Population Pharmacokinetic-Pharmacodynamic Modelling of Antimalarial Treatment

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From now on, I'll connect the dots my own way.

Calvin

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ABSTRACT

Malaria is one of the most important tropical diseases, with hundreds of millions of cases every year. The current recommended treatment is an artemisinin based combination therapy (ACT), which has shown good efficacy. However, differences in exposure have been observed in children and pregnant women for some antimalarial drugs. Interactions might also change the outcome of the treatment. Recently resistance development has been noted, which further underlines the importance to optimise these treatments. In this thesis, a nonlinear mixed-effects modelling approach has been used to optimise the treatment with ACT. The aims were to optimise the treatment with piperaquine, and to investigate the interactions between the antimalarial drug combination artemether-lumefantrine and antiretroviral therapy. The pharmacokinetics of piperaquine during pregnancy was investigated, and no difference in exposure was found. However, a difference in exposure was found in children, and a new optimised dose regimen for children and adults were derived. A significant difference in clinical outcome was found between three sites in Cambodia. Potential interactions between antimalarials and antiretrovirals were investigated and a significant difference in the exposure of lumefantrine was found when combined with the three antiretroviral drugs efavirenz, nevirapine or lopinavir, and new doses for artemether-lumefantrine were simulated. Exposure of nevirapine was also found to differ when combined with artemether-lumefantrine, and a new dose suggestion was simulated. In conclusion, this thesis has optimised the treatment of piperaquine and the co-treatment of artemether-lumefantrine and efavirenz, nevirapine and ritonavir boosted lopinavir.

Keywords: Malaria, pharmacometrics, HIV, drug-drug interactions, paediatrics, pregnancy, dose optimisation

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SAMMANFATTNING PÅ SVENSKA

Malaria är fortfarande ett stort problem i tropiska länder, speciellt i Afrika söder om Sahara. Malaria är en infektionssjukdom som orsakas av parasiter av släktet plasmodium. Världshälsoorganisationen (WHO) har beräknat att det år 2012 inträffade ca 207 miljoner fall av malaria i världen. Malaria behandlas oftast med en artemisinin baserad kombinationsterapi. Dessa kombinationsterapier innehåller ett artemisinin derivat, som finns kvar i kroppen en kort tid, och ett långtidsverkande läkemedel som finns kvar i kroppen dagar eller t.o.m. månader.

Denna avhandling syftar till att optimera behandlingen av malaria med störst tyngdpunkt på två långtidsverkande läkemedel: piperakin och lumefantrin. Behandlingen med lumefantrin och dess artemisinin derivat artemeter, har undersökts när de givits samtidigt med HIV-läkemedlen efavirenz, nevirapin och lopinavir. När två eller flera läkemedel ges samtidigt så kan de förändra varandras effekt. Dessutom har avhandlingen undersökt vad som händer med efavirenz och nevirapin när de ges tillsammans med artemeter-lumefantrin. Undersökningarna av alla dessa behandlingar gjordes med hjälp av matematiska och statiska modeller, samt simuleringar utifrån dessa modeller.

Mängden piperakin som kroppen exponeras för visade sig inte ändras mellan gravida och icke-gravida kvinnor. Dock fanns en stor skillnad i exponering mellan barn och vuxna och mellan friska och sjuka. Utifrån den framtagna matematiska modellen så utfördes simuleringar för att ta fram en ny behandlings rekommendation för piperakin som gäller för både barn och vuxna. I undersökningen av interaktionerna mellan artemeter-lumefantrin och HIV läkemedel så visade det sig att exponeringen för malaria behandlingen ändrades, oavsett vilket av de tre HIV läkemedlen som man gav. Dessutom förändrades expoeringen av nevirapin när man gav artemeter-lumefantrin samtidigt. Simuleringar utfördes och nya doser togs fram för att få samma exponering som man får när läkemedlen ges var för sig. Slutligen undersöktes den kliniska effekten av piperakin i tre proviner i Kambodja, och det visade sig att effekten skilde sig mellan provinserna.

Denna avhandling har optimerat behandlingen av malaria med piperakin samt sambehandlingen av malaria och HIV med artemeter-lumefantrin och HIV-läkemedel.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. **Hoglund RM**, Adam I, Hanpithakpong W, Ashton M, Lindegardh N, Day NP, White NJ, Nosten F, Tarning J. A population pharmacokinetic model of piperaquine in pregnant and non-pregnant women with uncomplicated *Plasmodium falciparum* malaria in Sudan. *Malaria Journal. 2012 Nov 29;11(1):398.*

II. **Hoglund RM**, WWARN pooled analysis group, Tarning J. Meta-analysis of the population pharmacokinetics of piperaquine; a revised dose regimen. (*In manuscript*)

III. **Hoglund RM**, Amaratunga C, Song L, Sreng S, Lim P, Suon S, Day NP, White NJ, Fairhurst R, Tarning J. Population pharmacokinetics and pharmacodynamics of piperaquine in Cambodian patients with drug-resistant *P. falciparum* malaria. (*In manuscript*)

IV. **Hoglund RM**, Byakika-Kibwika P, Lamorde M, Merry C, Ashton M, Hanpithakpong W, Day NP, White NJ, Äbelö A, Tarning J. Artemether-lumefantrine coadministration with antiretrovirals; population pharmacokinetics and dosing implications. *British Journal of Clinical Pharmacology. 2014 Oct 8*

V. **Hoglund RM**, Byakika-Kibwika P, Lamorde M, Merry C, Ashton M, Hanpithakpong W, Day NP, White NJ, Äbelö A, Tarning J. The impact of artemether-lumefantrine therapy on the population pharmacokinetics of efavirenz and nevirapine (*In manuscript*)

CONTENT

ABBREVIATIONS

ACT	Artemisinin-based combination therapy
AIDS	Acquired immunodeficiency syndrome
CD4	Helper T lymphocyte
CYP	Cytochrome P450
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
IC50	Inhibitory concentration at the half maximum effect
IPED	Individual prediction
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
UGT	Uridine diphosphoglucurosyltransferas
WHO	World Health Organisation

DEFINITIONS IN SHORT

Pharmacokinetics	What the body does to the drug [1].
Pharmacodynamics	What the drug does to the body [1].
Pharmacometrics	Branch of science concerned with mathematical models of biology, pharmacology, disease, and physiology used to describe and quantify interactions between xenobiotics and patients, including beneficial effects and side effects resultant from such interfaces [2].
Population pharmacokinetics	the study of the variability in plasma drug concentrations between individuals when standard dosage regimens are administered [3].

1 INTRODUCTION

Malaria and human Immunodeficiency Virus (HIV) are two important infectious diseases. Malaria still claims nearly 2000 deaths each day and is one of the infectious diseases which claims most lives each year [4]. HIV is a life-long infection with approximately 34 million people infected worldwide [5]. This thesis focuses on optimising and individualising current treatment options for both malaria and HIV. Children, under the age of five, and pregnant women are the two most vulnerable groups to malaria, and treatment of both of these groups have been addressed in this thesis.

The thesis consists of five different research papers and has been divided into two main parts. In the first part, the pharmacokinetic and pharmacodynamic properties of the antimalarial drug piperaquine have been investigated (paper I-III). In the second part, the interactions between antimalarial drugs and antiretroviral drugs have been investigated (paper IV-V). In the first paper the pharmacokinetic properties of piperaquine, with focus on differences between pregnant and non-pregnant women, have been investigated (Paper I). To optimise treatment of piperaquine in children a large meta-analysis, consisting of ten different clinical studies, was performed (Paper II). To investigate the potential spread of drug resistance to antimalarial therapy a time-to-event analysis of clinical outcome linked to piperaquine concentration were conducted (Paper III). The interaction between antimalarial therapy and antiretroviral drugs were divided into two studies; in the first one, it was investigated how artemether, lumefantrine and their respective metabolites, dihydroartemisinin and desbutyl-lumefantrine, were affected by concomitant treatment with efavirenz, nevirapine and ritonavir-boosted lopinavir (Paper IV); in the second paper it was investigated how efavirenz and nevirapine was influenced by concomitant treatment with artemether-lumefantrine (Paper V).

The chapters in this thesis are organized as follow. Chapter 1 offers an introduction to the field and familiarizes the reader with the theory behind the methodology used. Chapter 2 presents the broad aim of the thesis. Chapter 3 presents the methodology in detail and describes the populations and study designs of the different studies. Chapter 4 and 5 describes the results and discuss the impact of the findings in this thesis, respectively. Chapter 6 and 7 presents the main conclusions of the thesis and future perspectives.

1.1 Malaria

Malaria is an infectious disease caused by plasmodium parasites. It is estimated that there were 207 million (95% uncertainty range: 135-287 million) cases (infections) of malaria, and 627,000 (473,000-789,000) deaths in malaria, in 2012 [4].

Five different malaria species infect humans; *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi.* The parasites are all transmitted to humans by the female *Anopheles* mosquito. Of these five species, *P. falciparum* and *P. vivax* are the most common. *P. falciparum* causes the most serious infections and is more likely to lead to severe malaria and death compared with *P. vivax* malaria. The focus of this thesis will be *P. falciparum* malaria.

A malaria infection can be categorised as either uncomplicated or severe. The uncomplicated stage is characterised by flue like symptoms (i.e. fever, headache and chills). If untreated, a malaria infection could proceed to a severe state which has an increased risk of organ failure and death. The World Health Organisation (WHO) has listed several clinical symptoms of severe malaria, including: severe anaemia (haematocrit <15% or haemoglobin <50 g/l in the presence of parasite count above 10,000/µl) and organ failure [6–8]. Cerebral malaria is the most serious complication of severe malaria, characterised by impaired consciousness and a high risk of death [7, 9]. Nearly all cases of severe malaria are reported to be caused by *P. falciparum.*

Malaria is today found in the tropical areas of the world with the highest incidence in Africa. Approximately 90% of all deaths caused by malaria are estimated to be located in Africa, south of Sahara [4].

1.1.2 The parasite life-cycle

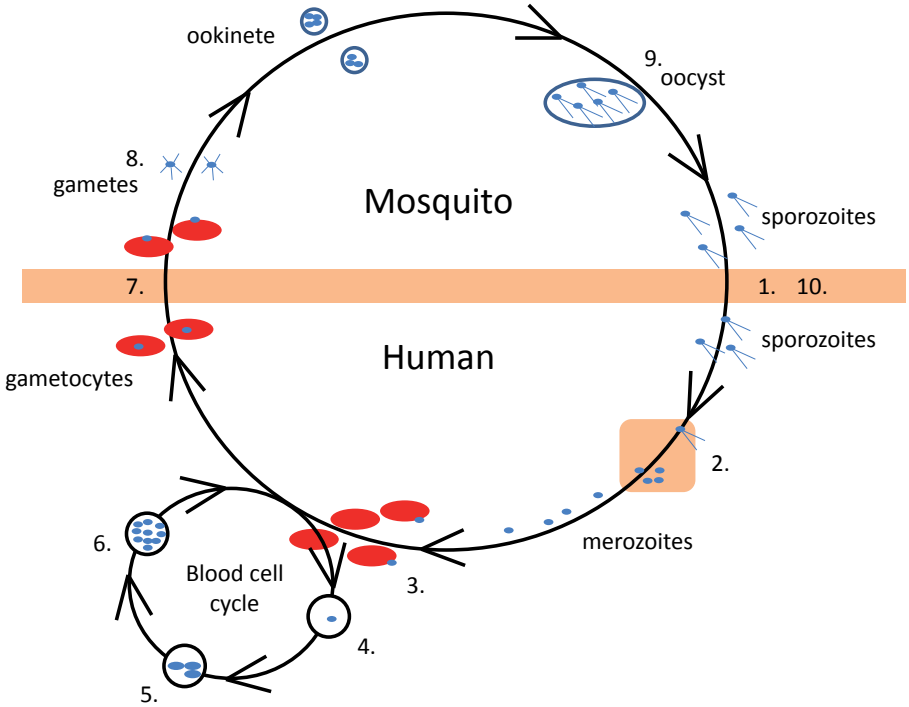

Figure 1. The life-cycle of the malaria parasite.

The plasmodium parasite undergoes life cycles in two different hosts, in mosquitos and in humans. The life-cycle in humans starts when being bitten by an infected female Anopheles mosquito [5], [6]; the bite injects malaria sporozoites into the human bloodstream (Figure 1. #1). These sporozoites rapidly invade hepatocytes (Figure 1. #2) where they mature into schizonts (for *P. falciparum* the liver stage lasts approximately five days). When the hepatocytes bursts, merozoites are released into the blood stream where they can infect erythrocytes (Figure 1. #3). In the erythrocytes the malaria parasite undergoes asexual replication (which takes approximately two days for *P. falciparum*) in which an early ring stage of malaria (immature trophozoites, Figure 1. #4) develops into trophozoites (Figure 1. #5) and blood schizonts (Figure 1. #6). These schizonts rupture the erythrocytes and releases new merozoites into the bloodstream, ready to infect new erythrocytes. The synchronised 48 hour cycle and the rupture of erythrocytes is responsible for the characteristic fever symptoms of malaria. The released merozoites enter a new asexual replication stage or a sexual blood stage where they mature

into gametocytes (males and females). Gametocytes are the sexual form of the plasmodium parasite, which can be consumed by another mosquito, thereby spreading the disease (Figure 1. #7). The malaria parasite also undergoes a lifecycle within the mosquito, different from that in humans. In mosquitoes, the imbibed gametocytes form gemetes (Figure 1. #8) which eventually develops into zygotes and sequentially into oocytes (Figure 1. #9) [6]. The oocytes form sporozoites which can be released from the saliva gland into a human during a blood meal (Figure 1. #10).

1.1.3 Malaria in children and pregnant women

Adults living in high-transmission areas of malaria have been gradually exposed to the disease for a long time, resulting in a developed semi-immunity to malaria (acquired immunity) [10]. Two groups have an increased sensitivity to malaria: children and pregnant women.

Children lack the acquired immunity of adults. This gives them an increased risk of symptomatic malaria, progression to the severe state of the disease and/or to die of the disease. Of the total deaths in malaria, 77% are estimated to be in children under the age of five. In 2012 it was estimated that approximately 1300 children died, each day, due to malaria [4].

Pregnant women have an increased risk to contract a malaria infection and to proceed to the severe state of the disease [11–14]. A malaria infected pregnant woman also has an increased risk of fetal loss, of dying or of low birth weight of the new-born baby, which increase the risk of death and complications later in life. During pregnancy the placenta is developed, which does not possess the acquired immunity. Therefore, women lose parts of the acquired immunity during a pregnancy [11, 12]. Also, the immune system in pregnant women is down regulated to not reject the foetus, which could partly explain the loss of acquired immunity [13]. It has been shown that pregnant women have twice the risk to contract an infection compared with non-pregnant women [14]. Linday *et al.* presents three possible explanations for this; pregnant women, in later stages of pregnancy, produces more exhaled breath compared with non-pregnant women (21% more), and some of the compounds in human breath could be attracting the mosquitoes (e.g. carbon dioxide [15]); the body temperature of pregnant women was found to be higher compared with non-pregnant women, and this increase in body temperature would increase the release of volatile substances from the skin, which could attract mosquitoes; pregnant women are twice as likely to leave the bed net at night to visit the bathroom compared with non-pregnant women, and this would increase the exposure to mosquitoes.

1.1.4 Anti-malarial therapy

Today, the main treatment of uncomplicated malaria is artemisinin-based combination therapy (ACT) consisting of one artemisinin derivative and a long-lasting partner-drug. In 2012 a total of 331 million treatments of ACT were delivered [4]. These combinations have shown great efficacy and are used as first-line treatment worldwide [16]. ACT reduces the number of parasites by approximately one hundred million-fold during the three days of treatment [17]. The artemisinins have a rapid effect and a short elimination half-life, and eliminates the majority of the parasite biomass. If not all parasites are killed they will start to regrow which will result in a return of the clinical symptoms of malaria (recrudescent malaria). The partner drugs have different mechanisms of action and often have a much longer half-life and are responsible for killing the residual parasites and thereby prevent recrudescent malaria. The partner drug also minimizes the risk of resistance development against the artemisinins [18]. The focus in this thesis is on two different ACTs: dihydroartemisinin-piperaquine and artemether-lumefantrine. Dihydroartemisinin-piperaquine has been the first line treatment in western Cambodia since 2008, because of emerging drug resistance against artesunate-mefloquine [19, 20]. Artemether-lumefantrine has been used as first line treatment in many countries, especially in Africa south of Sahara. Artemether-lumefantrine is the most common ACT used and account for 77% of all ACTs used.

1.1.5 Resistance

Western Cambodia has traditionally been a hot spot for developing drug resistance against antimalarial drugs. Both resistance against chloroquine and sulfadoxine/pyrimethamine was first seen here [21, 22]. Recently, a lower efficacy of artemisinin has been noted in Western Cambodia and later in other parts of South-East Asia [23]. In 2009 Dondorp *et al.* identified artemisinin resistance by noting an significant increased malaria parasite clearance time in Palin, in western Cambodia, compared to Wang Pha in northwestern Thailand (84 and 48 hours, respectively) [24]. In addition, resistance against the partner drugs has also been suspected. Recent studies have seen a lowered efficacy of piperaquine [25]. If this resistance would spread, it would severely limit our ability to treat malaria, which in turn would lead to an increased number of cases of severe malaria and deaths. Attempts have been made to identify a molecular marker for artemisinin resistance, and a recent study has suggested the K13-propeller to be associated with artemisinin resistance [26].

1.2 HIV

HIV is an infectious disease caused by the HIV-1 and HIV-2 viruses, with HIV-1 being the most common. HIV is spread across the globe. In Africa, south of Sahara, approximately 23.5 million people was infected in 2011[27]. The HIV-infection is divided into three stages. The first stage is the initial acute phase, in which the patient suffers from flue like symptoms, like rashes and fever. This phase is characterized by a rapid increase of viruses and a steep decline in CD4-cell counts [28]. The second step is a latent phase, which could last months or years. This phase is initiated by a decline in virus levels followed by a slow increase in the numbers of viruses and a slow decline in CD4-cell count [28]. The third and final stage (also known as acquired immunodeficiency syndrome, AIDS), is characterized by an increase in virus replication and very low CD-4 cell counts. This stage is terminal and the patient will die due to opportunistic infections [28].

1.2.1 HIV therapy (HAART)

As of today, no cure of HIV exists and the available therapies are designed to supress the disease, and to prolong life and increase life quality. HIV is treated by a combination treatment called highly active antiretroviral therapy (HAART). In this treatment, nucleoside reverse transcriptase inhibitors (NRTI) are combined either with a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor. The number of people receiving antiretroviral therapy worldwide, increased from 300,000 in 2002 to 6,650,000 in 2010 and the number of yearly deaths due to AIDS decreased from 2.0 million in 2002 to 1.8 million in 2010 [5]. However, in 2010 it was estimated that over 50% of the people who lives with HIV, does not receive antiretroviral therapy [5].

1.3 Drugs

1.3.1 Piperaquine

Piperaquine is a highly lipophilic drug related to halofantrine and chloroquine. The mechanism of action of piperaquine is unknown. However, chloroquine, which is structurally related to piperaquine, acts by preventing the detoxification process of haematin, a by-product from the parasites metabolism of haemoglobin, resulting in an accumulation of toxic haematin-chloroquine complex in the parasites food-vacuole [29–33]. Piperaquine was developed in 1966, by Shanghai Research Institute of Pharmaceutical Industry. Piperaquine was used extensively for nearly 15 years until excluded

from the treatment guidelines due to problems with resistance. In the 2000s, piperaquine was once again introduced, this time in combination with the artemisinin derivative dihydroartemisinin. This combination is administrated as three doses over three days. Piperaquine is given as the salt piperaquine tetra-phosphate, and the dose of the ACT depends on the patient's weight. Piperaquine is structurally related to halofantrine, which exhibits a cardiotoxic effect [34]. Piperaquine, in combination with dihydroartemisinin, has been deemed safe [35, 36]. However, two recent studies have shown prolonged QT-intervals in patients and volunteers in Cambodia [37, 38].

Piperaquine is highly bound to plasma proteins (>99%), has low elimination clearance (<1.4 L/h/kg), and a high volume of distribution (>100 L/kg), resulting in a long terminal half-life of more than 18 days (18-28 days) [39, 40][41–44]. Piperaquine is mainly metabolised in the liver, forming five urine identified metabolites [45]. The pharmacokinetics of piperaquine has been thoroughly studied and body weight has been shown to especially impact the pharmacokinetics [44]. The absorption of piperaquine has been shown to vary between dosing occasions, possibly due to recovery of the patients. A recent study by Tarning et al. included 48 Thai women (24 pregnant and 24 matched non-pregnant). By using a population pharmacokinetic approach, they showed that the elimination clearance is increased by 45.0% and the relative bioavailability is increased by 46.8% in pregnant compared to non-pregnant women, resulting in no changes in the overall exposure [43]. Less is known about the pharmacodynamics. One study has been able to link clinical outcome with day 7 concentrations of piperaquine and the total piperaquine exposure [44]. Another study described the outcome of a dihydroartemisinin-piperaquine treatment of *P. vivax* malaria by linking it to the piperaquine concentrations through a time-to-event model describing the risk to get a relapsing malaria episode [46], yielding a inhibitory concentration at the half maximum effect (IC_{50}) of piperaquine of 6.92 ng/mL. A study by Price et al. studied the link between day 7 concentrations and therapeutic response and was able to identify a cut-off value at 30 ng/mL on day 7 after initiation of treatment [47]. A concentration below this cut-off increases the risk of failed treatment, with a relative risk of 1.69 and a hazard ration of 6.6.

1.3.2 Artemisinins

Artemisinin was isolated from the herb Artemisia annua L. in 1972 [48]. Several derivatives have been synthesized from artemisinin, e.g. artesunate, artemether and dihydroartemisinin. These are commonly called artemisinin derivatives or artemisinins. The mechanism of action for artemisinins is

unknown, but it has been hypothesised that the drug effect is dependent on the endoperoxide bridge as a likely pharmacophore in the molecular structure. Cleavage of this bridge by iron ions could result in radicals which could affect molecules in the malaria parasite [49]. The artemisinins have been deemed safe [50, 51]. The focus in this thesis is on artemether and dihydroartemisinin.

Artemether is metabolized through cytochrome P450 (CYP) 2B6, CYP3A4/5, CYP2C9 and CYP2C19 also with a small contribution of CYP2A6 into dihydroartemisinin through demethylation. Dihydroartemisinin is metabolized through glucuronidation via uridine diphosphoglucurosyltransferas (UGT) A1, UGT1A9 and UGT2B7 [52–54]. Artemether induces CYP2C19 and CYP3A4 [55] resulting in an auto-induction, where artemether induces its own metabolism. This has been shown for a 3 day artemether-lumefantrine treatment [56].

Artemether has a short half-life of approximately one hour (0.5-2.6 hours). The reported values on pharmacokinetic parameters varies greatly between studies, with an elimination clearance of 1.96-16 L/h/kg and an volume of distribution of 7.46-39.7 L/kg [57–62]. After administration of artemether, formed dihydroartemisinin has a similar half-life of 0.8-5.7 hours and an elimination clearance of 1.42-8.5 L/h/kg and a volume of distribution of 1.00-10.1 L/kg [57–62].

1.3.3 Lumefantrine and desbutyl-lumefantrine

Lumefantrine is structurally related to halofantrine and chloroquine, and is co-administrated with artemether in ACT. As with most antimalarials, the mechanism of action is unknown but might be similar to chloroquine's (prevent detoxification of haematin) [29–33]. Lumefantrine is metabolized into desbutyl-lumefantrine for which the elimination is unknown. Both lumefantrine and desbutyl-lumefantrine exhibits antimalarial effects [63]. Recent studies have indicated that the antimalarial efficacy of desbutyl-lumefantrine is greater than that of lumefantrine [63]. However, lumefantrine has 85-300 times higher exposure compared with desbutyl-lumefantrine and is probably responsible for most of the antimalarial-activity clinically [64, 65]. Lumefantrine exposure is enhanced more than five times when administrated with a fat rich diet, and it has also been shown that lumefantrine exhibits dose dependent absorption [66, 67]. Concerns of the safety of lumefantrine have been raised, due to its relation to halofantrine which exhibits cardiotoxicity, but a study has found lumefantrine to be safe at standard doses [34, 68].

Lumefantrine has a long half-life of 2.7-7.2 days and the formed metabolite desbutyl-lumefantrine has a half-life of 6.0 days [65, 66, 69, 70]. The metabolism of lumefantrine into desbutyl-lumefantrine is mediated by CYP3A4 [61]. The elimination clearance of lumefantrine is low (<0.14 L/h/kg) and the volume of distribution lies between 4.16 and 7.65 L/kg [65, 69, 70]. The pharmacokinetics of desbutyl-lumefantrine has not been well characterised. A previous study by Salman *et al.* identified a high elimination clearance (14 L/h/kg) and a very high volume of distribution (1700 L/kg). Lumefantrine inhibits CYP2D6 [55]. Previous studies have attempted to link day 7 lumefantrine drug concentrations to therapeutically outcome and identified two cut off values. Price *et al.* used a cut off of 175 ng/mL and found that patients with a day 7 concentration below this cut off will have an increased risk of recrudescent malaria [71]. In a study by Ezzet *et al.* 75% of the patients with day 7 concentrations above 280 ng/mL had a successful treatment while only 51% of the patients with concentration below this cut off recovered fully from the infection [72].

1.3.4 Efavirenz, nevirapine and lopinavir

Efavirenz is a NNRTI and is combined with two NRTIs in the treatment of HIV. WHO recommends efavirenz in combination with either tenofovir disoproxil fumarate /lamivudine (emtricitabine) or zidovudine/lamivudine as first line treatment in adults [73]. Previously, concerns have been raised regarding safety of efavirenz during pregnancy, but WHO removed this restriction in their latest recommendations [73]. However, some national guidelines have not yet changed [74].

Efavirenz is metabolized by several enzymes including CYP3A4 and CYP2B6. At the same time efavirenz induces both these enzymes as well, particularly CYP2B6, resulting in an increased metabolism over time [75–77]. The half-life of efavirenz after a single dose is 52-76 hours while the half-life at steady-state is 40-55 hours [78]. The volume of distribution has been found to differ between males and females and the bioavailability is different in healthy volunteers compared with patients [79].

Nevirapine is also an NNRTI and is combined with two NRTIs in the same manner as efavirenz. Nevirapine is still widely used in HAART and is also used during pregnancy if the CD4 count is below 250 cells/µL, and to prevent the transmission of the virus from the mother to the child during birth [80, 81].

Nevirapine induces CYP3A4 and CYP2B6 and is also metabolized by these enzymes [75, 77, 82, 83]. The resulting auto-induction gives a half-life of 14.4-55.3 hours at steady state, while it is approximately 45 hours after a single dose [84–92]. Nevirapine's pharmacokinetics has been thoroughly studied. In a recent study diet and body weights were identified as covariates influencing the pharmacokinetics [90].

Several studies have been performed to evaluate the impact of efavirenz and nevirapine on the efflux protein P-glycoprotein, and these studies have reached contradictory conclusions [93–97].

Lopinavir is a protease inhibitor and is usually combined with ritonavir. This combination inhibits CYP3A4 and UGTs 1A1, 1A3, 1A4, 1A6, 1A9, and 2B7, and also induces CYPs 1A2, 2B6, 2C9, and 2C19 [77, 98, 99]. Some attempts have been made to evaluate the anti-malarial impact of ritonavir boosted lopinavir [100–102]. The anti-malarial effect of lopinavir observed in one study might be an effect of an interaction with artemether-lumefantrine.

1.4 Pharmacokinetics and pregnancy

Several physiological changes take place during pregnancy. The gastric empting and the motility of the small intestine is reported to be reduced [103]. However, several studies reports no differences in the gastric emptying for pregnant healthy women [104–107]. The cardiac output, and consequently the blood flow, is increased during pregnancy. The plasma volume and the water content in the body are increased. In addition, the expression of several enzymes and plasma proteins are changed [103, 108].

These changes may influence the pharmacokinetics of several drugs. The reduction in the gastric emptying and intestine motility will prolong the absorption and might change the time to reach the maximum blood concentration. These changes will not have any direct effect on the bioavailability, but the bioavailability might be affected by other characteristics of pregnancy (e.g. vomiting). The increased plasma and water volume will increase the volume of distribution of the drugs, and the changes in enzyme expression might impact the elimination of the drugs. This could have consequences for the exposure of the drugs [103, 108]. In a pregnancy the plasma albumin concentration will decrease which might lead to an increased free drug concentration for albumin-bound drugs [103].

For anti-malarial drugs, several studies have tried to identify clinically important changes in the pharmacokinetics during pregnancy. Artesunate,

artemether, dihydroartemisinin, lumefantrine, sulphadoxine, atovaquone, proguanil and cycloguanil have all been shown to have a reduced exposure in pregnant women [43, 60, 70, 109–114]. In contrast, no differences in the exposure have been found for quinine, amodiaquine/desethylamodiaquine or piperaquine [43, 115].

1.5 HIV-malaria co-infection

HIV and malaria are both common diseases in Africa, south of Sahara. A consequence of the similar spread of the two diseases is a high risk of individuals contracting both diseases at the same time. HAART treatment of HIV is a lifelong treatment, it also very probable that people living in high transmission areas of malaria will contract a malaria infection. This leads to a situation when two diseases are treated at the same time, which might lead to the interactions between the involved drugs. It has also been shown that a HIV infection will increase the risk to progress to the severe state of malaria and also aggravates the malaria infection during pregnancy [116, 117].

1.6 Drug-drug interactions

One drug can affect the absorption, distribution, metabolism and/or elimination of another drug. The clinically most important interactions are those affecting the exposure of other drugs. An increased exposure may increase the risk of adverse events, while a lowered exposure may result in a failed treatment.

The interactions between antimalarial and antiretroviral drugs have been studied in the past. It has been shown that especially efavirenz will have a large impact on the outcome of the antimalarial therapy of artemether-lumefantrine, and also decreasing the exposure of the antimalarial drugs [39, 118]. The studies on nevirapine are more contradictive [118, 119]. Kredo *et al.* shows an increase in lumefantrine exposure and a lowering of the artemether and dihydroartemisinin exposures. Byakika-Kibwika *et al.* showed similar results for artemether and dihydroartemisinin, with a lowered exposure, but in contrast to Kredo *et al.* found a trend of lower lumefantrine exposure, however not significant.

The impact of artemether-lumefantrine on the pharmacokinetics of nevirapine has also been studied, with contradictive results. Svensson *et al.* did not find any impact of artemether-lumefantrine on the exposure of nevirapine, while

Byakika-Kibwika *et al.* showed a lowered exposure of nevirapine during the co-administration [90, 118].

Ritonavir boosted lopinavir has previously been shown to lower the number of recrudescent malaria episodes and to increase the exposure of lumefantrine [102, 120, 121].

1.7 Pharmacometrics

The field of pharmacometrics (quantitative pharmacology) started in the 1970s and has been of increased use and focus during the last decade. The aim of pharmacometrics is to describe and quantify interactions between a biological system and one or more drugs. This is done by developing mathematical and statistical models to describe the processes in the body. Models range from very simple models to very complex models, and the appropriate one depends on the objective of the analysis.

Traditional pharmacokinetics are inconvenient to utilize on large quantities of data, and/or if the data are sparse (2-3 samples per individual), and might result in inaccurate findings. A pharmacometric approach utilizes all the data, at all time points and for all individuals, thereby increasing the power to describe the system. The most commonly used program for population based pharmacokinetic and/or pharmacodynamic analysis is NONMEM, but other software such as Monolix and ADAPT are also available [122, 123].

Pharmacometric data are usually analysed through a non-linear mixed effect approach, which contains both fixed effects and random effects [124].

1.7.1 Pharmacometric models

Equation 1 presents the general equation for a non-linear mixed effect model:

$$y_{ij} = f(t_{ij}, g(\theta, \eta_{ij}, x_i, z_i)) + r(t_{ij}, g(\theta, \eta_i, x_i, z_i), \varepsilon_{ij}) \quad (1)$$

where y_{ij} is the observed value for individual i at observation j, $f()$ is the function describing the structural model (e.g. a one compartment disposition model with first order absorption), t_{ij} is the independent variable (often time), $g()$ is the vector function describing the parameters in the structural model for individual i at observation j, θ is the typical values of the parameters, η_i is the random effect for individual i, x_i is the vector of discrete design variables for individual i (e.g. dose) and z_i is the vector of covariate values for individual i used in the structural model. $r()$ is the function of the residual variability

(unknown variability) and ε_{ij} is the difference between the true observation and the predicted value for individual i at observation j.

The equation can be divided into three components: a structural model, a statistical model and a covariate model. The covariate model is sometimes ignored depending on the available data.

Structural model

The structural model in a pharmacokinetic (or pharmacodynamic) system describes the typical individual in a population. A pharmacokinetic structural model describes the absorption, distribution and elimination of a drug. Other types of structural model could describe for example enzymatic auto-induction. Equation 2 gives an example of this, describing a one-compartment disposition model after intravenous administration;

$$y_{ij} = \frac{x_{dose,i}}{\theta_V} \cdot e^{-\frac{\theta_{CL}}{\theta_V} * t_{ij}} \quad (2)$$

where $x_{dose,i}$ is the intravenous dose given for individual i, θ_V is the typical value of the volume of distribution and θ_{CL} is the typical value of the elimination clearance.

Statistical model

The statistical model consists of two main parts; the between-subject variability and the residual variability. The between-subject variability describes differences between the individual pharmacokinetic profile and the population profile, by identifying variability between subjects in different parameters [124]. This variability can be included in different ways of which an exponential model is most common (Equation 3):

$$P_{pi} = \theta_P \cdot e^{\eta_i} \quad (3)$$

where $P_{p,i}$ is the value of parameter P for individual i. θ_p is the typical value of parameter P and η_i is the between-subject variability for individual i. η_i is drawn from a normal distribution with the mean 0 and the variance ω^2(η_i ~N(0, ω^2)). This will result in an individual parameter which is log-normally distributed. The advantage of this, compared with an additive between-subject variability, is that the individual parameter estimate will never be below zero.

Without inclusion of IV data, the true bioavailability of an oral drug cannot be known. Therefore, all estimated clearance and volume parameters are the parameters divided by the bioavailability (e.g. CL/F or V/F, where F is the bioavailability). Since the bioavailability differs between individuals, it is still possible to estimate a between-subject variability on the bioavailability, even though it is not possible to estimate the bioavailability itself. The resulting parameter is not a true bioavailability, but a relative bioavailability showing how individuals differ from the population mean.

In some instances a parameter might take different values at different occasions in the same individual. This is known as between-occasion variability and is exemplified in equation 4 If the between-occasion variability is not taken into account it might result in biased parameter estimates [125].

$$P_{pik} = \theta_P \cdot e^{\eta_i + \kappa_k} \tag{4}$$

where κ_k is the between-occasion variability for occasion k. κ_k is drawn from a normal distribution with mean 0 and variance π^2. In the equation above the different occasions could for example be dosing occasions or different follow up visits.

If a pharmacometric analysis contains data from several (>20) different clinical studies, a third type of variability may be considered: the inter-study variability [126].

The residual variability describes the variability which cannot be explained in any other way. This could be due to model misspecification, error in the chemical analysis, error in sampling times, etc. The residual variability could be included in different ways. The most common is the additive residual error (Equation 5) and the proportional residual error (Equation 6).

$$y_{ij} = IPRED_{ijk} + \varepsilon_{ij} \tag{5}$$

$$y_{ij} = IPRED_{ijk} + IPRED \cdot \varepsilon_{ij} \tag{6}$$

where $IPRED_{ijk}$ is the predicted value (e.g. concentration) for individual i at observation j and occasion k.

Covariate identification

Covariates are chemical, physiological or demographic properties which affect the pharmacokinetics and/or pharmacodynamics of a drug. In a model

without covariates these effects are included into the between-subject variability or residual variability instead. There are multiple types of covariates and they can be divided into continuous or categorical covariates. Several continuous covariates are commonly collected in clinical trials, such as body weight, age and parasite count. Categorical covariates are properties which can take two or more distinct values. A common categorical covariate is sex, but another important covariate is pregnancy. Some covariates can be both categorical and continuous depending on how they are collected. For example, pregnancy is a categorical covariate if it is only known if the patient is pregnant or not. However, one could also include information on how many weeks the women has been pregnant (estimated gestational age) which would be a continuous covariate.

Adding covariates to a model can explain and gives mechanistic understanding of the between-subject variability in the model. Adding covariates could also help in identifying populations at risk for, for example, a failed treatment. Covariates can be included in different ways, for continuous covariates the most common is a linear model centered on the covariate mean (Equation 7):

$$P_i = \theta_P \cdot (1 + \theta_{\text{cov}} \cdot (COV_i - COV_m)) \quad (7)$$

where θ_{cov} is the estimated covariate impact on the parameter for each step in covariate value, COV_i is the covariate value for individual i and COV_m is the median covariate value in the studied population.

Categorical covariates could be included as in equation 8:

$$P_i = \theta_P \cdot (1 + \theta_{cat\_\text{cov}} \cdot (COV_{cat\_i})) \quad (8)$$

where $\theta_{\text{cat\_cov}}$ is the percentage difference in the parameter value between the different categories and $\text{COV}_{\text{cat\_}i}$ is the covariate indicating which category patient i belongs to.

Some covariates are commonly included with an allometric function (e.g. body weight), according to equation 9 [127, 128].

$$P_i = \theta_P \cdot \frac{COV_i}{COV_m}^{EXP} \quad (9)$$

where EXP is an exponent describing the slope of the relationship between the parameter and the covariate.

Usually covariates take a specific value throughout a trial but in some instances the covariate will change with time. This is called a time varying covariate and this can also be incorporated into a model [129].

1.7.2 Pooled analysis

A pharmacometric analysis gives the opportunity to pool large quantities from different clinical trials together, and in this way maximize the power to detect covariates and to identify complex models [130].

There are different approaches to analyse a pooled data set. The most common is to analyse all data simultaneously. Svensson et al. presented an alternative approach in which each study is given a score depending on how much information it contains [90]. Thereafter, the highest scoring study is analysed first and then the less informative studies are added one by one until all data have been included in the model.

1.7.3 Time-to-event model

The efficacy (pharmacodynamics) of a drug could be evaluated through a time-to-event analysis, also known as survival analysis. In a time-to-event analysis the probability of having a certain event at a specific time is modelled. An event could constitute a number of different things: a recrudescent malaria infection, recovery from disease, death etc. The risk of getting an event depends on the survival function, which is dependent on the cumulative hazard. In the case of recrudescent malaria the hazard would be the number of recrudescent infections per time unit and the cumulative hazard would be the total number of infections over a given time. Modelling of time-to event data is a new technique in the analysis of antimalarial drugs pharmacodynamics, first used by Tarning *et al.* [131].

The cumulative hazard is the accumulated hazard over time. This depends on a baseline hazard which could be modelled in several different ways. The most common is the exponential hazard model (Equation 10) which gives a constant hazard over time. The Weibull hazard model (Equation 11), is another way of describing the hazard, in which it will change over time. This model depends on a baseline hazard (λ) and a shape parameter (α). If the shape parameter is 1 the baseline hazard is constant, if it is greater than 1 the hazard will increase over time and if it is below 1 the hazard will decrease

over time. A third type of hazard model is the Gompertz model (Equation 12) in which the hazard will decrease over time.

$$h(t) = \lambda \tag{10}$$

$$h(t) = \lambda\alpha(\lambda t)^{\alpha-1} \tag{11}$$

$$h(t) = \lambda e^{-t(LN(2)/\alpha)} \tag{12}$$

where *h(t)* is the hazard at time *t*.

The survival function describes the probability to survive (not having an event) beyond time *t*, calculated according to equation 13.

$$S(t) = e^{(-\int_0^t h(u)du)} \tag{13}$$

where S(t) is the survival at time *t* and *h(u)* is the hazard at time *u* (between 0 and *t*).

It is possible to investigate the impact of the drug levels on the outcome of a treatment by linking the time-to-event model to a model for the pharmacokinetics of the drug. This is usually performed by adding an E_{max}-model to the hazard according to equation 14. This will result in a change in hazard with changes in drug concentrations, high drug concentrations will give a low hazard (a low chance of getting the event) and low drug concentrations will give a higher hazard (a higher chance to get an event),

$$h(t)_i = h(t) \cdot (1 - \frac{Conc_{it}{}^{\gamma}}{IC50^{\gamma} + Conc_{it}{}^{\gamma}}) \tag{14}$$

where $h(t)_i$ is the hazard for individual *i* at time *t*, $Conc_{it}$ is predicted concentration for individual *i* and time *t*, IC50 is the concentration which reduces the hazard with 50% and γ is the Hill-factor describing the shape of the curve. In the Weibull and Gompertz models it might be difficult to distinguish between the drug effect and the hazard without placebo data.

2 AIM

The aim of the thesis was to investigate the pharmacokinetic and pharmacodynamic properties of antimalarial treatment and to optimise the treatment in different populations. The aim can be divided into the following specific aims:

1. To investigate the impact of pregnancy on the pharmacokinetics of piperaquine.

2. To develop a new optimised dose regimen for piperaquine.

3. To characterize the difference in outcome for patients with multi-drug resistant malaria, in different provinces in Cambodia and to link these differences to the predicted drug concentrations of piperaquine.

4. To investigate the impact of concomitant antiretroviral therapy on the pharmacokinetics of artemether, lumefantrine, and their respective metabolites dihydroartemisinin and desbutyl-lumefantrine, and to simulate new doses if necessary.

5. To investigate the impact of concomitant treatment with artemether-lumefantrine on the pharmacokinetics of efavirenz and nevirapine, and to simulate new doses if necessary.

3 PATIENTS AND METHODS

3.1 Patients and study design

3.1.1 Effect of pregnancy on the pharmacokinetics of piperaquine

The study was conducted at the New Halfa teaching hospital in New Halfa, Sudan. The study was a parallel study with 12 age- and weight-matched pregnant and 12 non-pregnant women. All women received a three day treatment of dihydroartemisinin-piperaquine tetra-phosphate (Duo Cotecxin, 40 mg/320 mg tablets, Beijing Holley-Cotec Pharmaceuticals, Co., Ltd.) with the number of tablets based on the women's weight. All women had symptomatic uncomplicated *P. falciparum* malaria and gave their consent before being enrolled in the study. The pregnant women were in the second or third trimester. Blood samples were collected pre-dose and at 1.5, 4, 8, 24, 25.5, 28, 32, 48, 49, 50, 52, 56, 60, 72 h after the first dose and on days 5, 7, 14, 21, 28, 35, 42, 49, 56, 63 and 90. All samples were kept on ice and transported to Bangkok for chemical analysis [132]. The study received ethical approval from the College of Medical and Technical Studies, Khartoum, Sudan.

The plasma samples were analysed with liquid chromatography (agilent 1200) with a tandem mass-spectrometer detection (API 5000) according to a previous published study [133].

3.1.2 Pharmacokinetic and pharmacodynamic properties of piperaquine in a pooled analysis

Data from ten previously published studies were collected (through the worldwide antimalarial network) and pooled [41–44, 132, 134–138]. The data available from the ten studies are presented in Table 1. The patients were between 0.56 and 55 years of age and with body weights ranging between 6.3 and 81 kg. Both healthy volunteers and patients were included in the studies. All individuals received dihydroartemisinin-piperaquine tetra-phosphate, but the dose regimens differed between the different studies. In one study, the concentrations were measured in capillary blood while the concentrations in the rest of the studies were measured in venous blood. Both men and women were included in the studies. The chemical analysis and the lower limit of

quantification differed between the studies. The ten clinical trials in this analysis all had ethical approval from the concerned authorities, listed in Table 1.

3.1.3 Pharmacokinetic and pharmacodynamic properties of piperaquine and markers for resistance

This study was performed at three different sites in Cambodia, one in the west (Pursat province), one in the north (Preah province) and one in the east (Ratanikiri province). One-hundred four patients between 2 and 59 years of age were recruited. All patients were infected with *P. falciparum* malaria. Patients received a three day treatment of dihydroartemisinin-piperaquine tetra-phosphate (40/320 mg, Duo-Cotecxin®; Holleypharma, P.R. China). Capillary blood samples were collected pre-dosing and at days 7, 14, 21, 28, 35, 42, 49, 56, and 63 post-dosing. At these follow up visits any recrudescent malaria were diagnosed and noted. Patients with recrudescent malaria got a rescue treatment consisting of artesunate-mefloquine. The piperaquine concentrations in the capillary blood samples were analysed as presented in a previously published method [133]. The study received ethical approval from the Cambodia's National Ethics Committee for health research and the national Institute of Allergy and Infectious Diseases Institutional Review Board, United States of America.

3.1.4 Interaction between antimalarial and antiretroviral drugs

The data were collected from two studies conducted in Uganda. The first study included 31 individuals and had a parallel study design with two arms. The first arm was with HIV-infected patients who had not started their antiretroviral therapy yet. The patients in the second arm were already being treated with ritonavir boosted lopinavir, combined with two nucleoside reverse transcriptase inhibitors (zidovudine and didanosine, or tenofovir and emtricitabine, regional non-controlled procurement). Both arms received a single dose of 80 mg artemether and 480 mg lumefantrine (four tablets) (Coartem®, Novartis Pharma AG, Basel, Switzerland; Batch number: F0660) and samples were collected at 1, 2, 4, 6, 8, 12, 24, 48, and 72 hours post dose and analysed for artemether, dihydroartemisinin, and lumefantrine concentrations according to previously published methods [139, 140].

The second study was a cross-over study divided into three phases. All patients (n=58) were HIV-infected and had not started HIV-therapy yet. In

the first phase, all patients received a standard treatment of artemether-lumefantrine (80/480 mg twice daily for three days; Coartem®). Plasma samples were collected after the last dose. Thereafter, the HIV therapy was initiated which consisted of either efavirenz (600 mg) or nevirapine (200 mg), combined with the nucleoside reverse transcriptase inhibitors zidovudine and lamivudine. The HIV-therapy was administrated for approximately one month. In the second phase of the study, samples were collected after a dose of efavirenz or nevirapine. Samples were collected every two hours, up to twelve hours post dosing, when the next dose was given. For efavirenz, an additional sample was taken after 24 hours. The patients continued on their anti-retroviral therapy and received a three day treatment of artemether-lumefantrine in the third phase. Blood samples were collected after the last dose. The samples collected were analysed for artemether, dihydroartemisinin, lumefantrine, efavirenz and nevirapine concentrations. The chemical analyses of the antimalarial drugs were conducted in Bangkok, Thailand, and the anti-retroviral drugs in Kampala, Uganda. The analytical method for nevirapine has previously been published [93]. The analytical method for efavirenz is unpublished [118]. The lower limits of quantification for the analyses were 450 ng/mL for nevirapine and 110 ng/mL for efavirenz.

At a later time point, the samples were analysed for desbutyl-lumefantrine, and only a subset of all samples was chosen for this analysis (due to a later analysis). The chemical analysis method for desbutyl-lumefantrine has not been published but was performed using liquid chromatography (agilent1200) coupled with a tandem mass spectrometer, and has a lower limit of quantification of 1.0 ng/mL (see Paper IV for further details).

Ethical approvals for the two studies were received from the Uganda National HIV/AIDS Research Committee (ARC 056) and the Uganda national Council of Science and Technology (HS 195) and the study was also registered with ClinicalTrials.gov (NCT 00619944).

Table 1. Demographic data of study population for the pooled analysis.

Study	Population	Country	Number of subjects	Weight	Age	Female (%)	Pregnant (%)	Malaria infection	Dose Regimen	Number of samples	Samples per subject (median [range])	Venous/Capillary measurments	Ethics	Reference
1	Pregnant and non-pregnant women	Thailand	48	36-78	18-45	100	50	Pf	3 doses over 3 days	1,250	33[23-34]	Venous	MUTM 2007-111 and OxTREC 017-07	[43]
2	Pregnant and non-pregnant women	Sudan	24	44-81	16-43	100	50	Pf	3 doses over 3 days	589	26 [9-30]	Venous	College of Medical and Technical studies Khartoum	[132]
3	Adults	Vietnam	18	31-55	16-49	33.3	0	Pf	2 doses over 2 days	18	1	Venous	Vietnam People's Army Department of Military Medicine, Vietnam Ministry of Health and the ADHREC (No. 507/8)	[138]
4	Adult	Thailand	30	39-73	18-55	13.3	0	Pf	3 doses over 3 days	1,038	36[27-37]	Venous	OxTREC and the ECFTM	[42]
5	Adult	Vietnam	12	47.5-75	19-51	0	0	Pf	3 doses over 3 days	240	20	Venous	Review and Scientific Board of Military Hospital 175 and the ADHREC (No. 379/05)	[136]
6	Children and adults	Thailand	98	12-74	3-55	39.8	0	Pf	3 or 4 doses over 3 days	482	5[3-10]	Venous	ECFTM and the OxTREC	[41]
7	Children	Burkina Faso	236	8-34	2-10	44.7	0	Pf	3 doses over 3 days	2,471	11 [4-12]	Capillary	CEIM and the CHRUC	[44]
8	Children	Kenya	105	6.3-20.7	0.58-4.9	56.2	0	Pf	3 doses over 3 days	105	1	Venous	NKEMRIERC, the OxTREC and the ECHUSM	[134]
9	Healthy adults	Vietnam	24	56-70	18-29	0	0	Healthy	Single dose	824	17[16-18]	Venous	Review and Scientific Board of Central Military Hospital 108 and the ADHREC (No. 437/06)	[135]
10	Healthy adults	Vietnam	26	54-70	19-24	0	0	Healthy	Single dose and 3 doses over 3 days	495	19.5[18-20]	Venous	Review and Scientific Board of Central Military Hospital 108 and the ADHREC	[137]
Total:	NA	NA	621	6.3-81	0.58-55	45.6	5.80	NA	NA	7,512	11[1-37]	NA	NA	NA

Study is the number designated to the study. Female (%) represents the proportion of females in the study. Pregnant (%) represents the proportion of pregnant women of the total population in the study. Malaria infection indicates if the subject were healthy or sick, Pf stands for a *Plasmodium falciparum* infection. Venous/Capillary measurements indicates in what matrix the samples were taken. Ethics indicates who issued the ethical approval. OxTREC is the Oxford Tropical Research Ethics Committee, ADHREC is the Australian Defence Human Research Ethics Committee, ECFTM is the Faculty of Tropical Medicine Mahidol University Ethical Committee, CEIM is the Comite d'Ethique Institutionnel du centre Muraz, CHRUC is the Committee on Human Research of the University of California, NKEMRIERC is the National KEMRI Ethical Review Committee and ECHUSM is the Ethics Committee Heidelberg University School of Medicine.

3.2 Pharmacometric and statistical analyses

All studies were analysed using a nonlinear mixed-effects modelling approach. The data were analysed using the software NONMEM (v. VI-7.3) [141, 142]. PsN (Perl speaks NONMEM v. 3.4.2-3.5.3) and Pirana (v. 2.6.0) were used to facilitate the modelling process and to perform model diagnostics [143, 144]. The statistical program R (v. 2.14.2) with the package Xpose (v. 4.04-4.35) was used for statistical tests and graphical evaluations [145].

To increase numerical stability, the concentration measurements were transformed into their natural logarithms. Different disposition models were evaluated to describe the data. Depending on the data, one-, two-, three-, and four- compartment disposition models were investigated. Different absorption models were also evaluated: first-order absorption, first order absorption with lag time, zero-order absorption, sequential zero and first order absorption, and transit compartment models were investigated [146].

Between-subject variability was accounted for using exponential models. The residual error was described by an additive model on logarithmic data. The bioavailability was fixed to 100% but with an estimated between-subject variability to quantify the individual relative bioavailability. Between-occasion variability between dosing days was also evaluated if data allowed. Covariates were tested in a step-wise manner with a forward step (p=0.05) and a more stringent backward step (p = 0.01 or 0.001). Body weight was included as covariate on all clearance and volume parameters, modelled as an allometric function with an exponent of 0.75 and 1, respectively [127, 128].

Discrimination between models were based on the objective function values. The objective function value in NONMEM is proportional to –2*log likelihood of the observed data given the estimated parameter values. The difference in objective function value between two nested models is approximately χ^2 distributed. A drop of 3.84 in objective function value between two nested models, when adding one extra model parameter (one degree of freedom) is significant on a 5% significance level. Models were also evaluated graphically, through goodness-of-fit plots, and by simulation based diagnostics (visual predictive checks) [147]. If necessary, prediction correction and variance correction were used in the simulation based diagnostics [148]. The precision of final parameter estimates were evaluated by using non-parametric bootstraps. The shrinkage of the individual

estimates was calculated and evaluated to determine the reliability of the individual parameters estimates [149].

3.2.1 Effect of pregnancy on the pharmacokinetics of piperaquine

Due to the low number of patients in the study, a Monte Carlo mapped power (MCMP) analysis [150] was performed. This analysis was based on a previous model for pregnant and non-pregnant women in Thailand, to determine if the present design could identify the previously identified covariates [43]. The effect of pregnancy on the pharmacokinetics of piperaquine was analysed with a full covariate approach. A full covariate model was analysed, with pregnancy as a categorical covariate. The model was analysed in a bootstrap with 200 re-sampled data sets. Differences in secondary parameters, between pregnant and non-pregnant women, were evaluated with Mann-Whitney U-tests.

3.2.2 Pharmacokinetic and pharmacodynamic properties of piperaquine in a pooled analysis

All data were pooled and analysed simultaneously, the number of disposition models and absorption models that were evaluated were limited due to the long run times of the model. The difference between the capillary blood concentrations and the venous blood concentrations were described by a conversion factor, which was estimated according to equation 15:

$$Ipred_c = Ipred_v + \theta_s \cdot Ipred_v \tag{15}$$

where $Ipred_C$ is the individual predicted capillary concentration, $Ipred_V$ is the individual predicted venous concentration, and θ_S is the population scale parameter between the two biological matrices.

An enzyme maturation model was investigated to describe the elimination clearance in very small children (Equation 16):

$$P_i = \theta_{CL} \cdot \frac{AGE^{Hill}}{MF50 + AGE^{Hill}} \tag{16}$$

where P_i is the individual parameter estimate for the i^{th} subject, θ_{CL} is the population value of the elimination clearance, MF50 is maturation half-life, and *Hill* is the Hill-coefficient describing the slope of the maturation.

Several covariates were evaluated: weight, sex and disease effect, and the covariate effects were visualized using simulations in Berkley Madonna [151]. Simulations of different dose regimens (using 1000 stochastic simulations per kg of body weight in NONMEM) were performed to optimise the treatment in populations of interest.

3.2.3 Pharmacokinetic and pharmacodynamic properties of piperaquine and markers for resistance

Due to the sparse nature of the samples in this study, a prior approach were undertaken. The prior model was based on a previous model by Tarning *et al.* [42]. The prior model was developed on concentrations measured in venous blood. Therefore, the conversion term from the meta-analysis was added to the model. The pharmacodynamic data was modelled using a time-to-event approach, in which a constant hazard model, a Weibull model and a Gompertz model were evaluated to describe the baseline hazard. The patients were followed up at fixed time intervals and the exact time of a recrudescent malaria infection was therefore unknown. To handle this, the survival was modelled with interval censoring. The interval was assumed to start at the last malaria-free follow-up visit and end at the next follow-up visit (in which a recrudescent malaria infection was noted). Survival up until the start of the interval was calculated according to equation 17 and the cumulative survival during the interval was calculated according to equation 18.

$$S_t = e^{-CHZ_{t,s}} \tag{17}$$

$$S_{t,e} = 1 - e^{CHZ_{t,s} - CHZ_{t,e}} \tag{18}$$

where S_t is the survival until the start of the interval, $CHZ_{t,s}$ is the cumulative hazard (according to the hazard-model) up to the start of interval, $S_{t,e}$ is the survival during the interval and $CHZ_{t,e}$ is the cumulative hazard (according to the hazard-model) during the interval. Patients with no recrudescent malaria infection were censored at the end of the study (right censoring).

To link the pharmacokinetics and the pharmacodynamic model, the individual pharmacokinetic parameters (received from the prior model) were

imputed in the data set and the individual concentrations were affecting the baseline hazard through an E_{max}-model.

Several covariates were tried in the model (i.e. sex, age, initial parasite density, body weight, and study site). In addition, mixture models were evaluated on IC50 and on the baseline hazard, in which two different populations with different IC50 or baseline hazard estimated, to investigate potential resistance against piperaquine or dihydroartemisinin.

3.2.4 Interaction between antimalarial and antiretroviral drugs

The antimalarial and the anti-retroviral drug data were analysed separately.

The antimalarial data were transformed into molar units and metabolite models, describing both the parent drugs and the metabolites simultaneously, were fitted to the data. It was assumed for both artemether and lumefantrine that 100% of the drugs were converted into their respective metabolites dihydroartemisinin and desbutyl-lumefantrine. A previous study has indicated a time-dependency in the pharmacokinetics of artemether [56]. Therefore, an enzymatic auto-induction model was investigated to describe changes in apparent elimination clearance over time.

Missing concentration data were either omitted or fitted with the M3-method (where concentrations above the limit of quantification are modelled as continuous data, and data reported to be below the limit of quantification are modelled as categorical data, evaluating the probability that a concentration is below the limit of quantification at a certain time point after dose) [152]. These approaches were evaluated through simulation-based diagnostics focusing on predicted and observed fraction of censored drug measurments, and through evaluations of the final parameter estimates.

Concomitant drug effects of efavirenz, nevirapine, and lopinavir/ritonavir were investigated as a categorical covariate on elimination clearance and bioavailability, before any other covariate effects were evaluated.

The pharmacokinetics of efavirenz and nevirapine were modelled and concomitant treatment with artemether-lumefantrine was evaluated as a categorical covariate in the model.

Identified drug-drug interactions were evaluated with stochastic simulations (1000 simulations in NONMEM) and new dose regimens were suggested if

necessary. Lumefantrine exhibits a dose dependent absorption and this was taken into account by assuming that a 100% increase in dose only increased the area under the concentration-time curve by 70% [66].

4 RESULTS AND DISCUSSION

4.1 Effect of pregnancy on the pharmacokinetics of piperaquine

The changes in piperaquine concentrations over time were described by a three-compartment disposition model. The absorption phase was described by three transit compartments. In the final model, between-subject variability was included on elimination clearance and bioavailability, and between occasion variability was included on the mean transit time and on the bioavailability. The elimination clearance was estimated to 44 L/h, which is lower compared to previous studies [41, 43]. However, this was the first time piperaquine pharmacokinetics was evaluated in a pregnant African population, using a population pharmacokinetic approach, which might explain the differences. Other pharmacokinetic parameter estimates were in line with what have been reported in previous studies [43, 153–156]. Statistical analysis of the area under the concentration-time curve showed no difference in exposure between pregnant and non-pregnant women.

To evaluate the possibility to detect differences in the pharmacokinetics between pregnant and non-pregnant women, a Monte Carlo Mapped Power analysis was performed. A previous study identified a pregnancy effect on apparent elimination clearance and bioavailability [43]. The analysis showed that in order to identify the pregnancy effect on elimination clearance and bioavailability with 80% power, a total of 8 and 13 patients, respectively, were needed in each group (pregnant and non-pregnant women) (Figure 2). The current study included 12 women in each group, which is not enough to identify all previously reported covariates. Instead a full covariate model approach was applied. As seen in Figure 3. the clinical impact of pregnancy was found to be low. Pregnancy might have a relevant impact on intercompartmental clearance. However, this will not impact the exposure of the drug. It was also found in the full covariate approach that the apparent elimination clearance is somewhat different in the second and third trimester (see Paper I).

Final parameter estimates, relative standard errors and diagnostic plots are reported in Paper I.

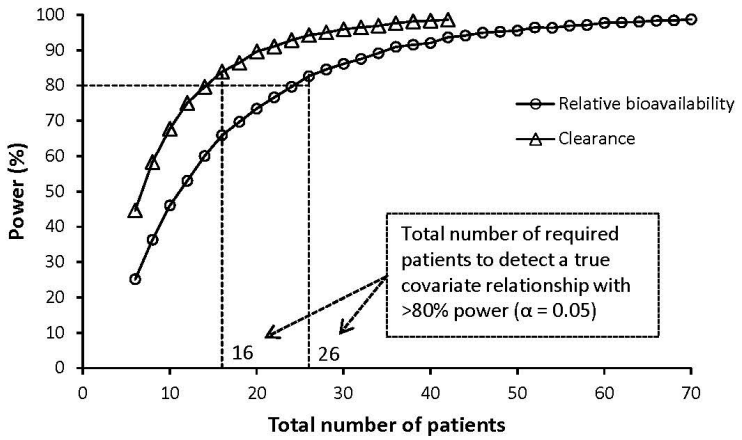

Figure 2. Monte-Carlo Mapped Power (MCMP) curve for identifying pregnancy as a covariate. Triangles represents the power curve for identifying pregnancy as covariate on apparent elimination clearance and circles the power curve for identifying pregnancy as a covariate on the relative bioavailability. The dotted black line represents 80% power. The inserted numbers are the total number of subjects needed to identify pregnancy as a covariate, given the used model and study sampling procedure.

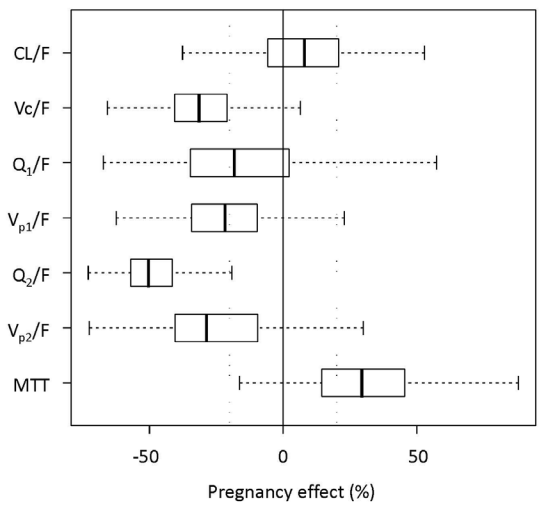

*Figure 3. Box (25th to 75th percentile) and whisker (1.5*interquartile range) plot of the full pregnancy-covariate model for piperaquine. Pregnancy was included as a categorical covariate and the solid black zero-line represents no covariate effect and the dotted black lines represent a covariate effect of ±20%. MTT is the mean transit time, Vc/F, Vp1/F and Vp2/F are the apparent volume of the central compartment, the first peripheral compartment and the second peripheral compartment, respectively. CL/F is the apparent elimination clearance and Q1/F and Q2/F are the apparent inter-compartmental clearances.*

4.2 Pharmacokinetic and pharmacodynamic properties of piperaquine in a pooled analysis

The pooled data was successfully described by a three-compartment disposition model with two transit compartments in the absorption phase. Between-subject variability was used on all parameters. Between occasion variability was added on the mean transit time and the bioavailability. The conversion factor between venous and capillary concentrations was estimated to 149%.

A disease effect was identified on mean transit time and the apparent elimination clearance. The mean transit time was 36.0% lower, and the elimination clearance 52.7% lower, in healthy individuals compared with patients, resulting in a higher exposure to piperaquine, and a slightly higher maximum concentration in healthy individuals than in patients. Weight was included as an allometrically scaled covariate to explain differences in all clearance and volume parameters. Diagnostic plots revealed that the between-occasion variability could not explain all the observed variability in the bioavailability between dosing occasions for piperaquine. An increase of the between occasion variability of 30.0% per dosing occasion was identified by estimation of an extra parameter. This change in bioavailability could be a result of changed absorption during the recovery from malaria.

Stochastic simulations of patients weighting between 5 and 100 kg, using the final model and the manufacturer's dose regimen, showed an under-exposure in small children (Figure 4). A new simple and optimised dose regimen was developed (Table 2), which resulted in adequate exposure in all weight groups without reaching higher maximum concentrations than those observed with the manufacturer's dose regimen.

Final parameter estimates, relative standard errors and diagnostic plots are reported in Paper II.

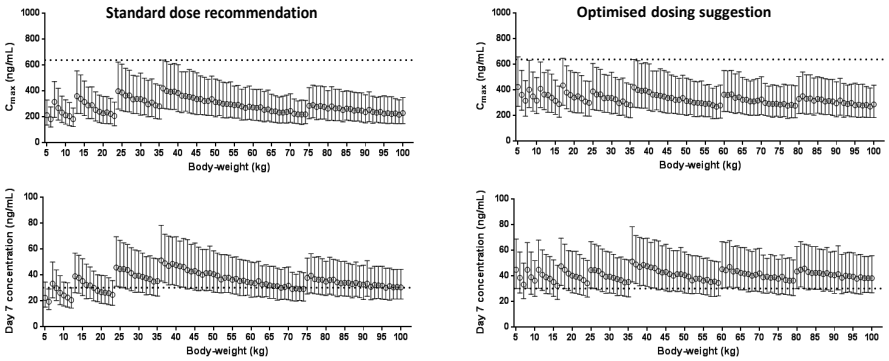

Figure 4. Stochastic simulation of two dose regimens of piperaquine. To the left, the simulations of the standard regimen are presented, and to the right, the simulations of the new optimised dose regimen are presented. The circles represent the median value and the vertical line represents the 50 %-simulation interval. The dashed horizontal lines represent the maximum concentration after a standard regimen (in the top row) and the threshold value of 30 ng/mL (in the bottom row).

Table 2. Dose regimens for piperaquine

Standard dose regimen			**Optimised dose regimen**		
Body weight (kg)	No. tablets/day	PQP/day (mg/kg)	Body weight (kg)	No. tablets/day	PQP/day (mg/kg)
5-6	0.25	13.3-16.0	5-7	0.5	22.9-32.0
7-12	0.5	13.3-22.9	8-10	0.75	24.0-30.0
13-23	1	13.9-24.6	11-16	1	20.0-29.1
24-35	2	18.3-26.7	17-24	1.5	20.0-28.2
36-74	3	13.0-26.7	25-35	2	18.3-25.6
75-100	4	12.8-17.1	36-59	3	16.3-26.7
			60-79	4	16.2-21.3
			80-100	5	16-20.0

No. tablets/day is the daily number of tablets. PQP/day is the total daily dose of piperaquine phosphate (mg/kg).

4.3 Pharmacokinetic and pharmacodynamic properties of piperaquine and markers for resistance

A total of 39 patients (236 samples) from study site 1, 33 patients (271 samples) from study site 2 and 32 patients (242 samples) from study site 3 were used in the analysis. In total 13.5% of the patients in the study had a recrudescent malaria infection; 25.6% in western Cambodia, 12.1% in northern Cambodia, and no failures in eastern Cambodia.

The three-compartment disposition model, including the conversion factor between venous and capillary concentrations found in the meta-analysis (Paper II), successfully described the capillary concentrations from this study.

The occurrence of recrudescent malaria was described by a constant hazard model, where the drug concentrations affected the hazard through an E_{max}-model. Estimating the hill coefficient in the E_{max}-model did not improve the fit, and it was therefore fixed to one throughout the analysis. Of the evaluated covariates, a difference between study sites was significant on both the IC50 and on the baseline hazard, with nearly the same drop in objective function value. Adding the covariate effect on IC50 resulted in a slightly lower objective function and was therefore included in the final model (Table 3). No other covariates were found significant. A difference in IC50 between the study sites might indicate piperaquine resistance. However, more data and/or *in vivo* IC50 values are needed to confirm this. Simulation based diagnostics (visual predictive checks) from the final model, stratified on the different study sites, is presented in (Figure 5).

Table 3. Parameter estimates from the pharmacokinetic-pharmacodynamic time-to-event model

	Population estimate [RSE %]	95% CI[a]
Baseline hazard (recrudescent infections/hour)	0.000832 [116]	0.000275-0.00833
$IC50_{site1}$ (ng/mL)	10.4 [123]	0.429-71.2
$IC50_{site2}$ (ng/mL)	2.77 [203]	0.167-31.8
$IC50_{site3}$ (ng/mL)	0 [0]	0
Hill	1 *fix*	

Where $IC50_{site1}$ is the half maximal inhibitory concentration at study site 1, $IC50_{site2}$ is the half maximal inhibitory concentration at study site 2, $IC50_{site3}$ is the half maximal inhibitory concentration at study site 3, and Hill is the factor describing the slope in the E_{max} model. The 95% confidence intervals (CI) are given as the 2.5 to 97.5 percentiles of bootstrap estimates.

[a.] Based on 952 successful resampled bootstraps runs (out of 1000)

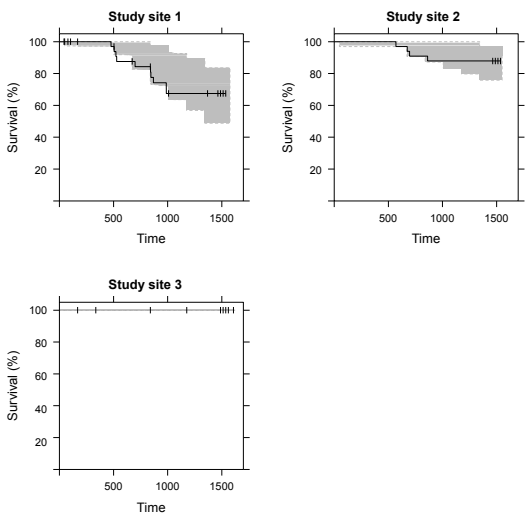

Figure 5. Visual predictive checks of the time-to-event model, stratified on study sites. The solid black line represents the observed survival. The shaded area represents the 95% confidence interval of the survival from the model (2000 simulations).

4.4 The influence of HIV-therapy on the pharmacokinetics of artemether-lumefantrine

Lumefantrine and desbutyl-lumefantrine concentrations were described by a metabolite model with two disposition compartments describing lumefantrine and one disposition compartment describing desbutyl-lumefantrine. Between-subject variability was included on apparent elimination clearance, mean transit time of the absorption phase, and the relative bioavailability. The three different antiretroviral therapies (efavirenz, nevirapine, and ritonavir boosted lopinavir) were included as categorical covariates. Efavirenz increased the elimination clearance of lumefantrine by 72.6%, nevirapine lowered the relative bioavailability of lumefantrine by 24.8%, and ritonavir boosted lopinavir lowered the apparent elimination clearance of lumefantrine by 62.1 and increased the elimination of desbutyl-lumefantrine by 392%.

The increase in lumefantrine elimination clearance when co-administrated with efavirenz is most likely a result of increased expression of CYP3A4, resulting in an increase in the hepatic extraction. The decrease in lumefantrine bioavailability when co-administrated with nevirapine might be a result of induction of intestinal P-glycoprotein expression or induction of intestinal CYP3A4. Lopinavir/ritonavir inhibits CYP3A4 activity [98], which results in the seen decreased elimination clearance of lumefantrine and consequently an increased exposure. In addition, the elimination clearance of desbutyl-lumefantrine was increased by lopinavir/ritonavir treatment in this study, resulting in decreased exposure to desbutyl-lumefantrine. The mechanism of elimination for desbutyl-lumefantrine is not known, and therefore the effect of lopinavir/ritonavir could be a result of changes in the elimination of desbutyl-lumefantrine or it could be a consequence of changes in other, unknown, metabolites of lumefantrine.

Artemether and dihydroartemisinin concentrations were also described with a metabolite model. However, this time a one-compartment disposition model was used to describe both the parent drug and the metabolite. Between-subject variability was included on elimination clearance of both artemether and dihydroartemisinin, and also on the absorption parameters (mean transit time and relative bioavailability). Concomitant treatment with efavirenz decreased the bioavailability of artemether by 71.5%. Nevirapine decreased

the bioavailability of artemether by 66.3%, and the elimination clearance of dihydroartemisinin by 44.5%. Ritonavir boosted lopinavir increased the elimination clearance of both artemether by 32.8% and dihydroartemisinin by 143%.

The previous findings on interaction between efavirenz/nevirapine and P-glycoprotein are contradictory. An in-vitro study showed that efavirenz and nevirapine induce the expression of P-glycoprotein [97], which could explain the observed decrease in relative bioavailability by these antiretroviral drugs. Another explanation could be induction of intestinal CYP3A4 enzymes, although this would preferentially affect artemether. Nevirapine also decreased the elimination clearance of dihydroartemisinin, but not to the same extent as it decreased the bioavailability of artemether, resulting in a total decrease in the exposure to dihydroartemisinin. The decrease in dihydroartemisinin elimination clearance in the present study is difficult to explain since nevirapine has not been reported to affect the UGT-system. The present study showed similar results compared with earlier studies with the exception of the study by Kredo *et al.* in which they present a trend towards increased exposure [119]. This might be explained by between-subject variability, different study sizes (36 compared with 89 in this study) and different study designs (parallel compared with cross-over in this study).

Lopinavir/ritonavir increased the elimination clearance of both artemether and dihydroartemisinin resulting in decreased exposures. Lopinavir/ritonavir induces other CYP enzymes such as CYP2B6, CYP2C9, and CYP2C19. Induction of CYP enzymes seems the most likely explanation for the observed increase in the elimination clearance of artemether. The increase in dihydroartemisinin elimination clearance is unexpected since lopinavir/ritonavir inhibits several UGT enzymes which would be expected to result in a decreased clearance. The increased clearance is so far unexplained but it could be a consequence of lopinavir dependent induction of other unknown metabolic pathways of artemether.

New dose regimens were suggested, based on stochastic simulations from the final models, and boxplots of drug exposure for the different scenarios are presented in Figure 6. The patients in the present study were not infected with malaria which could change the dose recommendation. Therefore, new studies are needed in malaria and HIV co-infected populations.

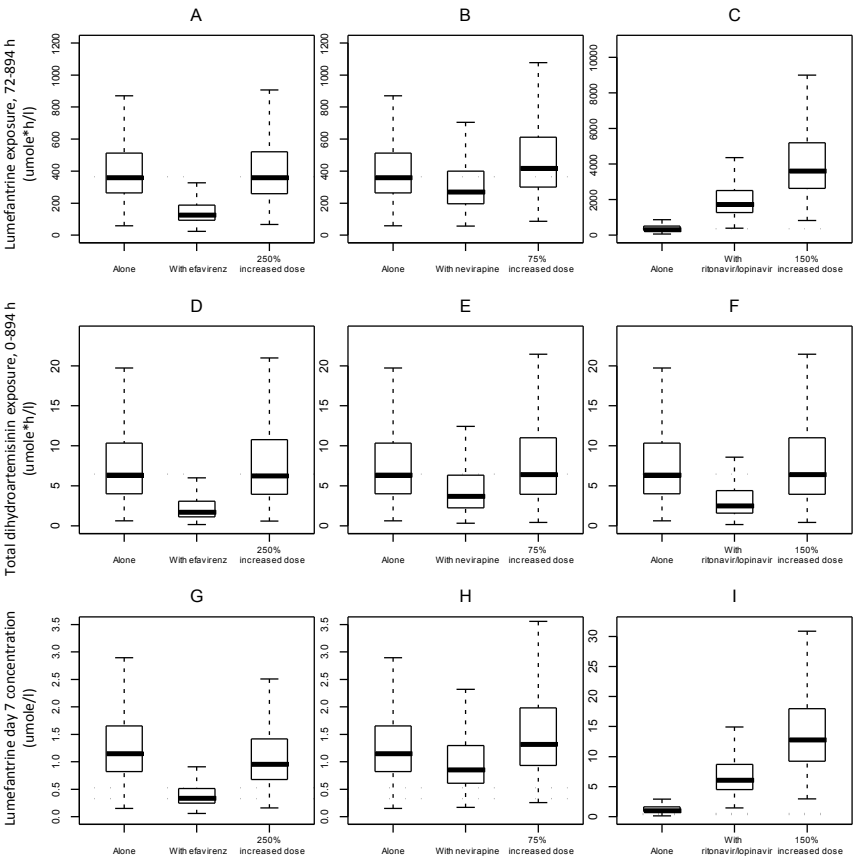

Figure 6. Box (25th to 75th percentile) and whisker (1.5×interquartile range) plot of dose simulations. The top row illustrates the simulated terminal exposures (AUC) from 72 hours to infinity for lumefantrine when given alone, in combination with HIV-treatment and after an adjusted dose regimen [efavirenz (A), nevirapine (B) and lopinavir/ritonavir (C)]. The middle row illustrates the simulated exposures (AUC) from 0 hours to infinity for dihydroartemisinin when given alone, in combination with HIV-treatment and after an adjusted dose regimen [efavirenz (D), nevirapine (E) or lopinavir/ritonavir (F)]. The bottom row illustrates the simulated day 7 concentrations for lumefantrine when given alone, in combination with HIV-treatment and after an adjusted dose regimen [efavirenz (G), nevirapine (H) or lopinavir/ritonavir (I)]. The dotted lines in the top and middle rows represent the standard exposures when the anti-malarial treatment is given alone. The dotted lines in the bottom row represent previously defined day 7 cut-off concentration for therapeutic failure of 280 ng/mL and 175 ng/mL.

4.5 The influence of antimalarial-therapy on the pharmacokinetics of nevirapine and efavirenz

Efavirenz concentrations were described by a two-compartment disposition model. Concomitant treatment with artemether-lumefantrine did not have an effect on the pharmacokinetics of efavirenz, and simulation of new doses was therefore not necessary.

Nevirapine concentrations were described by a one-compartment disposition model. Data in the absorption phase was lacking and a two transit-compartment absorption model was used, based on a previous meta-analysis [90]. Concomitant treatment with artemether-lumefantrine had a significant effect on the elimination clearance, resulting in an increase in elimination clearance of 65.4%, possibly due to artemether mediated induction of CYP3A4.

Due to the short treatment duration of antimalarial therapy (3 days), this should be of limited clinical significance but could have an impact in vulnerable populations, such as pregnant women. Therefore, new dose simulations, based on two different scenarios, were performed. In the first scenario it was assumed that the elimination clearance increased linearly from its uninduced steady-state value, and reached the new induced clearance value after the last artemether-lumefantrine dose. Thereafter, the elimination clearance was constant for the rest of the week. In this scenario an increased nevirapine dose of 100% over the three days of antimalarial therapy would result in standard exposure during the week (Figure 7). In the second scenario it was assumed that the new induced clearance was just constant for one day before it started to return to the uninduced value (same slope as during the increase). In this scenario, an increased nevirapine dose of 50% would be enough to yield a standard exposure (Figure 8). These two scenarios need to be evaluated in a population co-infected with HIV and malaria.

Final parameter estimates, relative standard errors and diagnostic plots are reported in Paper V.

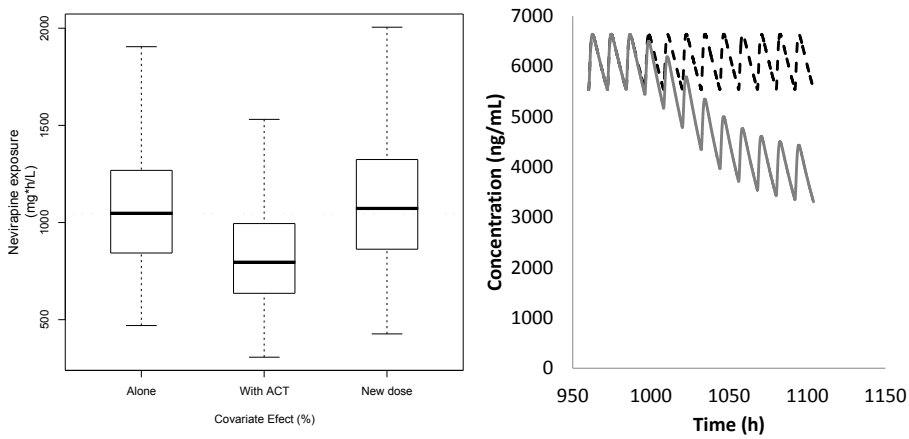

Figure 7. Simulation of drug exposure of nevirapine, when given together with artemether-lumefantrine, assuming an increase of nevirapine elimination clearance, reaching a constant value on day 3 of antimalarial therapy. Box (25th to 75th percentile) and whisker (1.5×interquartile range) plot (left) illustrating how the exposure (accumulated area under the time-concentration curve over 1 week of treatment at steady state) of nevirapine changes when given alone (without artemether-lumefantrine, starting on day two), when given together with artemether-lumefantrine and when increasing the dose with 100% during the three days that artemether-lumefantrine was given. The right graph shows the steady-state concentration-time profile of nevirapine over one week of treatment when given alone (dotted black line) and when given together with artemether-lumefantrine (solid grey line).

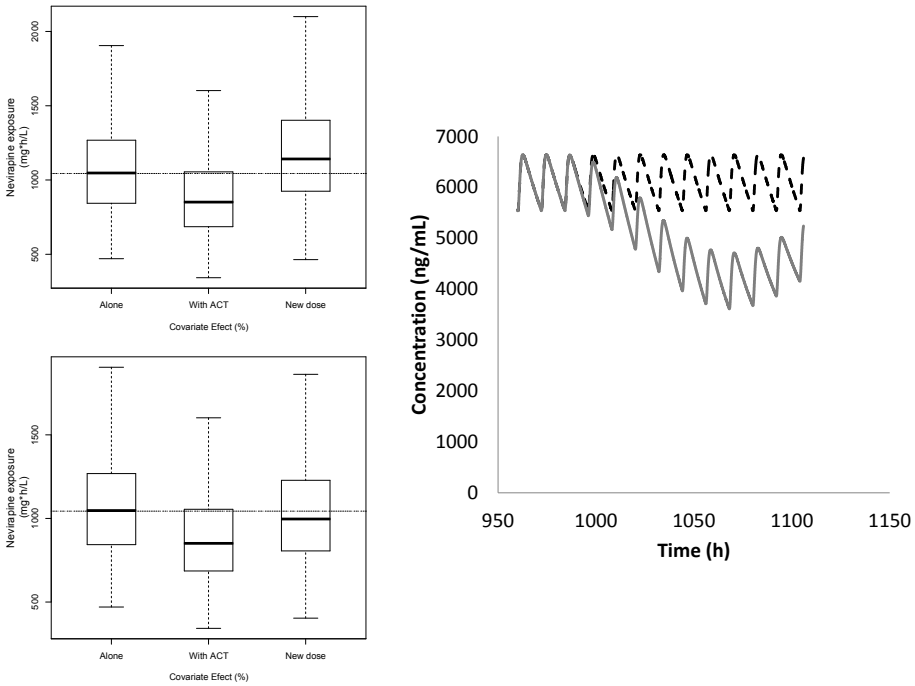

Figure 8. Simulation of drug exposure of nevirapine when given together with artemether-lumefantrine, assuming an increase of nevirapine elimination clearance, reaching a constant value on day 3 of antimalarial therapy, keeping that value for 24 hours, and thereafter, decrease linearly back towards the un-induced value. Box (25^{th} to 75^{th} percentile) and whisker (1.5×interquartile range) plot (left) illustrating how the exposure (accumulated area under the time-concentration curve over 1 week of treatment at steady state) of nevirapine changes when given alone (without artemether-lumefantrine, starting on day two), when given together with artemether-lumefantrine, and when increasing the dose with 100% (top) and 50% (bottom) during the three days that artemether-lumefantrine was given. The right graph shows the concentration-time profile of nevirapine over one week of treatment when given alone (dotted black line), and when given together with artemether-lumefantrine (solid black line).

5 GENERAL DISCUSSION

Dosing of antimalarial drugs has often been decided by trial and error, and not evidence-based. Recently, several studies and analyses have been performed to optimise antimalarial treatments in different populations [43, 44, 70, 131]. In this thesis, pharmacokinetic and pharmacodynamic modelling approaches have been utilized to: (1) optimise antimalarial therapy in pregnant women and children (the two most vulnerable groups), (2) investigate drug resistance, and (3) evaluate the impact of concomitant HAART therapy, both regarding the impact on the pharmacokinetics of the antimalarial treatment and regarding the impact on the pharmacokinetics of the antiretroviral treatment.

Pregnant women are especially vulnerable to malaria and the pharmacokinetics of several antimalarial drugs has been shown to change during pregnancy [43, 60, 70, 109–114]. The first study, included in this thesis, investigated the differences between pregnant and non-pregnant women regarding the pharmacokinetics of piperaquine. No differences were found in the exposure of the drug, which indicates that no dose adjustment is necessary. To further investigate the impact of pregnancy, a full covariate approach was conducted. This approach gives an opportunity to investigate which parameters are changed in pregnant women, compared to non-pregnant women. The approach revealed that the most probable change is a decrease in intercompartmental-clearance. This will have no impact on the total drug exposure but might have a small impact on the day 7 concentration (which sometimes is used to evaluate the treatment). However, a difference in day 7 concentrations between pregnant and non-pregnant women was not observed in this study. Furthermore, the difference in elimination clearance between different stages of pregnancy was evaluated, showing that the clearance increases between the second and third trimester of pregnancy, but this will not impact the exposure, and most likely not the clinical outcome.

Pregnancy is a risk factor in malaria, but the most vulnerable group, in regards to malaria infection and symptoms are children. In the second study, included in this thesis, an optimised dose regimen for piperaquine was developed. One thousand three hundred deaths out of the nearly 2000 deaths due to malaria each day, occurs in children [4]. Young children has an increased risk of a failed treatment, and they also have a lower drug exposure compared with adults [44, 157]. This shows the importance of optimising the treatment in children. Pharmacokinetic studies are often small, which limits the power to correctly describe the pharmacokinetics and the differences

between different groups. To maximize the power, data from several clinical studies was analysed simultaneously. Body weight had a significant impact on the pharmacokinetics, resulting in drug exposure profiles indicating an under-dosing in children. These findings are similar to what has previously been reported by Tarning *et al.* [44]. A new dose regimen for piperaquine was developed, to optimise the drug exposure for all body weights. The new dose regimen had to be simple, without too many dose bands. Today's dose regimen is safe, but increasing the maximum concentration further could increase the risk of serious side-effects such as QT-prolongation. Therefore, it was important to not increase the maximum concentration in the new dose regimen above the maximum concentration reached by the old dose regimen. The new dose regimen consists of eight dose intervals based on body weight. Simulations show that the new regimen will result in good exposure for all weight groups, and will not increase the maximum concentration reached by the old regimen. It should thereby result in a better treatment of malaria with dihydroartemisinin-piperaquine.

Due to the emergence of multi-drug resistance in Southeast Asia, especially against artemisinin derivates, the treatment guidelines have recently been changed. The combination dihydroartemisinin-piperaquine has been used in western Cambodia, but recently the efficacy of this combination has been failing [25]. In the third study in this thesis, the efficiency of dihydroartemisinin-piperaquine has been compared between three different sites in Cambodia through a time-to-event modelling approach. A time-to-event approach gives the possibility to characterize the outcome of a treatment, and to link the pharmacokinetics of the drug to the outcome (in this case recrudescent). There was a pronounced difference in outcome between the three study sites, and the categorical covariate 'study site' was found to be significant on both the baseline hazard and the IC50 of recrudescent. The difference between the sites could be a consequence of resistance to either piperaquine and/or dihydroartemisinin, but more data is needed to evaluate and investigate this further.

Due to the similar spread of malaria and HIV, co-infection and co-treatment of these two diseases will be common. It is therefore important to investigate drug-drug interaction between ACT and HAART, to ensure an adequate treatment. In this thesis, a considerable impact on the exposure of dihydroartemisinin and lumefantrine was found when concomitantly treated with HAART. Especially the HIV-drug efavirenz will lower the exposure of the ACT-drugs. A dose increase of 250% will result in adequate exposure of both lumefantrine and dihydroartemisinin. This is taking into consideration the dose dependent absorption of lumefantrine by raising the lumefantrine

exposure with just 70% for a 100% increase in dose. Nevirapine also had a limited impact on the ACT drugs and a dose increase of 75% was enough to receive standard exposure. The interaction between artemether-lumefantrine and ritonavir boosted lopinavir shows a different pattern compared to the rest of the studied interactions. When combined with ritonavir boosted lopinavir the exposure to lumefantrine increase while the exposure to dihydroartemisinin will decrease. This will result in a scenario in which the outcome should be as good or better compared to give artemether-lumefantrine alone and therefore no changes in dose should be necessary.

There is also a possibility that the antimalarial drugs can affect the exposure of the antiretroviral drugs, which was explored in the last study included in this thesis. Since the ACT is given during only three days, while HAART is a lifelong treatment, the potential interactions should have limited consequences on the HAART therapy. However, it might have consequences for risk groups. The developed model identified a statistically significant impact of concomitant artemether-lumefantrine treatment on the nevirapine clearance. Nevirapine is used to prevent the transfer of the HIV virus from the mother to the child [81], and a decreased nevirapine exposure could, during these circumstances, have severe consequences. Depending on which of the drugs or metabolites in the ACT treatment that are causing the interaction (this cannot be identified with the present study design), an increase of the nevirapine dose of 50-100% during the three days of ACT would be enough to receive standard exposure.

It is important to note that the two interaction studies investigated in this thesis were conducted in HIV patients without malaria. A malaria infection could change the pharmacokinetics of the different drugs and it is therefore important to evaluate the new suggested dose regimens in a population with both malaria and HIV. The interactions could also change with different doses. The proposed dose increases for artemether-lumefantrine, when administrated with efavirenz or nevirapine, could increase the induction of nevirapine (or efavirenz) elimination clearance and vice versa.

This thesis has focused on optimising anti-malarial treatment with piperaquine, and on investigating ACT-HAART interactions. The work shows that even though some pharmacokinetic parameters are different between pregnant and non-pregnant women, there is no need to change the dose regimen of piperaquine for pregnant women. A new dose regimen of piperaquine, based on body weight, was suggested to assure adequate drug exposure for children. Drug resistance patterns for dihydroartemisinin-piperaquine in Cambodia were explored. New doses for artemether-

lumefantrine co-administrated with HAART were developed, and new dose regimens for nevirapine when combined with ACT were developed. These findings will improve the treatment of malaria for different populations.

6 CONCLUSION

This thesis has investigated the pharmacokinetic and pharmacodynamic impact of antimalarial treatment. The first part investigated and optimised the treatment with piperaquine and the second part found considerable interactions between antiretroviral and antimalarial treatment. The following conclusions can be drawn:

- No clinical relevant impact of pregnancy on the pharmacokinetics of piperaquine was found.

- A new simple and optimised treatment regimen for piperaquine was developed.

- A time-to-event model for the risk of getting recrudescent malaria, in three different provinces in Cambodia was developed and a pharmacokinetic model for piperaquine was linked to the outcome model.

- Considerable impact on the pharmacokinetics of artemether, lumefantrine, and their respective metabolites dihydroartemisinin and desbutyl-lumefantrine, was found when individuals were concomitantly treated with efavirenz, nevirapine, or ritonavir boosted lopinavir. New doses have been developed for these antimalarial drugs when used at the same time as the antiretroviral drugs.

- A difference in the elimination clearance of nevirapine was found when administrated at the same time as artemether-lumefantrine. New doses for this interaction were developed.

7 FUTURE PERSPECTIVES

During the last years, drug resistance against the currently recommended treatment of malaria has been noted [23]. Optimising the antimalarial treatment would potentially slow the spread of resistance. Pharmacometrics gives an excellent opportunity to do this by maximizing the use of information from clinical trials. In this thesis, this approach has been used to optimise the treatment of malaria with piperaquine. The findings (no difference in exposure for pregnant compared with non-pregnant women, and a new dose regimen of piperaquine), may improve the current treatment with piperaquine, especially in children, and hopefully prolong the lifetime of dihydroartemisinin-piperaquine as a useful antimalarial drug combination.

Both malaria and HIV will, for the foreseeable future, continue to be common infectious diseases, especially in Africa south of Sahara. The findings in this thesis give the opportunity to increase the cure rate of malaria treated with artemether-lumefantrine, when combined with efavirenz or nevirapine. Further clinical studies and pharmacometric analyses of these interactions are necessary to fully optimise the changes in treatmen.

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REFERENCES

1. Benet LZ (1984) Pharmacokinetics: Basic Principles and Its use as a tool in drug metabolism". In: Horning J, Mitchell M (eds) Drug Metab. Drug Toxic. Raven Press, New York, NY, USA, pp 199–211

2. Barrett JS, Fossler MJ, Cadieu KD, Gastonguay MR (2008) Pharmacometrics: a multidisciplinary field to facilitate critical thinking in drug development and translational research settings. J Clin Pharmacol 48:632–49

3. Aarons L (1991) Population pharmacokinetics: theory and practice. Br J Clin Pharmacol 32:669–70

4. WHO (2013) World malaria report: 2013. Geneva, Switzerland

5. WHO (2011) Progress report 2011: Global HIV/AIDS response. Geneva, Switzerland

6. (2012) Management of severe malaria. Geneva, Switzerland

7. (2000) Severe falciparum malaria. World Health Organization, Communicable Diseases Cluster. Trans R Soc Trop Med Hyg 94 Suppl 1:S1–90

8. Trampuz A, Jereb M, Muzlovic I, Prabhu RM (2003) Clinical review: Severe malaria. Crit Care 7:315–23

9. Idro R, Marsh K, John CC, Newton CRJ (2010) Cerebral malaria: mechanisms of brain injury and strategies for improved neurocognitive outcome. Pediatr Res 68:267–74

10. Bull PC, Lowe BS, Kortok M, Molyneux CS, Newbold CI, Marsh K (1998) Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. Nat Med 4:358–60

11. Smereck J (2010) Malaria in Pregnancy: Update on Emergency Management. J Emerg Med. doi: S0736-4679(10)00298-2 [pii] 10.1016/j.jemermed.2010.04.029 [doi]

12. Schantz-Dunn J, Nour NM (2009) Malaria and pregnancy: a global health perspective. Rev Obs Gynecol 2:186–192

13. Abrams ET, Meshnick SR (2009) Malaria during pregnancy in endemic areas: a lens for examining maternal-fetal conflict. Am J Hum Biol 21:643–650

14. Lindsay S, Ansell J, Selman C, Cox V, Hamilton K, Walraven G (2000) Effect of pregnancy on exposure to malaria mosquitoes. Lancet 355:1972

15. Gillies MT (1980) The Role of Carbon Dioxide in Host-Finding by Mosquitoes (Diptera: Culicidae): A Review.

16. Nosten F, Brasseur P (2002) Combination therapy for malaria: the way forward? Drugs 62:1315–29

17. Nosten F, White NJ (2007) Artemisinin-based combination treatment of falciparum malaria. Am J Trop Med Hyg 77:181–92

18. White NJ (1999) Delaying antimalarial drug resistance with combination chemotherapy. Parassitologia 41:301–308

19. Denis MB, Davis TM, Hewitt S, Incardona S, Nimol K, Fandeur T, Poravuth Y, Lim C, Socheat D (2002) Efficacy and safety of dihydroartemisinin-piperaquine (Artekin) in Cambodian children and adults with uncomplicated falciparum malaria. Clin Infect Dis 35:1469–1476

20. Rogers WO, Sem R, Tero T, Chim P, Lim P, Muth S, Socheat D, Ariey F, Wongsrichanalai C (2009) Failure of artesunate-mefloquine combination therapy for uncomplicated Plasmodium falciparum malaria in southern Cambodia. Malar J 8:10

21. Verdrager J (1986) Epidemiology of the emergence and spread of drug-resistant falciparum malaria in South-East Asia and Australasia. J Trop Med Hyg 89:277–89

22. Payne D (1987) Spread of chloroquine resistance in Plasmodium falciparum. Parasitol Today 3:241–6

23. Klein EY (2013) Antimalarial drug resistance: a review of the biology and strategies to delay emergence and spread. Int J Antimicrob Agents 41:311–7

24. Dondorp AM, Nosten F, Yi P, et al (2009) Artemisinin resistance in Plasmodium falciparum malaria. N Engl J Med 361:455–67

25. Leang R, Barrette A, Bouth DM, Menard D, Abdur R, Duong S, Ringwald P (2013) Efficacy of dihydroartemisinin-piperaquine for treatment of uncomplicated Plasmodium falciparum and Plasmodium vivax in Cambodia, 2008 to 2010. Antimicrob Agents Chemother 57:818–26

26. Ariey F, Witkowski B, Amaratunga C, et al (2014) A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. Nature 505:50–5

27. The Joint United Nations Programme on HIV/AIDS (2012) Global fact sheet.

28. Coffin JM, Hughes SH, Varmus HE (1997) Course of Infection with HIV and SIV.

29. Slater AF Chloroquine: mechanism of drug action and resistance in Plasmodium falciparum. Pharmacol Ther 57:203–35

30. Sullivan DJ, Matile H, Ridley RG, Goldberg DE (1998) A common mechanism for blockade of heme polymerization by antimalarial quinolines. J Biol Chem 273:31103–7

31. Sullivan DJ, Gluzman IY, Russell DG, Goldberg DE (1996) On the molecular mechanism of chloroquine's antimalarial action. Proc Natl Acad Sci U S A 93:11865–70

32. Egan TJ, Ross DC, Adams PA (1994) Quinoline anti-malarial drugs inhibit spontaneous formation of beta-haematin (malaria pigment). FEBS Lett 352:54–7

33. Pagola S, Stephens PW, Bohle DS, Kosar AD, Madsen SK (2000) The structure of malaria pigment beta-haematin. Nature 404:307–10

34. Wesche DL, Schuster BG, Wang WX, Woosley RL (2000) Mechanism of cardiotoxicity of halofantrine. Clin Pharmacol Ther 67:521–9

35. Karunajeewa H, Lim C, Hung TY, Ilett KF, Denis MB, Socheat D, Davis TM (2004) Safety evaluation of fixed combination piperaquine plus dihydroartemisinin (Artekin) in Cambodian children and adults with malaria. Br J Clin Pharmacol 57:93–99

36. Hanboonkunupakarn B, Ashley EA, Jittamala P, et al (2014) Open-label crossover study of primaquine and dihydroartemisinin-piperaquine pharmacokinetics in healthy adult thai subjects. Antimicrob Agents Chemother 58:7340–6

37. Manning J, Vanachayangkul P, Lon C, et al (2014) Randomized, Double-Blind, Placebo-Controlled Clinical Trial of a Two-Day Regimen of Dihydroartemisinin-Piperaquine for Malaria Prevention Halted for Concern over Prolonged Corrected QT Interval. Antimicrob Agents Chemother 58:6056–67

38. Lon C, Manning JE, Vanachayangkul P, et al (2014) Efficacy of two versus three-day regimens of dihydroartemisinin-piperaquine for uncomplicated malaria in military personnel in northern Cambodia: an open-label randomized trial. PLoS One 9:e93138

39. Huang L, Parikh S, Rosenthal PJ, Lizak P, Marzan F, Dorsey G, Havlir D, Aweeka FT (2012) Concomitant efavirenz reduces pharmacokinetic exposure to the antimalarial drug artemether-lumefantrine in healthy volunteers. J Acquir Immune Defic Syndr 61:310–6

40. Sim IK, Davis TM, Ilett KF (2005) Effects of a high-fat meal on the relative oral bioavailability of piperaquine. Antimicrob Agents Chemother 49:2407–2411

41. Tarning J, Ashley EA, Lindegardh N, Stepniewska K, Phaiphun L, Day NP, McGready R, Ashton M, Nosten F, White NJ (2008) Population pharmacokinetics of piperaquine after two different treatment regimens with dihydroartemisinin-piperaquine in patients with Plasmodium falciparum malaria in Thailand. Antimicrob Agents Chemother 52:1052–1061

42. Tarning J, Lindegardh N, Lwin KM, Annerberg A, Kiricharoen L, Ashley E, White NJ, Nosten F, Day NPJ (2014) Population pharmacokinetic assessment of the effect of food on piperaquine bioavailability in patients with uncomplicated malaria. Antimicrob Agents Chemother 58:2052–8

43. Tarning J, Rijken MJ, McGready R, Phyo AP, Hanpithakpong W, Day NPJ, White NJ, Nosten F, Lindegardh N (2012) Population pharmacokinetics of dihydroartemisinin and piperaquine in pregnant and nonpregnant women with uncomplicated malaria. Antimicrob Agents Chemother 56:1997–2007

44. Tarning J, Zongo I, Somé FA, et al (2012) Population pharmacokinetics and pharmacodynamics of piperaquine in children with uncomplicated falciparum malaria. Clin Pharmacol Ther 91:497–505

45. Tarning J, Bergqvist Y, Day NP, Bergquist J, Arvidsson B, White NJ, Ashton M, Lindegårdh N (2006) Characterization of human urinary metabolites of the antimalarial piperaquine. Drug Metab Dispos 34:2011–9

46. Tarning J, Thana P, Phyo AP, Lwin KM, Hanpithakpong W, Ashley EA, Day NPJ, Nosten F, White NJ (2014) Population Pharmacokinetics and Antimalarial Pharmacodynamics of Piperaquine in Patients With Plasmodium vivax Malaria in Thailand. CPT pharmacometrics Syst Pharmacol 3:e132

47. Price RN, Hasugian AR, Ratcliff A, et al (2007) Clinical and pharmacological determinants of the therapeutic response to dihydroartemisinin-piperaquine for drug-resistant malaria. Antimicrob Agents Chemother 51:4090–4097

48. Klayman DL (1985) Qinghaosu (artemisinin): an antimalarial drug from China. Science 228:1049–55

49. Golenser J, Waknine JH, Krugliak M, Hunt NH, Grau GE (2006) Current perspectives on the mechanism of action of artemisinins. Int J Parasitol 36:1427–41

50. Li GQ, Guo XB, Fu LC, Jian HX, Wang XH (1994) Clinical trials of artemisinin and its derivatives in the treatment of malaria in China. Trans R Soc Trop Med Hyg 88 Suppl 1:S5–6

51. Sagara I, Rulisa S, Mbacham W, et al (2009) Efficacy and safety of a fixed dose artesunate-sulphamethoxypyrazine-pyrimethamine compared to artemether-lumefantrine for the treatment of uncomplicated falciparum malaria across Africa: a randomized multi-centre trial. Malar J 8:63

52. Navaratnam V, Mansor SM, Sit NW, Grace J, Li Q, Olliaro P (2000) Pharmacokinetics of artemisinin-type compounds. Clin Pharmacokinet 39:255–70

53. Kokwaro G, Mwai L, Nzila A (2007) Artemether/lumefantrine in the treatment of uncomplicated falciparum malaria. Expert Opin Pharmacother 8:75–94

54. Ilett KF, Ethell BT, Maggs JL, et al (2002) Glucuronidation of dihydroartemisinin in vivo and by human liver microsomes and expressed UDP-glucuronosyltransferases. Drug Metab Dispos 30:1005–12

55. Khoo S, Back D, Winstanley P (2005) The potential for interactions between antimalarial and antiretroviral drugs. AIDS 19:995–1005

56. Van Agtmael MA, Cheng-Qi S, Qing JX, Mull R, van Boxtel CJ (1999) Multiple dose pharmacokinetics of artemether in Chinese patients with uncomplicated falciparum malaria. Int J Antimicrob Agents 12:151–8

57. Ali S, Najmi MH, Tarning J, Lindegardh N (2010) Pharmacokinetics of artemether and dihydroartemisinin in healthy Pakistani male volunteers treated with artemether-lumefantrine. Malar J 9:275

58. Lefèvre G, Bindschedler M, Ezzet F, Schaeffer N, Meyer I, Thomsen MS (2000) Pharmacokinetic interaction trial between co-artemether and mefloquine. Eur J Pharm Sci 10:141–51

59. Van Agtmael MA, Gupta V, van der Graaf CA, van Boxtel CJ (1999) The effect of grapefruit juice on the time-dependent decline of

artemether plasma levels in healthy subjects. Clin Pharmacol Ther 66:408–14

60. Tarning J, Kloprogge F, Piola P, et al (2012) Population pharmacokinetics of Artemether and dihydroartemisinin in pregnant women with uncomplicated Plasmodium falciparum malaria in Uganda. Malar J. doi: 10.1186/1475-2875-11-293

61. Lefèvre G, Carpenter P, Souppart C, Schmidli H, McClean M, Stypinski D (2002) Pharmacokinetics and electrocardiographic pharmacodynamics of artemether-lumefantrine (Riamet) with concomitant administration of ketoconazole in healthy subjects. Br J Clin Pharmacol 54:485–92

62. Mosha D, Guidi M, Mwingira F, Abdulla S, Mercier T, Decosterd LA, Csajka C, Genton B (2014) Population pharmacokinetics and clinical response for artemether-lumefantrine in pregnant and nonpregnant women with uncomplicated Plasmodium falciparum malaria in Tanzania. Antimicrob Agents Chemother 58:4583–92

63. Wong RPM, Salman S, Ilett KF, Siba PM, Mueller I, Davis TME (2011) Desbutyl-lumefantrine is a metabolite of lumefantrine with potent in vitro antimalarial activity that may influence artemether-lumefantrine treatment outcome. Antimicrob Agents Chemother 55:1194–8

64. Hatz C, Soto J, Nothdurft HD, et al (2008) Treatment of acute uncomplicated falciparum malaria with artemether-lumefantrine in nonimmune populations: a safety, efficacy, and pharmacokinetic study. Am J Trop Med Hyg 78:241–7

65. Salman S, Page-Sharp M, Griffin S, Kose K, Siba PM, Ilett KF, Mueller I, Davis TME (2011) Population pharmacokinetics of artemether, lumefantrine, and their respective metabolites in Papua New Guinean children with uncomplicated malaria. Antimicrob Agents Chemother 55:5306–13

66. Ashley EA, Stepniewska K, Lindegårdh N, et al (2007) Pharmacokinetic study of artemether-lumefantrine given once daily for the treatment of uncomplicated multidrug-resistant falciparum malaria. Trop Med Int Health 12:201–8

67. Ashley EA, Stepniewska K, Lindegårdh N, Annerberg A, Kham A, Brockman A, Singhasivanon P, White NJ, Nosten F (2007) How much fat is necessary to optimize lumefantrine oral bioavailability? Trop Med Int Health 12:195–200

68. Van Vugt M, Ezzet F, Nosten F, Gathmann I, Wilairatana P, Looareesuwan S, White NJ (1999) No evidence of cardiotoxicity during antimalarial treatment with artemether-lumefantrine. Am J Trop Med Hyg 61:964–7

69. Tarning J, Kloprogge F, Dhorda M, Jullien V, Nosten F, White NJ, Guerin PJ, Piola P (2013) Pharmacokinetic Properties of Artemether, Dihydroartemisinin, Lumefantrine, and Quinine in Pregnant Women with Uncomplicated Plasmodium falciparum Malaria in Uganda. Antimicrob Agents Chemother 57:5096–103

70. Kloprogge F, Piola P, Dhorda M, et al (2013) Population Pharmacokinetics of Lumefantrine in Pregnant and Nonpregnant Women With Uncomplicated Plasmodium falciparum Malaria in Uganda. CPT pharmacometrics Syst Pharmacol 2:e83

71. Price RN, Uhlemann A-C, van Vugt M, et al (2006) Molecular and pharmacological determinants of the therapeutic response to artemether-lumefantrine in multidrug-resistant Plasmodium falciparum malaria. Clin Infect Dis 42:1570–7

72. Ezzet F, Mull R, Karbwang J (1998) Population pharmacokinetics and therapeutic response of CGP 56697 (artemether + benflumetol) in malaria patients. Br J Clin Pharmacol 46:553–61

73. WHO (2013) Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach. Geneva, Switzerland

74. Ministry of Health (Uganda) (2009) National Antiretroviral Treatment Guidelines for Adults, Adolescents, and Children.

75. Erickson DA, Mather G, Trager WF, Levy RH, Keirns JJ (1999) Characterization of the in vitro biotransformation of the HIV-1 reverse transcriptase inhibitor nevirapine by human hepatic cytochromes P-450. Drug Metab Dispos 27:1488–95

76. Robertson SM, Maldarelli F, Natarajan V, Formentini E, Alfaro RM, Penzak SR (2008) Efavirenz induces CYP2B6-mediated hydroxylation of bupropion in healthy subjects. J Acquir Immune Defic Syndr 49:513–9

77. Fellay J, Marzolini C, Decosterd L, Golay KP, Baumann P, Buclin T, Telenti A, Eap CB (2005) Variations of CYP3A activity induced by antiretroviral treatment in HIV-1 infected patients. Eur J Clin Pharmacol 60:865–73

78. Squibb B-M SUSTIVA®(efavirenz) product information leaflet. http://packageinserts.bms.com/pi/pi_sustiva.pdf. Accessed 9 Jul 2014

79. Mukonzo JK, Nanzigu S, Rekić D, Waako P, Röshammar D, Ashton M, Ogwal-Okeng J, Gustafsson LL, Aklillu E (2011) HIV/AIDS patients display lower relative bioavailability of efavirenz than healthy subjects. Clin Pharmacokinet 50:531–40

80. Natarajan U, Pym A, McDonald C, Velisetty P, Edwards SG, Hay P, Welch J, de Ruiter A, Taylor GP, Anderson J (2007) Safety of nevirapine in pregnancy. HIV Med 8:64–9

81. Guay LA, Musoke P, Fleming T, et al (1999) Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. Lancet 354:795–802

82. Back D, Gibbons S, Khoo S (2003) Pharmacokinetic drug interactions with nevirapine. J Acquir Immune Defic Syndr 34 Suppl 1:S8–14

83. Lamson M, MacGregor T, Riska P, Erickson D, Maxfield P, Rowland L, Gigliotti M, Robinson P, Azzam S, Keirns J (1999) Nevirapine induces both CYP3A4 and CYP2B6 metabolic pathways. Clin Pharmacol Ther 65:137–137

84. Chou M, Bertrand J, Segeral O, et al (2010) Population pharmacokinetic-pharmacogenetic study of nevirapine in HIV-infected Cambodian patients. Antimicrob Agents Chemother 54:4432–9

85. Dailly E, Raffi F, Perré P, Martin J, Deslandes G, Jolliet P (2009) Influence of darunavir coadministration on nevirapine

pharmacokinetics in HIV-infected patients: a population approach. HIV Med 10:586–9

86. De Maat MMR, Huitema ADR, Mulder JW, Meenhorst PL, van Gorp ECM, Beijnen JH (2002) Population pharmacokinetics of nevirapine in an unselected cohort of HIV-1-infected individuals. Br J Clin Pharmacol 54:378–85

87. Elsherbiny D, Cohen K, Jansson B, Smith P, McIlleron H, Simonsson USH (2009) Population pharmacokinetics of nevirapine in combination with rifampicin-based short course chemotherapy in HIV- and tuberculosis-infected South African patients. Eur J Clin Pharmacol 65:71–80

88. Kappelhoff BS, van Leth F, MacGregor TR, Lange J, Beijnen JH, Huitema ADR (2005) Nevirapine and efavirenz pharmacokinetics and covariate analysis in the 2NN study. Antivir Ther 10:145–55

89. Sabo JP, Lamson MJ, Leitz G, Yong CL, MacGregor TR (2000) Pharmacokinetics of nevirapine and lamivudine in patients with HIV-1 infection. AAPS PharmSci 2:E1

90. Svensson E, van der Walt J-S, Barnes KI, Cohen K, Kredo T, Huitema A, Nachega JB, Karlsson MO, Denti P (2012) Integration of data from multiple sources for simultaneous modelling analysis: experience from nevirapine population pharmacokinetics. Br J Clin Pharmacol 74:465–76

91. Zhou XJ, Sheiner LB, D'Aquila RT, Hughes MD, Hirsch MS, Fischl MA, Johnson VA, Myers M, Sommadossi JP (1999) Population pharmacokinetics of nevirapine, zidovudine, and didanosine in human immunodeficiency virus-infected patients. The National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group Protocol 241 Investigators. Antimicrob Agents Chemother 43:121–8

92. Viramune® (nevirapine) Tablets.

93. Almond LM, Edirisinghe D, Dalton M, Bonington A, Back DJ, Khoo SH (2005) Intracellular and plasma pharmacokinetics of nevirapine in human immunodeficiency virus-infected individuals. Clin Pharmacol Ther 78:132–42

94. Burhenne J, Matthée A-K, Pasáková I, Röder C, Heinrich T, Haefeli WE, Mikus G, Weiss J (2010) No evidence for induction of ABC transporters in peripheral blood mononuclear cells in humans after 14 days of efavirenz treatment. Antimicrob Agents Chemother 54:4185–91

95. Chandler B, Almond L, Ford J, Owen A, Hoggard P, Khoo S, Back D (2003) The effects of protease inhibitors and nonnucleoside reverse transcriptase inhibitors on p-glycoprotein expression in peripheral blood mononuclear cells in vitro. J Acquir Immune Defic Syndr 33:551–6

96. Oswald S, Meyer zu Schwabedissen HE, Nassif A, et al (2012) Impact of efavirenz on intestinal metabolism and transport: insights from an interaction study with ezetimibe in healthy volunteers. Clin Pharmacol Ther 91:506–13

97. Störmer E, von Moltke LL, Perloff MD, Greenblatt DJ (2002) Differential modulation of P-glycoprotein expression and activity by non-nucleoside HIV-1 reverse transcriptase inhibitors in cell culture. Pharm Res 19:1038–45

98. Yeh RF, Gaver VE, Patterson KB, Rezk NL, Baxter-Meheux F, Blake MJ, Eron JJ, Klein CE, Rublein JC, Kashuba ADM (2006) Lopinavir/ritonavir induces the hepatic activity of cytochrome P450 enzymes CYP2C9, CYP2C19, and CYP1A2 but inhibits the hepatic and intestinal activity of CYP3A as measured by a phenotyping drug cocktail in healthy volunteers. J Acquir Immune Defic Syndr 42:52–60

99. Zhang D, Chando TJ, Everett DW, Patten CJ, Dehal SS, Humphreys WG (2005) In vitro inhibition of UDP glucuronosyltransferases by atazanavir and other HIV protease inhibitors and the relationship of this property to in vivo bilirubin glucuronidation. Drug Metab Dispos 33:1729–39

100. Porter KA, Cole SR, Eron JJ, et al (2012) HIV-1 protease inhibitors and clinical malaria: a secondary analysis of the AIDS Clinical Trials Group A5208 study. Antimicrob Agents Chemother 56:995–1000

101. Parikh S, Gut J, Istvan E, Goldberg DE, Havlir D V, Rosenthal PJ (2005) Antimalarial activity of human immunodeficiency virus type 1 protease inhibitors. Antimicrob Agents Chemother 49:2983–5

102. Achan J, Kakuru A, Ikilezi G, et al (2012) Antiretroviral agents and prevention of malaria in HIV-infected Ugandan children. N Engl J Med 367:2110–8

103. Dawes M, Chowienczyk PJ (2001) Drugs in pregnancy. Pharmacokinetics in pregnancy. Best Pr Res Clin Obs Gynaecol 15:819–826

104. Wong CA, Loffredi M, Ganchiff JN, Zhao J, Wang Z, Avram MJ (2002) Gastric emptying of water in term pregnancy. Anesthesiology 96:1395–400

105. Chiloiro M, Darconza G, Piccioli E, De Carne M, Clemente C, Riezzo G (2001) Gastric emptying and orocecal transit time in pregnancy. J Gastroenterol 36:538–43

106. Macfie AG, Magides AD, Richmond MN, Reilly CS (1991) Gastric emptying in pregnancy. Br J Anaesth 67:54–7

107. Whitehead EM, Smith M, Dean Y, O'Sullivan G (1993) An evaluation of gastric emptying times in pregnancy and the puerperium. Anaesthesia 48:53–7

108. Dean M, Stock B, Patterson RJ, Levy G (1980) Serum protein binding of drugs during and after pregnancy in humans. Clin Pharmacol Ther 28:253–261

109. Green MD, van Eijk AM, van Ter Kuile FO, Ayisi JG, Parise ME, Kager PA, Nahlen BL, Steketee R, Nettey H (2007) Pharmacokinetics of sulfadoxine-pyrimethamine in HIV-infected and uninfected pregnant women in Western Kenya. J Infect Dis 196:1403–1408

110. McGready R, Stepniewska K, Edstein MD, Cho T, Gilveray G, Looareesuwan S, White NJ, Nosten F (2003) The pharmacokinetics of atovaquone and proguanil in pregnant women with acute falciparum malaria. Eur J Clin Pharmacol 59:545–552

111. McGready R, Stepniewska K, Lindegardh N, Ashley EA, La Y, Singhasivanon P, White NJ, Nosten F (2006) The pharmacokinetics of artemether and lumefantrine in pregnant women with uncomplicated falciparum malaria. Eur J Clin Pharmacol 62:1021–1031

112. McGready R, Ashley EA, Moo E, Cho T, Barends M, Hutagalung R, Looareesuwan S, White NJ, Nosten F (2005) A randomized comparison of artesunate-atovaquone-proguanil versus quinine in treatment for uncomplicated falciparum malaria during pregnancy. J Infect Dis 192:846–853

113. McGready R, Stepniewska K, Ward SA, Cho T, Gilveray G, Looareesuwan S, White NJ, Nosten F (2006) Pharmacokinetics of dihydroartemisinin following oral artesunate treatment of pregnant women with acute uncomplicated falciparum malaria. Eur J Clin Pharmacol 62:367–371

114. Tarning J, McGready R, Lindegardh N, et al (2009) Population pharmacokinetics of lumefantrine in pregnant women treated with artemether-lumefantrine for uncomplicated Plasmodium falciparum malaria. Antimicrob Agents Chemother 53:3837–3846

115. Abdelrahim II, Adam I, Elghazali G, Gustafsson LL, Elbashir MI, Mirghani RA (2007) Pharmacokinetics of quinine and its metabolites in pregnant Sudanese women with uncomplicated Plasmodium falciparum malaria. J Clin Pharm Ther 32:15–19

116. French N, Nakiyingi J, Lugada E, Watera C, Whitworth JA, Gilks CF (2001) Increasing rates of malarial fever with deteriorating immune status in HIV-1-infected Ugandan adults. AIDS 15:899–906

117. Whitworth J, Morgan D, Quigley M, Smith A, Mayanja B, Eotu H, Omoding N, Okongo M, Malamba S, Ojwiya A (2000) Effect of HIV-1 and increasing immunosuppression on malaria parasitaemia and clinical episodes in adults in rural Uganda: a cohort study. Lancet 356:1051–6

118. Byakika-Kibwika P, Lamorde M, Mayito J, et al (2012) Significant pharmacokinetic interactions between artemether/lumefantrine and efavirenz or nevirapine in HIV-infected Ugandan adults. J Antimicrob Chemother 67:2213–21

119. Kredo T, Mauff K, Van der Walt JS, Wiesner L, Maartens G, Cohen K, Smith P, Barnes KI (2011) Interaction between artemether-lumefantrine and nevirapine-based antiretroviral therapy in HIV-1-infected patients. Antimicrob Agents Chemother 55:5616–23

120. Byakika-Kibwika P, Lamorde M, Okaba-Kayom V, et al (2012) Lopinavir/ritonavir significantly influences pharmacokinetic exposure of artemether/lumefantrine in HIV-infected Ugandan adults. J Antimicrob Chemother 67:1217–23

121. German P, Parikh S, Lawrence J, Dorsey G, Rosenthal PJ, Havlir D, Charlebois E, Hanpithakpong W, Lindegardh N, Aweeka FT (2009) Lopinavir/ritonavir affects pharmacokinetic exposure of artemether/lumefantrine in HIV-uninfected healthy volunteers. J Acquir Immune Defic Syndr 51:424–9

122. Bauer RJ, Guzy S, Ng C (2007) A survey of population analysis methods and software for complex pharmacokinetic and pharmacodynamic models with examples. AAPS J 9:E60–83

123. S.L. Beal L. B. Sheiner AJB NONMEM Users Guides. Ellicott City, Maryland, USA

124. Sheiner LB, Rosenberg B, Melmon KL (1972) Modelling of individual pharmacokinetics for computer-aided drug dosage. Comput Biomed Res 5:411–59

125. Karlsson MO, Sheiner LB (1993) The importance of modeling interoccasion variability in population pharmacokinetic analyses. J Pharmacokinet Biopharm 21:735–50

126. Laporte-Simitsidis S, Girard P, Mismetti P, Chabaud S, Decousus H, Boissel JP (2000) Inter-study variability in population pharmacokinetic meta-analysis: when and how to estimate it? J Pharm Sci 89:155–67

127. Anderson BJ, Holford NH (2008) Mechanism-based concepts of size and maturity in pharmacokinetics. Annu Rev Pharmacol Toxicol 48:303–332

128. Anderson BJ, Holford NHG (2009) Mechanistic basis of using body size and maturation to predict clearance in humans. Drug Metab Pharmacokinet 24:25–36

129. Wählby U, Thomson AH, Milligan PA, Karlsson MO (2004) Models for time-varying covariates in population pharmacokinetic-pharmacodynamic analysis. Br J Clin Pharmacol 58:367–77

130. Riley RD, Lambert PC, Abo-Zaid G (2010) Meta-analysis of individual participant data: rationale, conduct, and reporting. BMJ 340:c221

131. Tarning J, Chotsiri P, Jullien V, et al (2012) Population pharmacokinetic and pharmacodynamic modeling of amodiaquine and desethylamodiaquine in women with Plasmodium vivax malaria during and after pregnancy. Antimicrob Agents Chemother 56:5764–73

132. Adam I, Tarning J, Lindegardh N, Mahgoub H, McGready R, Nosten F (2012) Pharmacokinetics of piperaquine in pregnant women in Sudan with uncomplicated Plasmodium falciparum malaria. Am J Trop Med Hyg 87:35–40

133. Lindegardh N, Annerberg A, White NJ, Day NP (2008) Development and validation of a liquid chromatographic-tandem mass spectrometric method for determination of piperaquine in plasma stable isotope labeled internal standard does not always compensate for matrix effects. J Chromatogr B Anal Technol Biomed Life Sci 862:227–236

134. Borrmann S, Sasi P, Mwai L, et al (2011) Declining responsiveness of Plasmodium falciparum infections to artemisinin-based combination treatments on the Kenyan coast. PLoS One 6:e26005

135. Chinh NT, Quang NN, Thanh NX, Dai B, Geue JP, Addison RS, Travers T, Edstein MD (2009) Pharmacokinetics and bioequivalence evaluation of two fixed-dose tablet formulations of dihydroartemisinin and piperaquine in Vietnamese subjects. Antimicrob Agents Chemother 53:828–31

136. Nguyen DVH, Nguyen QP, Nguyen ND, Le TTT, Nguyen TD, Dinh DN, Nguyen TX, Bui D, Chavchich M, Edstein MD (2009)

Pharmacokinetics and ex vivo pharmacodynamic antimalarial activity of dihydroartemisinin-piperaquine in patients with uncomplicated falciparum malaria in Vietnam. Antimicrob Agents Chemother 53:3534–7

137. Nguyen TC, Nguyen NQ, Nguyen XT, Bui D, Travers T, Edstein MD (2008) Pharmacokinetics of the antimalarial drug piperaquine in healthy Vietnamese subjects. Am J Trop Med Hyg 79:620–3

138. Thanh NX, Trung TN, Phong NC, Quang HH, Dai B, Shanks GD, Chavchich M, Edstein MD (2012) The efficacy and tolerability of artemisinin-piperaquine (Artequick®) versus artesunate-amodiaquine (Coarsucam™) for the treatment of uncomplicated Plasmodium falciparum malaria in south-central Vietnam. Malar J 11:217

139. Annerberg A, Singtoroj T, Tipmanee P, White NJ, Day NPJ, Lindegårdh N (2005) High throughput assay for the determination of lumefantrine in plasma. J Chromatogr B Analyt Technol Biomed Life Sci 822:330–3

140. Hanpithakpong W, Kamanikom B, Dondorp AM, Singhasivanon P, White NJ, Day NPJ, Lindegardh N (2008) A liquid chromatographic-tandem mass spectrometric method for determination of artesunate and its metabolite dihydroartemisinin in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 876:61–8

141. Beal SL, Sheiner LB (1982) Estimating population kinetics. Crit Rev Biomed Eng 8:195–222

142. S.L. Beal L. B. Sheiner AJB (1992) NONMEM users guides. NONMEM project group, University of Califonia. San Fransisco, CA

143. Keizer RJ, van Benten M, Beijnen JH, Schellens JHM, Huitema ADR (2011) Piraña and PCluster: a modeling environment and cluster infrastructure for NONMEM. Comput Methods Programs Biomed 101:72–9

144. Lindbom L, Ribbing J, Jonsson EN (2004) Perl-speaks-NONMEM (PsN)--a Perl module for NONMEM related programming. Comput Methods Programs Biomed 75:85–94

145. Jonsson EN, Karlsson MO (1999) Xpose--an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. Comput Methods Programs Biomed 58:51–64

146. Savic RM, Jonker DM, Kerbusch T, Karlsson MO (2007) Implementation of a transit compartment model for describing drug absorption in pharmacokinetic studies. J Pharmacokinet Pharmacodyn 34:711–726

147. Holford N (2005) A degenerative predictive check. Popul. approach Gr. Eur. absract 73:

148. Bergstrand M, Hooker AC, Wallin JE, Karlsson MO (2011) Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. AAPS J 13:143–51

149. Savic RM, Karlsson MO (2009) Importance of shrinkage in empirical bayes estimates for diagnostics: problems and solutions. AAPS J 11:558–569

150. Vong C, Bergstrand M, Nyberg J, Karlsson MO (2012) Rapid sample size calculations for a defined likelihood ratio test-based power in mixed-effects models. AAPS J 14:176–86

151. Krause A, Lowe PJ (2014) Visualization and communication of pharmacometric models with berkeley madonna. CPT pharmacometrics Syst Pharmacol 3:e116

152. Beal SL (2001) Ways to fit a PK model with some data below the quantification limit. J Pharmacokinet Pharmacodyn 28:481–504

153. Hung TY, Davis TM, Ilett KF, Karunajeewa H, Hewitt S, Denis MB, Lim C, Socheat D (2004) Population pharmacokinetics of piperaquine in adults and children with uncomplicated falciparum or vivax malaria. Br J Clin Pharmacol 57:253–262

154. Karunajeewa HA, Ilett KF, Mueller I, Siba P, Law I, Page-Sharp M, Lin E, Lammey J, Batty KT, Davis TME (2008) Pharmacokinetics and efficacy of piperaquine and chloroquine in Melanesian children with uncomplicated malaria. Antimicrob Agents Chemother 52:237–43

155. Sim I-K, Davis TME, Ilett KF (2005) Effects of a high-fat meal on the relative oral bioavailability of piperaquine. Antimicrob Agents Chemother 49:2407–11

156. Salman S, Page-Sharp M, Batty KT, Kose K, Griffin S, Siba PM, Ilett KF, Mueller I, Davis TME (2012) Pharmacokinetic comparison of two piperaquine-containing artemisinin combination therapies in Papua New Guinean children with uncomplicated malaria. Antimicrob Agents Chemother 56:3288–97

157. (2013) The effect of dosing regimens on the antimalarial efficacy of dihydroartemisinin-piperaquine: a pooled analysis of individual patient data. PLoS Med 10:e1001564; discussion e1001564