akan berpengaruh terhadap hak anda untuk mendapatkan pelayanan kesehatan yang sudah baku di klinik.

Setiap saat anda/anak anda dapat mengundurkan diri dari penelitian dan masih akan mendapatkan pengobatan seperti biasanya.

Jika anda masih mempunyai beberapa pertanyaan mengenai penelitian ini, anda dapat menghubungi Dr. Rini di 0901-301881/883 atau dokter peneliti di klinik.

Pada kasus kegawatan anda harus segera kembali ke klinik atau jika diluar jam kerja anda harus segera ke bagian gawat darurat Rumah Sakit Mitra Masyarakat dan memberitahukan dokter bahwa anda adalah partisipan penelitian.

Jika anda mempunyai keluhan mengenai penelitian, silahkan melapor ke Dr. Liliana Kurniawan, Kepala Badan Komite Etik Jakarta. Telpon: 021-4259860.

Jika anda ingin mengetahui hasil penelitian: anda dapat menulis surat ke Direktur Medis RSMM. Tidak akan ada satupun nama pasien yang tercantum dalam setiap laporan yang dibuat dan yang akan dipublikasikan kemudian.

LEMBAR PERSETUJUAN

Formulir Consent untuk ORANG DEWASA yang berpartisipasi pada penelitian malaria:

"Sejauh mana keberhasilan pengobatan malaria di daerah Timika?"

"Bagaimana orang dapat terlindungi dari malaria?"

Anda dapat menolak untuk ikut dalam penelitian ini

Saya telah membaca dan memahami informasi yang telah diberikan, dan telah diberi kesempatan untuk mendiskusikan hal ini dan menanyakan beberapa hal.

Saya setuju jika sampel darah saya akan dipergunakan untuk penelitian seperti yang tertulis dalam lembaran informasi.

Saya mengerti bahwa saya tidak harus berpartisipasi dalam penelitian ini.

Jika saya tidak berpartisipasi dalam penelitian ini saya masih akan menerima pelayanan kesehatan baku yang diterapkan di klinik.

Saya ……setuju untuk berpartisipasi pada penelitian tersebut diatas.

Sebagai peserta penelitian ini saya setuju untuk dilakukan pengambilan darah dari ujung jari selama follow up.

Saya SETUJU / TIDAK SETUJU untuk diambil darahnya pada saat berpartisipasi dalam penelitian, hari ke 7 dan hari terakhir selama masa follow up (hari 28 atau 42).

Saya SETUJU / TIDAK SETUJU untuk diambil darah dan bahwa darah saya akan disimpan untuk kemungkinan penggunaannya dimasa yang akan datang dalam penelitian mengenai bagaimana orang terkena, berespon atau terlindungi dari malaria. (* dapat di coret sesuai pilihan)

Saya memahami bahwa sampel darah saya yang disimpan tidak akan dipergunakan untuk keperluan lain.

Saya menyadari bahwa saya dapat mengundurkan diri dari penelitian kapan saja sesuai pilihan saya.

Tanda tangan pasien:

……

Tanggal:.. ……

Saksi:

Nama……..

Tanda tangan ……..

Formulir Consent UNTUK ANAK yang berpartisipasi dalam penelitian malaria:

"Sejauh mana keberhasilan pengobatan malaria di daerah Timika?"

"Bagaimana orang dapat terlindungi dari malaria?"

Anda dapat menolak untuk ikut dalam penelitian ini

Saya telah membaca dan memahami informasi yang telah diberikan, dan telah diberi kesempatan untuk mendiskusikan hal ini dan menanyakan beberapa hal.

Saya setuju jika sampel darah anak saya akan dipergunakan untuk penelitian sesuai yang tertulis dalam formulir informasi.

Saya mengerti bahwa kami tidak harus berpartisipasi dalam penelitian ini. ***Jika kami tidak berpartisipasi dalam penelitian ini kami masih akan menerima pelayanan kesehatan baku yang diterapkan di klinik.***

Saya sebagai ORANG TUA/PENGASUH

Dari ……..menyetujui bahwa

anak saya dapat diikutkan dalam penelitian tersebut diatas.

Sebagai peserta penelitian ini saya setuju untuk dilakukan pengambilan darah dari ujung jari selama follow up.

Saya SETUJU / TIDAK SETUJU anak saya untuk diambil darahnya pada saat berpartisipasi dalam penelitian, hari ke 7 dan hari terakhir selama masa follow up (hari 28 atau 42).

Saya SETUJU / TIDAK SETUJU anak saya untuk diambil darah dan bahwa darah saya akan disimpan untuk kemungkinan penggunaannya dimasa yang akan datang dalam penelitian mengenai bagaimana orang terkena, berespon atau terlindungi dari malaria. (* dapat di coret sesuai pilihan)

Saya memahami bahwa sampel darah anak saya yang disimpan tidak akan dipergunakan untuk keperluan lain.

Saya menyadari bahwa saya dapat mengundurkan diri dari penelitian kapan saja sesuai pilihan saya.

Tanda Tangan Orang Tua :…….

Tanggal: ……

Saksi mata:

Nama: ……

Tanda Tangan: ……..