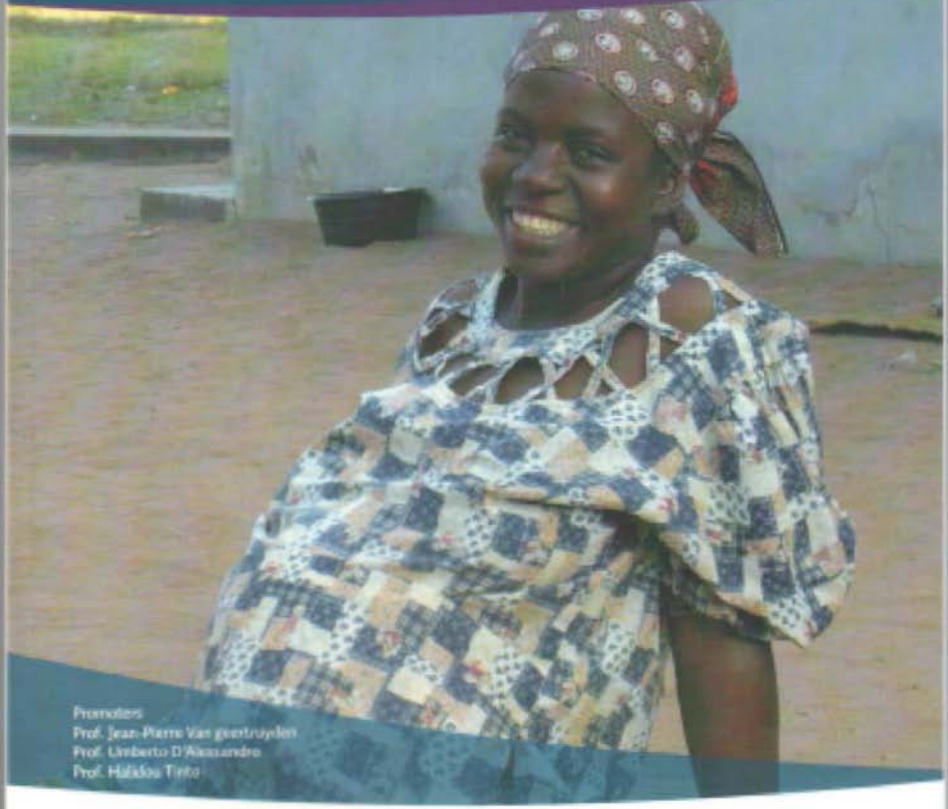
Case management and control of malaria in pregnancy: Case of Burkina Faso

Dissertation submitted for the degree of Doctor of Philosophy in Medical Sciences at the University of Antwerp to be defended by

Marc Christian Tahita



Promoters
Prof. Jean-Pierre Van geertruyden
Prof. Umberto D'Alessandro
Prof. Halidou Tinto

Global Health Institute
Department of Epidemiology and Social Medicine
Faculty of Medicine and Health Sciences
University of Antwerp
Antwerp 2017

Global Health Institute
University of Antwerp

Department of Epidemiology and Social Medicine

Faculty of Medicine and Health Sciences

University of Antwerp

&

Institute of Tropical Medicine, Antwerp

Case management and control of malaria in pregnancy: Case of Burkina Faso

Dissertation submitted for the degree of Doctor of Philosophy in Medical Sciences

at the University of Antwerp to be defended by

Marc Christian Tahita

Promoters:

Prof. Jean-Pierre Van geertruyden

Prof. Umberto D'Alessandro

Prof. Halidou Tinto

Doctoral committee

Promoter: Prof. Jean-Pierre VanGeertruyden, Prof Umberto Dalessandro, Prof Halidou Tinto

Chair Examining Committee: Prof. Joost Weyler

Chair Public Defense: **Prof. Joost Weyler**

University of Antwerp

Jury Members:

Prof. Yves Jacquemyn

University of Antwerp

Prof. Michet Cot

IRD, Laboratoire de parasitologie, Faculté de pharmacie, Paris, France

Prof. Daniel Chandramohan

London School of Hygiene and Tropical Medicine (LSHTM)

Prof. Jean-Pierre VanGeertruyden

University of Antwerp

Prof. Umberto Dalessandro

Medical Research Council, The Gambia (Gambie), London School of Hygiene and Tropical Medicine (LSHTM)

Prof. Halidou Tinto

Institut de Recherche en Sciences de la Santé (IRSS)/CNRST

Table of Contents

List of abbreviations

ACT	Artemisinin-based combination therapy
AL	Artemether-lumefantrine
ANC	Antenatal clinic
AS	Artesunate
CI	Confidence interval
CMA	Centre médical avec antenne chirurgicale
CQ	Chloroquine
CSA	Chondroitin sulphate A
CSPS	Centre de santé et de promotion sociale
CTX	Cotrimoxazole
DHA	Dihydroartemisinin
DNA	Desoxyribo nucleic acid
HDSS	Health and Demographic Surveillance System
HRP2	Histidine-rich protein 2
IEs	Infected erythrocytes
IPTp	Intermittent preventive treatment during pregnancy
IRS	Indoor residual spraying
ITNs	Insecticide treated nets
IUGR	Intrauterine growth retardation
LBW	Low birth weight
LLIN	Long lasting insecticide-treated nets

LM	Lumefantrine
MIS	Malaria indicator survey
MDAQ	Monodesethylamodiaquine
MQ	Mefloquine
MoH	Ministry of health
NMCP	National malaria control programme
PCR	Polymerase chain reaction
PQ	Piperaquine
QN	Quinine
RDTs	Rapid diagnostic tests
SP	Sulfadoxine-pyrimethamine
SSA	Sub-Saharan Africa
Vs	Versus

Samenvatting

Achtergrond en motivering

Malaria, de meest voorkomende parasitaire ziekte bij de mens, is zowel vermijdbaar als behandelbaar, maar veroorzaakt nog steeds een enorme ziekte en sterfte bij kinderen en zwangere vrouwen. Malaria is voornamelijk een gezondheidsprobleem in lageloonlanden. In sub-Sahara Afrika zijn er jaarlijks meer dan 32 miljoen zwangerschappen in malaria-endemische gebieden. Indien er geen zwangerschap-specifieke malaria interventies zouden zijn, bevallen er 12 miljoen zwangere vrouwen (45% van alle bevallingen) van een baby blootgesteld aan malaria infectie. Dit zou leiden tot ongeveer 900.000 baby's met een laag geboortegewicht als gevolg van vroeggeboorte of intra-uteriene groeiachterstand.

In Burkina Faso is malariatransmissie seizoensgebonden en voornamelijk tijdens de maanden augustus-december overlappend met het regenseizoen (juli-oktober). Het hoogtepunt van het malariaseizoen treedt meestal op in september-oktober. Malaria is een van de meest voorkomende redenen voor het bezoeken van de gezondheidscentra. De meest voorkomende vectoren zijn *Anopheles gambiae sensu stricto, Anopheles funestus* en *Anopheles arabiensis*. *Plasmodium falciparum* (*P. falciparum*) is de voornaamste malariaparasiet. *P. malariae* en *P. ovale* worden ook waargenomen in respectievelijk 3-8% en 0,5-2% van de gevallen, en hebben ook schadelijke effecten op de moeder en hun nakomelingen. Ze gaan echter meestal samen met *P.falciparum* malaria tijdens de zwangerschap

In malaria-endemische gebieden in sub-Sahara Afrika, beveelt de WGO aan om tijdens de zwangerschap insecticide geïmpregneerde muggennetten te gebruiken en om intermitterende preventieve behandeling (IPTp) met sulfadoxine-pyrimethamine in het tweede en derde trimester te nemen. Sulfadoxine-pyrimethamine wordt goed getolereerd, is veilig in het tweede en derde trimester, betaalbaar, overal verkrijgbaar, en kan worden gegeven in één

enkele dosis, waardoor gesuperviseerde behandeling tijdens prenatale consultaties mogelijk is. IPTp met sulfadoxine-pyrimethamine is zeer effectief voor het verminderen van de consequenties van malaria infecties tijdens de zwangerschap, maar wordt bedreigd door het ontstaan en de wijde verspreiding van antimalaria resistentie. Twee cruciale factoren voor een succesvolle behandeling zijn het vroegtijdig opsporen van de malaria parasiet en een behandeling met een efficiënt antimalariamiddel. Tijdens het tweede en derde trimester van de zwangerschap, raadt de WGO een snelle en efficiënte behandeling aan met een op artemisinine gebaseerde combinatie behandeling (ACT). De malariaopsporing tijdens de zwangerschap is echter niet evident want de meeste besmette vrouwen zijn asymptomatisch in endemische gebieden.

In deze thesis worden de klinische presentatie van malaria tijdens de zwangerschap, de preventie ervan met behulp van IPTp, en d*e* in vivo en ook de ex vivo efficiëntie van de aanbevolen tegen antimalariamiddelen onderzocht. De belangrijkste doelstellingen waren (1) het evalueren van het nut van klinische tekenen en symptomen in de diagnose van malaria tijdens zwangerschap, (2) het bepalen van de prevalentie van moleculaire merkers van SP resistentie bij asymptomatische en symptomatische malaria-geïnfecteerde zwangere vrouwen in Burkina Faso, (3) het 'ex vivo' bepalen van de huidige werking van anti-malaria middelen aanbevolen door de NMCP in Burkina Faso (4) het in vivo bepalen van de doeltreffendheid en veiligheid van ACTs gebruikt voor ongecompliceerde malaria bij zwangere vrouwen in Afrika en ten slotte (5) het beoordelen van de relevantie van de aanbevelingen van de NMCP voor de behandeling van malaria tijdens de zwangerschap in Burkina Faso.

Methoden

Wij voerden een longitudinale studie uit waarin aan alle zwangere vrouwen die zich presenteerden op een routine prenatale consultatie (ANC) of in de polikliniek, werd gevraagd

om deel te nemen. Tijdens de consultatie en na het bekomen van een schriftelijke geïnformeerde toestemming, werden vrouwen ingedeeld in een symptomatische groep of een controlegroep. De vrouwen behorende tot de symptomatische groep hadden ten minste één van de volgende tekenen of symptomen: een temperatuur van ≥37.5 ° C (gemeten door een elektronische thermometer) of koorts gedurende de voorafgaande 48 uur, hoofdpijn, bleekheid, gewrichts- of spierpijnen, convulsies, braken, duizeligheid, malaise, moeheid, vergrote lever of milt. De vrouwen behorende tot de controlegroep hadden dus geen enkel symptoom. Voor elk ziektegeval werden 2 controlegevallen geselecteerd gepaard op pariteit (0, 1-3, ≥4), zwangerschapsduur (gemeten via de fundushoogte) en het seizoen (geselecteerd binnen een maand na het desbetreffende ziektegeval). Ook bepaalden wij de prevalentie van moleculaire merkers voor SP resistentie bij asymptomatische en symptomatische malaria-geïnfecteerde zwangere vrouwen in Burkina Faso.

De tweede studie maakte deel uit van een multicenter studie (Burkina Faso, Ghana, Malawi en Zambia) (ClinicalTrials.gov-ID: NCT00852423) die de werkzaamheid en veiligheid van vier anti-malaria behandelingen onderzocht, namelijk dihydroartemisinin–piperaquine (DHA-PQ), mefloquine-artesunaat (MQAS), artesunaat-amodiaquine (ASAQ) en artemether-lumefantrine (AL), in *falciparum* malaria-geïnfecteerde zwangere vrouwen. Zwangere vrouwen werden opgenomen in het proces als zij voldaan aan de volgende criteria: zwangerschap ≥16 weken, *P. falciparum* mono-infectie met eender welke densiteit met of zonder symptomen, hemoglobine ≥7 g/dl, verblijf binnen de gezondheidszone van het centrum en de bereidheid om te bevallen in het gezondheidscentrum. Een ex vivo studie over de medicamenteuze gevoeligheid van parasiet isolaten van zwangere vrouwen was ingebed in de klinische trial site in Burkina Faso. Voor de ex vivo studie was de opneming beperkt tot vrouwen met een parasietconcentratie van ten minste 100/µl.

Resultaten

Nut van klinische tekenen en symptomen voor de diagnose van malaria tijdens de zwangerschap

Zeshonderd zwangere vrouwen werden gerekruteerd (200 symptomatische en 400 controle gevallen), de meeste van hen in het tweede en derde trimester van de zwangerschap. Malaria prevalentie werd gemeten via microscopie in 49,0% van de symptomatische gevallen en 39,5% bij de controlegroep (p=0,03). Onder de symptomatische malaria-geïnfecteerde vrouwen, was leeftijd gerelateerd aan malariainfectie en hadden vrouwen van <20 jaar meer risico dan die van ≥35 jaar oud. Koorts, geschiedenis van koorts, hoofdpijn en duizeligheid hadden elk een positief voorspellende waarde (PPV) van rond de 50%, terwijl voor alle anderen de PPV ruim lager was. De hoogste PPV werden gevonden bij het combineren van koorts en duizeligheid (61,5%, 95%CI:35–82) en koorts en braken (66,7%, 95%CI:20-93). Als koorts was gebruikt voor de diagnose van malaria zou 47.2% van de koorts vrouwen onnodig worden behandeld. Ook zou 46,8% van de vrouwen zonder koorts geen behandeling hebben gehad voor hun malaria infectie.

Prevalentie van moleculaire merkers voor SP resistentie bij asymptomatische en symptomatische malaria-geïnfecteerde zwangere vrouwen in Burkina Faso

Onder de 600 zwangere gerekruteerde vrouwen, werden 256 gediagnosticeerd met malaria door microscopie. Na genotypering, had meer dan de helft van de stalen de *dhfr* C59R (61,2%, 156/255) en/of de S108N (55,7%, 142/255) mutaties terwijl slechts 12,2% (31/255) de N51I mutatie hadden; geen I164L-mutatie werd gevonden. Er zijn 6 verschillende *dhfr* allelen; de prevalentie van het *dhfr* "wild type" was 30,2% (77/255). Onder de gemuteerde allelen, was de dubbele NRNI-mutatie de meest voorkomende (35,7%, 91/255), gevolgd door de drievoudige mutatie IRNI (11,4%, 29/255). Meer dan een derde van de stalen (34,2%, 79/231) droeg de *dhps* A437G mutatie maar geen van hen had de K540E mutatie.

Antimalariamiddelen: ex vivo werkzaamheid van antimalariamiddelen in zwangerschap

In totaal hadden 90 isolaten (83,3%) interpreteerbare resultaten voor ten minste één van de studiemedicaties, overeenkomende met een slaagpercentage van 80% voor de cultuur. Onder de medicijnen die momenteel gebruikt worden had mefloquine (MQ) de hoogste proportie (9,2%) van resistente parasieten (meetkundige gemiddelde IC50=1.1nM; 95%CI:0,8-1,7), gevolgd door monodesethylamodiaquine (MDAQ) (8,0%; meetkundige gemiddelde IC50=1.5nM; 95%CI:1.0-2.2) en kinine (QN) (4,4%; meetkundige gemiddelde IC50=34.2nM;95%CI:24.1-48,5). De medijcinen waren even actief tegen de chloroquine CQ-gevoelige als CQ-resistente stalen. Er was een aanzienlijke positieve correlatie tussen de gevoeligheid van dihydroartemisinin (DHA) en zowel MQ als CQ, en tussen CQ en lumefantrine (LUM) en tussen MDAQ en MQ.

Werkzaamheid en veiligheid van ACTs bij gebruik bij zwangere vrouwen in Afrika

ACTs hebben bewezen om effectief te zijn bij de behandeling van ongecompliceerde malaria tijdens de zwangerschap. Zij toonden een goed werkzaamheidsprofiel wat de bestaande WGO aanbevelingen bevestigd. Veiligheid, een andere belangrijke parameter, varieerde tussen de behandelingen. Vrouwen behandeld met de combinaties ASAQ of MQAS hadden meer bijwerkingen dan met AL en DHA-PPQ. AL, werd, hoewel doeltreffend, geassocieerd met een hoger risico van nieuwe infecties als gevolg van de korte eliminatietijd van LUM. DHA-PPQ was effectief, had een lange profylactisch effect, en een aanvaardbaar veiligheidsprofiel.

Conclusies en aanbevelingen

Detectie van malaria-infecties tijdens de zwangerschap: Is klinische diagnose nuttig?

Tekenen en symptomen waren niet discriminant voor malaria-infecties. Beperking van de behandeling tot symptomatische zwangere vrouwen zou een slechte strategie zijn ter vermindering van de morbiditeit en de mortaliteit veroorzaakt door malaria. Alle zwangere

vrouwen moeten hetzij systematisch worden gescreend voor malaria infectie en nadien behandeld indien positief of SP worden gegeven op elk gewenst moment dat ze een gezondheidscentrum bezoeken, met minstens één maand tussen 2 dosissen.

Preventie van Malaria in zwangerschap: werkzaamheid van SP in Burkina Faso

De prevalentie van de triple mutant, een bekende resistentie in vitro tegen pyrimethamine, was relatief laag waardoor SP nog steeds kan bruikbaar is als IPTp in Burkina Faso. Ervan uitgaande dat besmette zwangere vrouwen meestal met lage parasitaire densiteit hebben, zal SP de malaria-infecties klaren op het moment van de behandeling. En in alle zwangere vrouwen, ook de niet geïnfecteerde, zal de SP hen enkel weken beschermen.

Antimalariamiddelen: ex vivo efficiëntie werkzaamheid van antimalariamiddelen tijdens de zwangerschap

De weinige resistente isolaten tegen MDAQ suggereren dat de combinatie ASAQ doeltreffend is tijdens de zwangerschap. Relatieve lage IC50 werden ook gevonden voor de twee andere ACT partner behandeling: LUM en MQ. Dit is een indicatie dat de combinaties AL en ASMQ hier ook effectieve behandelingen zijn.

ACTs voor de behandeling van zwangeren met malaria

DHA-PPQ en AL hadden beiden een goed veiligheids-en werkzaamheidsprofiel.

Motivering en relevantie voor de nationale malaria controle-richtlijnen in Burkina Faso

ASAQ had het beste behandelingsprofiel in Burkina Faso. Het werd echter minder goed getolereerd dan AL. AL had daarentegen een beter veiligheidsprofiel maar met een hoger risico op reinfectie. DHAPQ met goed behandelingsprofiel en zijn lange profylactische bescherming na behandeling is een geode kandidaat voor een eerstelijnsbehandeling. Ten laatste, vermelden we dat de IPTp-SP nog steeds een optie is in Burkina Faso daar de parasite resitentie hier nog steeds zeer laag is.

English Summary

Background and rationale

Malaria, the most common human parasitic disease, is both preventable and treatable, but continues to disproportionately affect children and pregnant women. Malaria continues to be a major health problem in low-income countries. In sub-Saharan Africa, more than 32 million pregnancies occur annually in malaria-endemic regions. In the absence of pregnancy-specific interventions, 12 million live-born deliveries (45% of all live-born deliveries) would be exposed to malaria infection, which would cause an estimated 900 000 low birthweight deliveries due to preterm labour and intrauterine growth retardation.

In Burkina Faso, malaria transmission is seasonal, during the months of August-December, and overlaps with the rainy season (July-October). The peak for malaria cases usually occurs in September-October. Malaria is one of the most common reasons for attending a health facility. The more common vectors are *Anopheles gambiae sensu stricto*, *Anopheles funestus* and *Anopheles arabiensis*. *Plasmodium falciparum* (*P. falciparum*) is the predominant malaria parasite and *P. malariae* and *P. ovale* are observed in 3-8% and 0.5-2% of malaria cases, respectively, most of them co-infected with *Pf.* Malaria in pregnancy is known to cause adverse effects on the mother and their offspring.

The WHO recommends the use of long-lasting insecticidal nets throughout pregnancy in malaria-endemic regions in sub-Saharan Africa, as well as intermittent preventive treatment (IPTp) with sulfadoxine–pyrimethamine in the second and third trimesters. Sulfadoxine–pyrimethamine is well tolerated, safe in the second and third trimesters, affordable, widely available, and can be given as a single dose, allowing for directly observed therapy at antenatal clinics. IPTp with sulfadoxine–pyrimethamine is very effective for reducing adverse outcomes of malaria during pregnancy, but is threatened by the emergence of widespread

parasite resistance. The WHO also recommends prompt and efficient case management with artemisinin-based combination treatment (ACT) during the second and third trimester of pregnancy. Two keys factors for successful case management are the early detection of malaria, and their treatment with efficient antimalarial drugs. The detection of malaria during pregnancy is a challenge, however, as in endemic areas most infected women are asymptomatic.

In this thesis, the clinical presentation of malaria, the prevention using IPTp, the in vivo and also the ex vivo efficacies of the recommended antimalarial drugs were investigated. The main objectives were, (1) to evaluate the usefulness of clinical signs and symptoms in malaria diagnosis during pregnancy, (2) to determine the prevalence of molecular markers of SP resistance in asymptomatic and symptomatic malaria-infected pregnant women in Burkina Faso, (3) to determine 'ex vivo' the current efficacy of antimalarial drugs recommended by the NMCP in Burkina Faso, (4) to determine the efficacy and safety of ACTS used for uncomplicated malaria in pregnant women in Africa, and finally (5) to evaluate the pertinence of the NMCP recommendations for the treatment of malaria in pregnancy in Burkina Faso.

Methods

We carried out a longitudinal study in which all pregnant women attending either the routine antenatal care (ANC) or the outpatient clinic were asked to participate. During the visit and after having obtained written informed consent, women were divided into cases or controls. Cases had at least one of the following signs and symptoms: temperature ≥37.5°C (measured by electronic thermometer) or history of fever in the previous 48 hours, headache, pallor, arthromyalgia, convulsions, vomiting, dizziness, malaise, fatigue, enlarged liver, or enlarged spleen. Controls had none of these symptoms. For each case, two controls, matched by parity (0, 1–3, ≥4), gestational age (measured by fundal height) and seasonality (recruited within one

month from the corresponding case) were recruited. Also, we determined the prevalence of molecular markers of SP resistance in asymptomatic and symptomatic malaria-infected pregnant women in Burkina Faso.

The second study was part of a multi-centre (Burkina Faso, Ghana, Malawi, and Zambia) trial (ClinicalTrials.gov ID: NCT00852423) investigating the efficacy and safety of four anti-malarial treatments, namely dihydroartemisinin–piperaquine (DHAPQ), mefloquine-artesunate (MQAS), artesunate-amodiaquine (ASAQ) and artemether-lumefantrine (AL), in *falciparum* malaria-infected pregnant women. Pregnant women were included in the trial if they fulfilled the following criteria: gestation ≥16 weeks, *P. falciparum* mono-infection at any density with or without symptoms, haemoglobin ≥7 g/dl, residence within the health facility catchment's area, and willingness to deliver at the health facility. An ex vivo study on the drug sensitivity of isolates from pregnant women was nested into the trial in Burkina Faso. For the ex vivo study the inclusion was limited to women with a parasite density of at least 100/µl.

Key Results

Usefulness of clinical signs and symptoms for the diagnosis of malaria during pregnancy

Six hundred (200 case and 400 matched controls) pregnant women were included, most of whom were in the second and third trimester of pregnancy. Malaria prevalence by microscopy was 49.0% among cases and 39.5% among controls (p = 0.03). Among malaria-infected women with symptoms, age was significantly related to malaria infection, with women <20 years more at risk than those ≥35 years old.

Fever, history of fever, headache, and dizziness each had a positive predictive value (PPV) around 50%, while for all the others the PPV was much lower. The highest PPVs were found when combining fever and dizziness (61.5%, 95%CI:35–82), and fever and vomiting (66.7%,

95%CI:20–93). If fever had been used to diagnose malaria, 47.2% of the febrile women would have been unnecessarily treated. Also 46.8% of the non-febrile women would have missed treatment for their malaria infection.

Prevalence of molecular markers of SP resistance in asymptomatic and symptomatic malaria-infected pregnant women in Burkina Faso

Among the 600 pregnant women recruited, 256 were diagnosed with malaria by microscopy. After genotyping, 61.2% (156/255) of the samples had the *dhfr* C59R and/or the S108N (55.7%, 142/255) mutations while only 12.2% (31/255) had the N51I mutation; no I164L mutation was found. There were 6 different *dhfr* alleles; the prevalence of the *dhfr* wild type was 30.2% (77/255).

Among the mutant alleles, the double mutation NRNI was the most frequent (35.7%, 91/255), followed by the triple mutation IRNI (11.4%, 29/255). More than a third of the samples (34.2%, 79/231) carried the *dhps* mutations A437G but none of them had the mutation K540E.

Antimalarials drugs: ex vivo efficacy of antimalarials in pregnancy

A total of 90 isolates (83.3%) had interpretable results for at least one of the study drugs, giving a culture success rate of 80%. Among the drugs currently in use, mefloquine (MQ) had the highest prevalence of resistant isolates (geometric mean IC50 = 1.1 nM; 95% CI 0.8–1.7) with 9.2%, followed by monodesethylamodiaquine (MDAQ) (geometric mean IC50 = 1.5; 95% CI 1.0–2.2) with 8.0%, and then quinine (QN) (geometric mean IC50 = 34.2; 95% CI 24.1–48.5) with 4.4%. Most drugs were equally active against the chloroquine (CQ)-sensitive and CQ-resistant isolates. There was a positive significant correlation between the sensitivity of dihydroartemisinin (DHA) and both MQ and CQ, between CQ and lumefantrine (LM) and between MDAQ and MQ.

Efficacy and safety of ACTs use in pregnant women in Africa

ACTs have proven to be effective in treating uncomplicated malaria in pregnancy. They have shown a good efficacy profile confirming the recommendations for their use. The results of safety, another parameter to take into account, varied between treatments. Women treated with the combinations ASAQ or MQAS had more adverse events than AL and DHAPQ. AL, although efficacious, was associated with a higher risk of re-infections due to the short elimination half-life of LM. DHAPQ had good efficacy, a long post treatment prophylaxis period and an acceptable safety profile.

Conclusions and recommendations

Detection of malaria infections during pregnancy: is clinical diagnosis (screening-based on signs and symptoms) useful?

Signs and symptoms were not discriminative for malaria infections. Restricting treatment to symptomatic pregnant women would be an inadequate strategy to reduce the morbidity and mortality associated with malaria. All pregnant women should be either systematically screened for malaria infection and treated if positive or given SP any time they visit a health facility, provided this is done at least one month apart.

Malaria in pregnancy prevention: efficacy of SP in Burkina Faso

The prevalence of the triple mutation known to confer resistance in vitro to pyrimethamine was relatively low indicating that SP can still be used as IPTp. Assuming that pregnant women are generally infected with low parasite densities, SP should clear malaria infections at the time of administration. For all pregnant women, including the non-infected, SP would protect them for a few weeks.

Antimalarials drugs: ex vivo efficacy of antimalarials in pregnancy

The few resistant isolates to MDAQ suggest that the combination ASAQ is efficacious during pregnancy. Relative low IC50 was also found with two partner drugs of ACT, LM and MQ. This is an indication that the combinations AL and ASMQ are efficacious treatments.

ACTs for the treatment of pregnant women with malaria

DHAPQ and AL have shown good safety and efficacy profiles.

Rationale and pertinence of the national malaria guidelines in Burkina Faso

Pregnant women should be systematically screened for malaria, by RDT or microscopy, when attending a health facility.

ASAQ and AL showed good efficacy profile confirming their choice by the NMCP as first line treatment. Finally, SP can still be used as IPTp in our context.

Chapter 1: Introduction to malaria

1.1 Global burden of malaria

Malaria is caused by the protozoan parasite Plasmodium. Five species are known to cause malaria in humans; *P. falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium knowlesi. Plasmodium* is among the most researched genera of parasites in the world (1). Despite substantial progress made in its control, malaria remains a major public health problem.

This thesis focuses on *P. falciparum* infection in pregnancy and will therefore not address the other malaria species mentioned above.

According to the latest World Health Organization (WHO) estimates, there were about 214 million cases of malaria in 2015 (with an uncertainty range of 149 million to 303 million) and an estimated 438 000 deaths (with an uncertainty range of 236 000 to 635 000) (2). Most of these cases (80%) and deaths (90%) occurred in sub-Saharan Africa (Figure 1), and most deaths (70%) were in children under 5 years of age. The malaria mortality rate, which takes into account population growth, is estimated to have decreased between 2000 and 2015 by 60% globally (2).

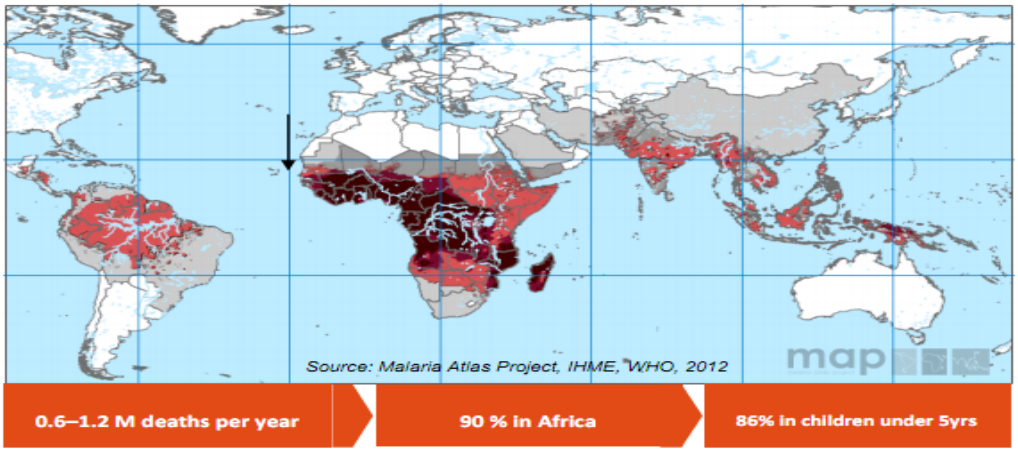


Figure 1: Countries with ongoing transmission of malaria, 2013

1.2 Clinical malaria in non-pregnant individuals

Clinical malaria is characterized by an acute febrile illness. The first symptoms, fever, headache, chills and vomiting, occur at least 7 days after the infectious mosquito bite. They may be mild and difficult to recognize as malaria (3). If not promptly treated, *P. falciparum* malaria may progress to severe illness and death. In children, severe malaria can present as severe anemia, respiratory distress due to metabolic acidosis, or cerebral malaria. Repeated infections in malaria endemic areas result in the development of partial immunity, which decreases the risk of severe malaria but does not prevent infection (4).

1.3 Malaria in pregnancy, pathogenesis and immunity

In addition to children under 5 years, pregnant women are also more vulnerable to malaria (5). Malaria infection in pregnancy may cause low birth weight, which increases the risk of death during the first year of life.

Adult women in areas of stable, high transmission usually have a high level of immunity against malaria (5). In areas with lower transmission this effect is less marked (3,4), and absent in areas at risk of epidemics (7). There is a physiological decrease of immunity during pregnancy, which makes women more susceptible to malaria (8). In addition, where malaria transmission is stable, primigravidae are at higher risk than multigravidae. This is due to the development of an immune response against parasites that specifically sequester in the placenta (7,8). Infected erythrocytes accumulate in the maternal vascular area of the placenta, the intervillous space, to much higher densities than in the peripheral circulation (11). Parasites expressing Chondroitin Sulphate A (CSA) - binding surface molecule Var2CSA can adhere to the placental tissue through the binding of Var2CSA with the placental adhesion receptor CSA. Some studies point to the existence of additional receptors (10,11). However, current evidence suggests that they are less important than CSA (14), which is present in the

placenta as a glycosaminoglycan side chain to the tissue anticoagulant thrombomodulin (15), and as part of a secreted low-sulphated aggrecan in the intervillous space, postulated to function as a reversible immobilizer of hormones, cytokines, and other molecules (16). Placental sequestration seems to occur throughout the intervillous space, by contrast with sequestration in other tissues, where infected erythrocytes are usually found in close apposition to the vascular wall (16). Such sequestration triggers substantial changes such as an increased number of maternal phagocytic cells, especially monocytes, in the intervillous spaces. Inflammation processes and obstruction of nutrients flowing through the placenta, from mother to child, are believed to be the causes of adverse events of malaria in pregnancy. The relation between placental malaria and birth weight was reviewed by Brabin and colleagues (17) in 1983. Briefly, chronic infections were most closely associated with decreased birth weight due to fetal growth restriction, whereas acute infection (especially with high parasite density) (16,17) was more closely associated with preterm delivery. In addition, chronic infection was also associated with lower maternal hemoglobin or severe anemia (18,19).

Younger maternal age (especially adolescence) is also an independent risk factor for malaria in pregnancy (i.e. young primigravidae and multigravidae are at greater risk of malaria and its adverse effects than older primigravidae or multigravidae) (22–26).

1.4 Clinical presentation of malaria in pregnancy (MiP)

First described in 1937 by Wickaramsuriya GAW (27), malaria in pregnancy (MiP) continues to be a major health issue. In malaria endemic settings, MiP results in adverse outcomes for both the woman and fetus (28–32). This was clearly highlighted by many studies describing its indirect morbidity burden (18,33–36). Malaria control relies on prompt diagnosis and effective treatment of malaria cases (37). However, studies on the importance of signs and

symptoms of malaria are scarce (33,38). It was assumed that in high transmission areas, infected pregnant women are often without symptoms because of their acquired immunity, while where transmission is low malaria infection would cause symptoms (39). Such assumption is based on few studies done during ANCs or on cross-sectional surveys which all tend to underestimate the frequency of malaria-related symptoms in pregnancy. In Sierra Leone (40) and Central Africa (41) the majority of pregnant women remained asymptomatic. In Malawi, in a trial that enrolled 4,187 pregnant women and evaluated 4 study treatments and prophylaxis regimen (3 arms on chloroquine and one arm on mefloquine), few women reported symptoms of fever or malaria, despite the fact that about 36% of them remained or became parasiteamic during the follow up, until delivery. At delivery, women with higher parasite density were not more likely to report fever than women with lower parasite density. Although fever was reported by 25% of women before enrolment, it was not associated with malaria infection. Relying on self-reported fever would have identified only 24% of infected women and less than half of the infected women with a parasite density of > 10,000 parasites per µL (42).

A few years later, a cross-sectional community-based survey enrolled 686 pregnant women in southern Mozambique (38). Conducted during two malaria transmission seasons, this study aimed at describing malaria prevalence and anemia and their relation to parity and age. Clinical malaria, defined as the presence of microscopic *P. falciparum* asexual parasitaemia plus fever, was uncommon in this study. The study design did not allow for collecting the outcome of pregnancy as women were examined only once, at the time of the survey. In low transmission areas, only a few studies describing the epidemiology of MiP addressed the issue of clinical presentation of malaria during pregnancy. In Central Sudan, Sayed et al investigated the impact of *P falciparum* infection in 550 parturient women. In this study pregnant women who were clinically suspected to have malaria were recruited (42). All

recruited pregnant women had at least 2 or more of the following symptoms: fever, rigors, headache, vomiting, diarrhea, abdominal and joint pains. The prevalence of malaria parasitaemia was almost 60%. The majority of women with clinical suspicion of malaria were found to be malaria positive using microscopy. This finding was in line with the assumption of the symptomatic character of malaria in low transmission area. In Ethiopia, Newman et al (7) conducted a study to assess the burden of MiP in both low (unstable) and high (stable) transmission settings. Malaria-infected women were more likely to have reported a fever during pregnancy than non-infected ones, particularly in areas of low transmission (61.5% vs. 10.9%, respectively; relative risk [RR], 5.7; $P < .001$). Moreover, most malaria infections were associated with a reported fever in the week before enrolment (53.9% vs. 4.2%; RR, 12.9; $P < .001$) in the unstable transmission area.

Existing studies that explored MiP clinical presentation are prone to bias due to the study design. In Nigeria, Nnaji et al failed to diagnose malaria in pregnancy using reported febrile illness as predictor (43). A total of 420 pregnant women attending their first ANCs were recruited in a case-control study. Nearly 58% of infected pregnant women and 54% of uninfected ones reported fever before their inclusion in the study. The sensitivity, specificity, PPV and PNV were 57.4%, 46%, 80.25% and 22%, respectively. The efficiency of reported febrile illness as a screening approach was 55%, meaning that almost half of the pregnant women without history of fever had a malaria infection. From these results, malaria diagnosis based on reported febrile illness would not be adequate to identify infected pregnant women (43). In Mozambique, a hospital-based descriptive study carried out between August 2003 and November 2005 aimed at characterizing the clinical presentation of malaria in African pregnant women and to evaluate the adequacy of case management based on clinical complaints (44). Only pregnant women attending the ANCs with clinical complaints were included. In total, 3,129 cases were recruited. Signs and symptoms suggestive of malaria,

although frequent, were not discriminative of malaria infection. The majority of women presenting a sign or symptom suggestive of malaria did not have parasitaemia and most of the signs and symptoms had low predictive values (less than 40%). Only current fever had a PPV of 50% but its prevalence was low (12%). Even combining several symptoms did not improve the PPV (less than 50%). This study focused only on women with predefined symptoms, and a control group was missing. Consequently, sensitivity and specificity of each symptom could not be estimated.

Another study was conducted in Ghana (45) with the objective to report signs and symptoms of malaria infection during pregnancy. The study included 900 pregnant women with confirmed peripheral parasitaemia and 223 malaria negative pregnant women as controls. Including a control group was important for this study as it allowed ascertaining the background incidence of signs and symptoms in pregnant women without malaria. Though only 7% of malaria-infected women had fever, all the symptoms were more frequent in infected than uninfected women. In addition, fever, headache, vomiting, and general malaise were strongly associated with malaria. The study concluded that in stable transmission areas, malaria in pregnancy is often symptomatic, a finding in contrast with previous results obtained in areas of stable malaria. Nevertheless, this study had some limitations and one should be cautious when interpreting these findings. Firstly, the existence of the control group, although important, could have actively influenced the report of the symptoms. In this study, pregnant women were informed that they were not malaria infected and would be included in a control group. This could have led to under-reporting of symptoms. Secondly, symptoms were actively collected and solicited, possibly explaining the high frequency of signs and symptoms.

In 2011, in Benin, the STOPPAM project assessed the clinical presentation of malaria infection during the whole duration of pregnancy (46). Initially, the STOPPAM project was

conceived to study the underlying immunopathological processes causing poor outcomes in MiP. Through this, a cohort of 982 pregnant women with clinical complaints was followed until delivery at ANCs and unscheduled visits. In the latter, fever (OR =5.2; $p < 0.001$), headache (OR = 2.1; $p = 0.004$), and shivering (OR = 3.1; $p < 0.001$) were significantly associated with malaria infection while during ANCs only headache was significantly associated with malaria infection. Although the STOPPAM project was not designed to assess the clinical presentation of malaria during pregnancy, the study was able to capture all clinical symptoms during ANCs and also unscheduled visits. Therefore, during ANCs the majority of malaria-infected pregnant women were symptomless (46).

References	**Setting**	**Enrolment criteria and sample size**	**Definition of malaria**	**Sample size**	**Predictors performance**	**Conclusion**	**Limitations**
Steketee et al (1996)	Malawi Rural area, High transmission area	Pregnant women at first ANCs with fever or history of fever	Parasitaemia	4187 pregnant women	Sensitivity: 24% Specificity: 71%	Fever inadequate predictor to identify Parasitaemic pregnant women	ANCs
Saute et al (2002)	Mozambique Rural area, High transmission area	Pregnant women with fever	Parasitaemia plus fever (T≥37.5°C)	686 pregnant women	3.4% of clinical malaria	Clinical malaria uncommon	Community based cross sectional
Ahmed et al (2002)	Central Sudan Low to moderate transmission area	Pregnant women in labor	Clinical presentation (fever, rigors, Headache…) with parasitaemia	550 pregnant Women	60% of clinical malaria	Malaria in pregnancy is usually symptomatic	Community based cross sectional study
Newman et al (2002)	Ethiopia High and low transmission area	Pregnant women at ANCs and delivery units	Reported fever during pregnancy or week of enrolment	962 pregnant women (249 high transmission and 713 low transmission)	More parasitaemic women reported fever in the week before enrolment than aparasitaemic (53.9% vs. 4.2%; RR: 12.9; P≤.001).	Malaria in pregnancy is mostly symptomatic	Community based cross sectional study
Nnaji et al (2007)	Nigeria High transmission area	Pregnant women attending ANCs	History of fever with parasitaemia	420 pregnant women	History fever Sensitivity: 57.4% Specificity: 46%, PPV: 80.25%	Low specificity of history of fever to predict malaria in	Cases control study

					PNV: 22%	pregnancy	
Bardaji et al (2008)	Mozambique High transmission area	Pregnant women attending ANCs with clinical complaints	*P. falciparum* parasitaemia with signs and symptoms suggestive of malaria	3129 visits analyzed	PPV current fever: 50%, 95%CI (45–56) History of fever 33%, 95%CI (31–35) Arthromyalgias 29%, 95%CI (28–31) Headache 28% 95%CI (27–30)	Clinical signs and symptoms frequents but are poor predictor of malaria infection	Community based cross sectional study
Tagbor et al (2008)	Ghana High transmission area	Pregnant women attending ANCs	*P. falciparum* parasitaemia	900 parasitaemic pregnant women and 223 aparasitaemic pregnant women	Parasitaemic *vs* non-parasitaemic History of fever: RR: 6.9 95%CI (4.1–11.5), P<0.0001 Vomiting: RR: 15.0 95%CI (3.7–60.4), P<0.0001	Malaria in pregnancy is often symptomatic	Randomized control trial to determine antimalarial drugs' efficacy and safety
Huynh et al (2011)	Benin High transmission area	Pregnant women attending ANCs	*P. falciparum* parasitaemia	Pregnant women with clinical complaints	Clinical symptoms associated with higher risk of positive slide: Fever (OR=5.2; p < 0.001), headache (OR = 2.1; p = 0.004), shivering (OR = 3.1; p < 0.001)	The majority of pregnant women were symptomless when infected	Longitudinal cohort study

1.5 Malaria in pregnancy - biological diagnosis

The non-specific nature of the clinical signs and symptoms of malaria, especially in pregnancy, may result in unnecessary treatment of malaria or non-treatment of other infectious diseases (47). Despite many strategies to prevent malaria in pregnancy, such as IPTp-SP and insecticide treated nets (ITNs), pregnant women are still vulnerable to malaria, and early detection and treatment with efficacious and safe treatments are essential. In malaria endemic areas, infected pregnant women are generally pauci-symptomatic or asymptomatic, often with low parasite density infections which may be difficult to diagnose (46–49). Various methods can be used for the diagnosis of malaria. Conventional microscopy and rapid diagnostic tests (RDTs) detecting circulating antigen are the most commonly used methods, though molecular methods are being considered (50–52).

1.5.1 Placental histology

Histological examination of a stained placental biopsy is the reference standard for diagnosing placental malaria at delivery with better sensitivity than peripheral and/or placental blood smears (53). Placental histology is able to detect sequestered parasites when none are detected in peripheral circulation (54).

Table 1: Classification of placenta pathology

Placenta pathology	**Description**
Not infected	No evidence of parasites pigment (haemozoin)
Active-acute	Parasites present, with absent or minimal pigment deposition within fibrin
Active-chronic	Parasites, with substantial amounts of pigment in fibrin or in cells
Past	Presence of pigment with no parasites

In practice, a full-thickness biopsy is collected from the maternal surface of the placenta approximately a third of the distance from the umbilical cord and the edge of the placental disc. This specimen (approximately 2.5 × 2.5 × 1 cm thick) should be collected as soon as possible after delivery and placed in 10% neutral buffered formalin [CONSORTIUM MIP, PERS. COMM.]. Infections are classified as acute, chronic or past infections (see Table 1). Although histology is an important tool for understanding disease processes in placental malaria, its use is associated with several challenges. Firstly, it cannot be performed before delivery and its cost makes it unsuitable for routine diagnosis. During the process of preparing the biopsy, the sample can be altered making the detection of infected erythrocytes difficult. Secondly, reading placenta slides may be difficult. Indeed, McGready (55) and Muehlenbachs (56) reported that women with malaria detected by weekly screening and treatment often have no histological changes at delivery. Thirdly, there is limited standardization between laboratories and limited infrastructure to process, score and analyze samples in some tropical settings (57).

1.5.2 Microscopy

The classic tool used to diagnose malaria is microscopy. Its most important shortcomings are relatively low sensitivity, particularly at low parasite densities, and the need for electricity. Although the expert microscopist may be able to detect densities as low as 5 parasites/µl, the detection threshold for an average microscopist would be around 50-100 parasites/µl (58). The sensitivity of microscopy is even lower in pregnant women, where peripheral blood smears may be negative even in the presence of parasites in the placenta (59). Microscopy of intervillous placental blood may have higher diagnostic sensitivity than that of peripheral blood, though placenta histology would perform better for the diagnosis of placental malaria (60). However, this method is unsuitable for routine diagnostics as it can be performed only

after delivery. The differentiation between the asexual and sexual parasite stages is possible by microscopy.

1.5.3 Immunochromatographic Tests/RDT

Rapid diagnostic tests (RDTs) are individual test kits that can detect *Plasmodium*-specific antigens in a drop of blood using lateral flow immunochromatography (59,60). RDTs offer a feasible alternative to microscopy, particularly for rural first-level health facilities. Indeed, they do not require a laboratory or special equipment, are simple to use with relatively little training, and provide, depending on the test kit, a positive or negative result within 20 minutes (52). Although commercial tests can detect several circulating malaria antigens, they cannot quantify parasitaemia or detect life-cycle stages (asexual vs sexual forms blood stages).

The Histidine-Rich Protein 2 (HRP-2) is a soluble antigen excreted by the parasite into the bloodstream. It is specific for *P. falciparum*; RDTs based on this antigen are the most commonly used ones. In case of placental malaria, these RDTs can detect circulating antigen. A disadvantage of the HRP-2 tests is the prozone effect, i.e. false negative results with extremely high parasite densities (61).

Other RDTs detect *P. falciparum*-specific parasite lactate dehydrogenase (*Pf*-pLDH). In contrast with HRP-2, *Pf*-pLDH is more rapidly cleared from the bloodstream and is not affected by the prozone effect. However, these tests also have some limitations (61,62). Indeed, pLDH is also produced by gametocytes which are not affected by many antimalarial drugs and may thus persist after clearance of asexual forms (63,64). Other commercial tests carry both an assay for genus specific aldolase enzyme and an HRP-2 assay, thus making them capable of distinguishing between falciparum and non-falciparum infections.

In a meta-analysis comparing RDT and PCR *vs* placental blood microscopy, PCR and RDT show higher sensitivity compared to placental microscopy (60). Moreover, taking peripheral

microscopy as "reference standard", the sensitivity and specificity of RDTs on peripheral blood were 81% [51-95 CI] and 94% [76-99 CI] respectively. RDTs seem to miss positive patients by microscopy, and this is not very reassuring for the value of RDTs to diagnose malaria in pregnancy.

The number of studies comparing RDT to histology is scarce, so firm recommendations cannot be made. Only one study on the performance of RDT was found. Brahmbhatt et al (65), assessed malaria and HIV mother-to-child transmission and evaluated the performance of RDT (HRP2-Aldolase) on peripheral blood *vs* histology. They reported a sensitivity of 57.5% [41-73 CI] and specificity of 89.5% [80-96 CI].

1.5.4 Molecular diagnostics tests

Recent developments in molecular biological technologies, e.g. polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), and nucleic acid sequence-based amplification (NASBA), have led to new strategies for malaria diagnosis.

Polymerase chain reaction (PCR)

Polymerase chain reaction, or PCR, is a laboratory technique used to make multiple copies of a DNA segment. PCR-based techniques have proven to be the most specific and sensitive diagnostic methods, particularly for malaria cases with low parasitemia or mixed infection (66). The PCR technique is used to confirm malaria infection, follow-up therapeutic response, and identify drug resistant parasites (67). Currently, PCR is the most commonly used molecular diagnostic for malaria with a detection limit of ~1 parasite/ µl depending on the assay type (66,67). PCR is more sensitive compared to microscopy and RDT, and therefore able to detect asymptomatic malaria carriers who may be targeted for treatment (68).

Moreover, PCR can help to detect drug-resistant parasites, mixed infections, and can be automated to process large numbers of samples (68,69). PCR provides high sensitivity and specificity in detecting low density of parasites. For this, PCR could have been a good

technique for the diagnosis of MiP (70). Although not yet routinely used as a diagnostic, PCR is increasingly being used for confirmatory diagnosis of returning travelers or migrants suspected of malaria in Western laboratories, and in some (reference) laboratories in endemic countries (71). However, its implementation in resource-limited endemic settings remains a challenge for malaria diagnosis or even routine clinical diagnostic usage, mainly due to the expensive consumables (72). Nowadays, differentiation between sexual and asexual forms in malaria, is possible using RNA amplification (73).

Other molecular tools

In order to combine the advantages of the PCR with the ability to use in field settings, new molecular diagnostic tools are under development. Nowadays, two main groups can be distinguished:

1. Isothermal amplification technologies and the PCR-nucleic acid lateral flow immunoassay (PCR-NALFIA).
2. Isothermal amplification technologies consist of two promising technologies: the nucleic acid sequence based amplification (NASBA) and the loop-mediated isothermal amplification (LAMP). These technologies do not require a thermocycler for amplification nor a gel imaging system for result reading (74).

LAMP is a novel semi-isothermal amplification technique that requires two temperatures for amplification. It is considered field-applicable because only a UV light source is required for a visual fluorescence based read-out and no PCR machine is required (74). However, there is still the need for DNA extraction. A major drawback of LAMP is that it cannot be performed in a multiplex format so 6 separate tests on a single blood sample are needed to do species differentiation (1 pan plasmodium and 5 species-specific reactions, depending on the setting). The reagent cost for LAMP is $0.40 –$0.70 per test, making LAMP less cost-effective than PCR (75,76).

NASBA is a semi-quantitative isothermal amplification technique with a fluorescent read-out. As POC technique it is prone to contamination and false-positive results, and requires more extensive sample preparation compared to LAMP. The cost of NASBA reagents is approximately $5–20 per test, making them at present too expensive for malaria diagnosis (77). However, because it is the most sensitive to low-level infections, NASBA has the potential to be especially useful as a screening tool despite a relatively high cost. NASBA will be really helpful to detect submicroscopic infections.

PCR-nucleic acid lateral flow immunoassay (PCR-NALFIA) is a rapid immuno-chromatographic test to detect labelled amplicon products on a nitrocellulose stick coated with specific antibodies (70). They use simple and cheap lateral flow read-out systems. To simplify the technique, a direct-on-blood system was developed to circumvent preamplification handling such as DNA extraction (74,78). In a recent review and compared to PCR, Roth et al reported sensitivity ranging from 87% (95% CI: 79-93) to 95% (95% CI: 89-98), and specificity from 82% (95% CI: 76-88) to 95% (95% CI: 93-97) (71). This good internal validity figures may constitute a real progress for MiP management as most infected pregnant women have low parasite densities. However, there are two major drawbacks. Firstly, the need for an electricity source to perform the PCR amplification and to maintain the cold chain for reagents storage makes this technique not suitable for field use and limits its utilization only to laboratories with key equipment (76,77). The same applies to microscopy which also needs an electricity source. Secondly, as for other molecular tools, a differentiation between asexual (disease) and sexual (transmission) stage of the parasite cannot be made (79). Using the test in combination with clinical presentation of the disease would have been a solution but malaria in pregnancy is mostly asymptomatic (38,49). PCR-NALFIA could be considered as a good option if solar energy system would be available.

1.6 Strategies to control malaria in pregnancy

Malaria control aims at reducing the disease burden to a level at which it is no longer a public health problem (80). The strategic approaches to malaria control in general, and which are also true for pregnant women are (81) vector control, i.e. long-lasting insecticidal nets (LLIN) and/or indoor residual spraying (IRS), chemoprevention, and case management.

1.6.1 Malaria prevention by vector control

LLINs and IRS are the most efficient and promoted applied interventions. Insecticide-treated nets (ITNs) are mosquito nets that have been treated by dipping in a pyrethroid insecticide solution, while LLINs refers to factory-treated mosquito nets made with a netting material that has insecticide incorporated into the fibers, or as a coating on the fibers. By reducing the contact human-vector and the lifespan of female mosquitoes, these interventions can have a substantial impact on transmission. Indeed, ITNs are estimated to reduce malaria mortality rates by 55% in children under 5 years of age in sub-Saharan Africa (82) and have been shown to reduce the incidence of malaria cases by 50% in a variety of settings (83). When ITNs are used by pregnant women, they are also efficacious in reducing maternal anemia, placental infection and low birth weight (84). That is why, since 2007, the WHO has recommended universal coverage with LLINs, rather than a predetermined number per household. IRS consists of the application of residual insecticides to the inner surfaces of dwellings, where many vector species of anopheline mosquitoes tend to rest after taking a blood meal (85). IRS is one of the primary vector control interventions for reducing and interrupting malaria transmission (85).

1.6.2 Chemoprevention and case management

Chemoprevention in pregnancy

The objective of this strategy is to prevent malarial illness by maintaining therapeutic antimalarial drug concentrations in the blood throughout the period of greatest malarial risk. Chemoprevention is particularly effective in pregnant women and young children. In pregnancy, preventive treatment is achieved by administering sulfadoxine-pyrimethamine at regular intervals. The antifolate combination sulfadoxine-pyrimethamine (SP) has been used widely to treat *falciparum* malaria for nearly half a century. Because of its low cost, safety, and prolonged post-treatment prophylactic effects (86), SP is currently being used as preventive therapy in vulnerable populations.

In pregnant women, evidence from four trials demonstrated the benefit of intermittent preventive treatment with SP. Based on these evidences, the WHO currently recommends IPTp with SP during the second and the third trimester, at least a month apart and any time women attend an antenatal clinic (87). These doses are given systematically without a diagnosis and are called intermittent preventive treatment during pregnancy (SP-IPTp). A recent meta-analysis by Radeva-Petrova shows that IPTp can reduce severe maternal anemia by 40%, the incidence of low birth weight by 27 %, and neonatal mortality by 38 %, although the evidence on neonatal mortality is less conclusive (88). Resistance to SP first appeared in Southeast Asia and South America in the 1970s (89), and soon followed in Africa, reaching Tanzania in 1982 (86,87) and West Africa by the late 1980s (88,89). This rapid evolution of resistance to antifolates is attributed to specific point mutations combined with the fact that these mutations may have already been present at a lower level. In addition, the use of antibacterial antifolate combination trimethoprim–sulfamethoxazole may have played a role in selecting resistant parasites. Due to the high SP and chloroquine resistance, most countries

have abandoned SP as first-line therapy in favor of artemisinin-based combination therapies (ACTs). Nevertheless, in these high-resistance areas, IPTp-SP is still associated with increases in birth weight and maternal haemoglobin (90–92). Indeed, ter Kuile et al (90) reviewed the effect of SP resistance on the efficacy of IPT-SP and concluded that 2 doses of SP as IPTp maintains its efficacy. Similar findings were reported by Coulibaly et al (91) during a multi-centre study in Burkina Faso and Mali. In this study, triple mutation (Ile51+Arg59+Asn108) was present in about half of infections while less than 2% of the samples recrudesced at day 42. More recently, a review by Desai et al (92) was conducted in 6 sites with different level of SP resistance (high, medium or low). They found that IPT-SP was associated with a 22% reduction in the risk of LBW, a lower risk of placental infection (23%) by impression smear, even in areas of high SP resistance. Possible explanations for maintained efficacy are acquired immunity contributing importantly to parasite clearance, which in turn influences the association of treatment outcome with different *dhfr* and *dhps* alleles (91). Secondly, SP may have some antibacterial and antifungical effects that may improve fetal growth and maternal health (93). Consequently, the relationship between SP level of resistance and its effectiveness in preventing LBW can be affected by the prevalence of others risk factors.

Case management artemisinin-based combination treatment (ACT)

Effective case management is one strategy recommended by the WHO to control MiP, in addition to insecticide-treated nets, indoor residual spraying (IRS) of insecticides, and intermittent preventive treatment (94). Prompt diagnosis and efficient treatment are paramount, although *P falciparum* drug resistance has narrowed the treatment options.

For the treatment of malaria in pregnancy during the second and third trimesters, the WHO recommends the use of ACTs. These combinations associate a fast-acting artemisinin

derivative that rapidly reduces the parasite biomass and gametocyte carriage (95,96), and slower-acting partner drug that clears the remaining parasites and provides post-treatment prophylaxis whose duration depends on its pharmacokinetic properties (96–98). The rationale for combining antimalarials with different mechanisms of action, such as in the ACTs, is to prevent the development of resistance or at least slow down its onset (99). In this section we will focus on the efficacy and safety of ACTs used in our main study for the treatment of uncomplicated malaria in pregnancy.

First trimester of pregnancy

The WHO recommends quinine plus clindamycin to be given for 7 days for uncomplicated *P. falciparum* malaria or artesunate plus clindamycin for 7 days if this treatment fails (99). Pre-studies in animals have highlighted the teratogenicity of ACTs during the first trimester (100–102); however, the same phenomenon has not been observed in humans. Nowadays, there is an increasing body of evidence of ACTs' safety during the first trimester of pregnancy. Manyando et al conducted a systematic review of the safety and efficacy of AL during pregnancy (103). In total, 1,103 reports of AL use in pregnant women, of which 212 during the first trimester. In line with these findings, others studies also confirmed the safety of ACTs use in the first trimester (104–109). The latest Malaria Policy Advisory Committee to the WHO, on the basis of >1,000 first trimester exposures to artemisinins have recommended to review the current guidelines (110).

Second and third trimester

Artemether-lumefantrine (AL)

AL is a fixed-dose combination of 20 mg of artemether and 120 mg of lumefantrine. AL is the most widely used ACT as first-line or second-line treatment for uncomplicated malaria worldwide (111). AL needs to be administered twice daily (the first doses given 8 hours apart)

for 3 days. In addition, the absorption of AL is enhanced by fatty foods (112,113) but the recommendation of taking it with food is not usually respected during pregnancy due to frequent anorexia, nausea, or vomiting (114). The complexity of the treatment together with the need of fatty foods is associated with poor adherence and compliance (115–117). This could lead to treatment failure and selection of resistant parasites. There are few studies assessing the efficacy and safety of AL in pregnancy given the fact that pregnant women are generally excluded from trials and also because ACTs is not recommended in the first trimester. Kaye et al in 2008, compared the safety and the clinical and the parasitological response of AL to chlorproguanil-dapsone in a randomized control trial (118). A high cure rate of 100% was found after 28-day of follow up. AL also show very good cure rate at a long follow up period. Indeed, Piola et al, randomly assigned 152 pregnant women in AL and quinine groups in a non-inferiority trial (119). At day 42, 99.3% of the AL group and 97.6% of the quinine group were cured. In addition, the number of adverse events was about half in the AL than in the quinine group. In contrast, lower cure rates of AL at day 42 were observed in a similar study in northern Thailand; 89.2% (82.3–96.1%) for artesunate alone and 82.0% (74.8–89.3%) for AL (120). Such difference was attributed to a lower immunity in women in Thailand compare to those of Uganda or to the multidrug resistance profile in Thailand.

Artesunate-amodiaquine (ASAQ)

ASAQ is presented as a fixed-dose combination of 100 mg of artesunate and 270 mg of amodiaquine base for three days treatment (121–124). The combination ASAQ has been extensively studied in children and adults, but there is few data on its use on pregnancy. So far, two studies investigated the efficacy of ASAQ for uncomplicated malaria in pregnancy (except PregACT study). Mutabingwa et al first compared the efficacy and safety of the combination ASAQ, SP+AQ, Chlorproguanil-Dapsone and SP (125). From a total of 1433 women screened, 272 met the inclusion criteria and were randomized to one of the four

regimens. The adjusted parasitological failure rate at day 28 was 18% for CD, 1% for SP+AQ and 4.5% for AQ+AS. More recently, Ukah et al conducted a trial comparing the efficacy of ASAQ *vs* AL for uncomplicated malaria during pregnancy (126). The day-28 cure rate was 98.5% for ASAQ and 94.4% for AL. There was no difference between the two regimens (p=0.138). In contrast, adverse events were more frequent in the ASAQ arm.

Artesunate-mefloquine (MQAS)

MQAS is a co-formulated tablet containing 100 mg of AS and 220 mg of MQ hydrochloride. Studies on the efficacy and safety of MQAS are surprisingly scarce. The only efficacy study was conducted in 1995-1997, a randomized trial comparing MQAS vs quinine for multidrug-resistant falciparum in pregnancy (127). MQAS regimen performed well compared to the 7-day quinine treatment. The overall efficacy of MQAS was 98.2% (95% CI 0-11) and that of quinine 67.0% (95% CI 43.3-90.8). In addition, the combination MQAS was associated with lower gametocytes carriage. Others studies on MQAS investigated the treatment's pharmacokinetic properties. A study conducted in Nanoro investigated whether MQ dosage should be adjusted for pregnant women. Twenty four pregnant and 24 non-pregnant women with P *falciparum* were recruited and followed until day-63 (128). This study shows that no dose adjustment is required in pregnancy. The efficacy rate at day 63 was 95.8% in pregnant women and 100% for the non-pregnant women. MQ taken alone is generally associated with adverse events (nausea, vomiting, dizziness, dysphoria…) (129–133).

Dihydroartemisinin-piperaquine (DHAPQ)

DHAPQ is presented as co-formulated tablet containing 40 mg dihydroartemisinin and 320 mg piperaquine. Increase piperaquine absorption can lead to QT prolongation and cardiac arrhythmias and thus treatment should be taken with an empty stomach (134). Studies on the efficacy of DHAPQ during pregnancy are rare. Apart of the pharmacokinetics (135–137),

which found DHAPQ to be effective during pregnancy, only one study documented the exposure of pregnant women with malaria to DHAPQ. The objective was to assess their safety in both mother and baby and impact on pregnancy outcome (138). During a six-year period, pregnant women were treated with one of the following antimalarial: DHAPQ, chloroquine plus sulfadoxine-pyrimethamine (CQSP) and quinine alone. This was in accordance with the local guidelines of malaria treatment recommending quinine plus clindamycin for patients with *falciparum* malaria and CQSP for women with non- *falciparum* malaria. In 2006, Papua Indonesia changed his policy with DHAPQ for uncomplicated malaria of any species while quinine + clindamycin was reserved for the first trimester. In those presenting with a febrile illness, DHAPQ resulted in a 2.5 fold greater likelihood of being discharged from hospital with an ongoing pregnancy compared to women receiving oral quinine. Data on the temporal analysis of adverse outcomes in pregnancy and early life following the policy change was published in 2015 in a separate article (139). Overall, the increased use of DHAPQ was associated with a 54% fall in the proportion of maternal malaria at delivery and a 98% decrease in congenital malaria (from 7.1% prior to 0.1% after policy change). Recently, DHAPQ was tested as antimalarial drug in the intermittent screening and treatment strategy (140–142).

1.7 Monitoring antimalarial drug resistance

As described above, the treatments used for IPTp and those used for case management are threatened by the emergence of drug resistance. It is therefore essential to monitor treatment efficacy. This can be done with in vivo tests, in vitro/ex vivo assays and molecular markers for drug resistance (143).

1.7.1 In vivo tests

In vivo tests remain the gold standard for monitoring antimalarial drug efficacy. They are also the primary source of information used by policy makers to shape recommendations for malaria chemotherapy and prophylaxis. In vivo methods can be used to measure therapeutic efficacy following standardized WHO protocols (144). Treatment outcomes can be classified as follows:

Early treatment failure (ETF): The development of danger signs for severe malaria on days 1, 2, or 3 in the presence of parasitaemia; parasitaemia on day 2 higher than the day 0 count regardless of axillary temperature; parasitaemia on day 3 with axillary temperature ≥37.5°C; parasitaemia on day 3 similar or greater than on day 0.

Late clinical failure (LCF): The development of danger signs for severe malaria after day 3 in the presence of parasitaemia, without previously meeting any of the criteria of ETF; presence of parasitaemia and axillary temperature ≥37.5°C or history of fever on any day from day 4 until the end of the follow up, without previously meeting any of the criteria of ETF.

Late parasitological failure (LPF): The presence of parasitaemia after day 3 at any day of the follow up and axillary temperature <37.5°C, without previously meeting any of the criteria for ETF or LCF.

Adequate Clinical and Parasitological Response (ACPR) is characterized by the absence of parasitaemia at the end of follow up day, regardless of axillary temperature without previously meeting any of the criteria for ETF, LTF, or LPF.

1.7.2 Ex vivo tests

Ex vivo assays measure the intrinsic susceptibility of malaria parasites to anti-malarial drugs, and establish baseline susceptibility of local parasite isolates to newly introduced drugs. Several techniques for ex vivo tests have been developed. These techniques include the WHO microtest, the isotopic based tests, the enzyme-linked immunosorbent assay (ELISA) and more recently the SYBR green-based tests and the cytometric tests (145,146). However, the availability of these techniques is limited in low-income countries due to financial cost, time consumption and sometimes problems related to the management of radioactive waste. Moreover, the lack of standardization of the non-radioactive methods is another problem (147). Recently, a rapid, reproducible and cheap alternative to radioisotopes was developed and tested successfully in West Africa in Ghanaian isolates (148). Though the SYBR Green I ex vivo assay offers advantages compared to the previous method, it still requires specific material and expertise commonly not available in field laboratories.

1.7.3 Molecular markers

The limitations of in vivo and ex vivo methods for measuring drug-resistant malaria and the elucidation of molecular mechanisms of resistance to some antimalarial drugs have led to considerable research on molecular markers for resistance. Polymorphisms in parasite genes that are associated with changes in drug responses have been used as molecular markers of resistance to some antimalarials and as a complement to ex vivo and clinical measures of resistance. For example, some markers such as the K76T alleles of the *P. falciparum* chloroquine-resistance transporter (pfcrt) (149–150), *dhfr*, *dhps* for SP (151–153) and increased copy number of the *P. falciparum* multi-drug-resistance-1 gene (*Pfmdr1*) (154) have all been associated with resistance to antimalarial drugs (155). Although molecular markers for resistance to the artemisinins and some of their newer partners are not well

defined yet, for the 'old' drugs, trends in prevalence of these resistance markers are useful predictors of treatment responses and can help guiding treatment policy (156).

1.8 Aim and outline of this thesis

This thesis studies three important aspects of MiP management: early recognition of malaria through a detailed study on the clinical presentation of malaria, ex vivo drug sensitivity profiles of different antimalarials used in MiP, and specific drug resistance to SP in Burkina Faso as an important parameter of the IPTp-SP intervention strategy.

Early diagnosis is extremely important to manage malaria cases among pregnant women. In areas of stable transmission, it is believed that infected pregnant women are usually asymptomatic. However, this is based on a few studies carried out among women attending antenatal clinics (ANCs) for routine examination and on cross-sectional community-based studies, which tend to underestimate the actual frequency of malaria-related symptoms (38). Therefore chapter 3 reports a study on the clinical signs and symptoms of malaria in a cohort of pregnant women.

IPTp reduces the prevalence of placental malaria, severe anemia among primigravidae, pre-term delivery (157,158), low birth-weight and improves neonatal survival (157–159). It is considered to be safe, efficacious and easy to administer at antenatal clinics (ANC) (160). IPTp with SP was introduced in Burkina Faso in 2005, and since then there have been few studies on the efficacy of IPTp. Chapter 4 reports the prevalence of the molecular markers of SP resistance.

Safe and efficient drugs are needed for the treatment of MiP. Malaria-infected pregnant women, who represent an important group at risk, are usually excluded from clinical trials. Consequently, there is limited data on the ex vivo efficacy of ACT used for the treatment of

malaria in pregnant women. Chapter 5 reports the ex vivo efficacy of antimalarial drugs recommended by the NMCP in Burkina Faso while in chapter 6 and 7, efficacy and safety of ACTs in Africa are investigated. The chapter 8 presents the general discussion of the thesis and chapter 9 the conclusions and recommendations.

All these studies were carried out in Burkina Faso. The country profile and important aspects of malaria in pregnancy in Burkina Faso are described in chapter 3.

1.9 Study Objectives

1.9.1 General objective

The general objective of this thesis is to provide information to decrease the burden of malaria among pregnant women in Burkina Faso.

1.9.2 Specific objectives

The specific objectives of this thesis are the following:

1. To evaluate the usefulness of clinical signs and symptoms for the diagnosis of malaria in pregnant women;
2. To determine the prevalence of molecular markers of SP resistance in asymptomatic and symptomatic malaria-infected pregnant women;
3. To determine 'ex vivo' the current efficacy of antimalarial drugs recommended by the NMCP in Burkina Faso;
4. To determine the safety and efficacy of three ACT for the treatment of malaria in pregnant women in Burkina Faso.

1.10 Overview of the thesis chapters

Chapter 1 provides a general description of malaria epidemiology, globally and in Sub Saharan Africa. We also present a review of studies on the clinical features of malaria, and treatment options of uncomplicated malaria.

Chapter 2 provides a description of the 'malaria in pregnancy' burden in Burkina Faso.

Chapter 3 presents the results of a prospective study documenting the clinical presentation of malaria among pregnant women and assesses their predictive value.

Chapter 4 discusses the efficacy of SP as IPTp through the assessment of the prevalence of molecular markers of SP resistance.

Chapter 5 presents the ex vivo sensitivity profile of isolates collected from pregnant women, an important group at risk.

Chapter 6 presents the result of a multi-centre study on the safety and efficacy of four artemisinin-based combination treatments in African pregnant women with malaria: Case of Burkina Faso

Chapter 7 discusses the site-specific results of PREGACT (Burkina Faso data)

Chapter 8 presents the general discussion

Chapter 9 presents the conclusions and further perspectives in malaria in pregnancy

1.11 References

1. White NJ. *Plasmodium knowlesi*: the fifth human malaria parasite. Clin Infect Dis. 2008;46(2):172–3.

2. WHO. Malaria, Fact sheet N°94 Reviewed April 2015.

3. WHO. Malaria fact sheet updated. 2015.

4. CDC. Malaria. 2015.

5. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoa K, et al. Epidemiology and burden of malaria in pregnancy. Lancet Infect Dis. 2007;7(2):93–104.

6. Nosten F, ter Kuile F, Maelankirri L, Decludt B, White NJ. Malaria during pregnancy in an area of unstable endemicity. Trans R Soc Trop Med Hyg. 1991;85(4):424–29.

7. Newman RD, Hailemariam A, Jimma D, Degifie A, Kebede D, et al. Burden of malaria during pregnancy in areas of stable and unstable transmission in Ethiopia during a nonepidemic year. J Infect Dis. 2003;187(11):1765–72.

8. Bayoumi NK, Elhassan EM, Elbashir MI, Adam I. Cortisol, prolactin, cytokines and the susceptibility of pregnant Sudanese women to *Plasmodium falciparum* malaria. Ann Trop Med Parasitol. 2009;103(2):111–17.

9. Duffy PE, Fried M. *Plasmodium falciparum* adhesion in the placenta. Curr Opin Microbiol. 2003;6(4):371–76.

10. Beeson JG, Reeder JC, Rogerson SJ, Brown GV. Parasite adhesion and immune evasion in placental malaria. Trends Parasitol. 2001;17(7):331–37.

11. Duffy PE. Immunity to malaria during pregnancy: different host, different parasite. In: Duffy PE, Fried M, eds. London: Taylor & Francis, 2001:70-126.

12. Flick K, Scholander C, Chen Q, Fernandez V, Pouvelle B, et al. Role of nonimmune IgG bound to PfEMP1 in placental malaria. Science. 2001;293(5537):2098–100.

13. Rasti N, Namusoke F, Chene A, Chen Q, Staalsoe T, et al. Nonimmune immunoglobulin binding and multiple adhesion characterize *Plasmodium falciparum*-infected erythrocytes of placental origin. Proc Natl Acad Sci. 2006;103(37):13795–800.

14. Fried M, Domingo GJ, Gowda CD, Mutabingwa TK, Duffy PE. *Plasmodium falciparum*:

Chondroitin sulfate A is the major receptor for adhesion of parasitized erythrocytes in the placenta. Exp Parasitol. 2006;113(1):36–42.

15. Salem HH, Maruyama I, Ishii HMP. Isolation and characterization of thrombomodulin from human placenta. J Biol Chem. 1984;10(259):12246–51.

16. Muthusamy A, Achur RN, Bhavanandan VP, Fouda GG, Taylor DW, et al. *Plasmodium falciparum*-infected erythrocytes adhere both in the intervillous space and on the villous surface of human placenta by binding to the low-sulfated chondroitin sulfate proteoglycan receptor. Am J Pathol. 2004;164(6):2013–25.

17. Brabin BJ. An analysis of malaria in pregnancy in Africa. Bull World Health Organ. 1983;61(6):1005–16.

18. Menendez C, Ordi J, Ismail MR, Ventura PJ, Aponte JJ, et al. The impact of placental malaria on gestational age and birth weight. J Infect Dis. 2000 ;181(5):1740–45.

19. Tako EA, Zhou A, Lohoue J, Leke R, Taylor DW, et al. Risk factors for placental malaria and its effect on pregnancy outcome in Yaounde, Cameroon. Am J Trop Med Hyg. 2005;72(3):236–42.

20. Shulman CE, Marshall T, Dorman EK, Bulmer JN, Cutts F, et al. Malaria in pregnancy: Adverse effects on haemoglobin levels and birthweight in primigravidae and multigravidae. Trop Med Int Heal. 2001 Oct;6(10):770–78.

21. Rogerson SJ, Pollina E, Getachew A, Tadesse E, Lema VM, et al. Placental monocyte infiltrates in response to Plasmodium falciparum malaria infection and their association with adverse pregnancy outcomes. Am J Trop Med Hyg. 2003;68(1):115–19.

22. Espinoza E, Hidalgo LCP. The effect of malarial infection on maternal-fetal outcome in Ecuador. J Matern Fetal Neonatal Med. 2005;18(2):101–5.

23. Leenstra T, Phillips-Howard PA, Kariuki SK, Hawley WA, Alaii JA, et al. Permethrin-treated bed nets in the prevention of malaria and anemia in adolescent schoolgirls in western Kenya. Am J Trop Med Hyg. 2003;68(4 Suppl):86–93.

24. Rogerson SJ, van den Broek NR, Chaluluka E, Qongwane C, Mhango CG, et al. Malaria and anemia in antenatal women in Blantyre, Malawi: a twelve-month survey. Am J Trop Med Hyg. 2000;62(3):335–40.

25. Marques PX, Saute F, Pinto VV, Cardoso S, Pinto J, et al. *Plasmodium* species mixed infections in two areas of Manhica district, Mozambique. Int J Biol Sci. 2005;1(3):96–102.

26. Walker-Abbey A, Djokam RRT, Eno A, Leke RFG, Titanji VPK, et al. Malaria in pregnant Cameroonian women: the effect of age and gravidity on submicroscopic and mixed-species infections and multiple parasite genotypes. Am J Trop Med Hyg. 2005;72(3):229–35.

27. Wickramasuriya GAW. Some observations on malaria occurring in association with pregnancy. J Obs Gynaecol. 1935;42:816–34.

28. Menendez C, D'Alessandro U, ter Kuile FO. Reducing the burden of malaria in pregnancy by preventive strategies. Lancet Infect Dis. 2007;7(2):126–35.

29. Eisele TP, Larsen DA, Anglewicz PA, Keating J, Yukich J, et al. Malaria prevention in pregnancy, birthweight, and neonatal mortality: A meta-analysis of 32 national cross-sectional datasets in Africa. Lancet Infect Dis. 2012;12(12):942–49.

30. Guyatt HL, Snow RW. Malaria in pregnancy as an indirect cause of infant mortality in sub-Saharan Africa. Trans R Soc Trop Med Hyg. 2001;95(6):569–76.

31. Guyatt HL, Snow RRW. Impact of malaria during pregnancy on low birth weight in sub-Saharan Africa. Clin Microbiol Rev. 2004;17(4):760–69.

32. Guyatt HL, Snow RW. The epidemiology and burden of Plasmodium falciparum-related anemia among pregnant women in sub-Saharan Africa. Am J Trop Med Hyg. 2001;64(1-2 Suppl):36–44.

33. Steketee RW, Wirima JJ, Slutsker L, Heymann DL, Breman JG. The problem of malaria and malaria control in pregnancy in sub-Saharan Africa. Am J Trop Med Hyg. 1996;55(1 Suppl):2–7.

34. Menendez C. Malaria during pregnancy. Curr Mol Med. 2006;6(2):269–73.

35. Greenwood B. The use of anti-malarial drugs to prevent malaria in the population of malaria-endemic areas. Am J Trop Med Hyg. 2004;70(1):1–7.

36. D'Alessandro U, Langerock P, Bennett S, Francis N, Cham K, et al. The impact of a national impregnated bed net programme on the outcome of pregnancy in primigravidae in The Gambia. Trans R Soc Trop Med Hyg. 1996;90(5):487–92.

37. Winstanley PA. Chemotherapy for *falciparum* malaria: the armoury, the problems and the prospects. Parasitol Today. 2000;16(4):146–53.

38. Saute F, Menendez C, Mayor A, Aponte J, Gomez-Olive X, et al. Malaria in pregnancy in rural Mozambique: The role of parity, submicroscopic and multiple *Plasmodium falciparum* infections. Trop Med Int Heal. 2002;7(1):19–28.

39. Nosten F, Rogerson SJ, Beeson JG, McGready R, Mutabingwa TK, et al. Malaria in pregnancy and the endemicity spectrum: What can we learn? Trends Parasitol. 2004;20(9):425–32.

40. GA Walton. On the control of malaria in Freetown, Sierra Leone. II. Control methods and the effects upon the transmission of Plasmodium falciparum resulting from the reduced abundance of Anopheles gambiae. Ann Trop Med Parasitol. 1949;43:117–39.

41. SCHWETZ J, PEEL M. Congenital malaria and placental infections amongst the negroes of Central Africa. Trans R Soc Trop Med Hyg. 1934;28:167–74.

42. Sayed MA, Somaya K. Abd AR, Ahmed A. Mohamedani, Ali B. Habour AAS. Malaria parasitemia during delivery. Saudi Med J. 2002;23(6):684–88.

43. Nnaji GA, Ikechebelu JI. An evaluation of the use of reported febrile illness in predicting malaria in pregnancy. J Obstet Gynaecol. 2007 Nov;27(8):791–94.

44. Bardají A, Sigauque B, Bruni L, Romagosa CC, Sanz S, et al. Clinical malaria in African pregnant women. Malar J. 2008;7:27.

45. Tagbor H, Bruce J, Browne E, Greenwood B, Chandramohan D, et al. Malaria in pregnancy in an area of stable and intense transmission: Is it asymptomatic? Trop Med Int Heal. 2008;13(8):1016–21.

46. Huynh BT, Fievet N, Gbaguidi G, Borgella S, Mévo B, et al. Malaria associated symptoms in pregnant women followed-up in Benin. Malar J. 2011;10(1):72.

47. Bhandari PL, Raghuveer CV, Rajeev A, Bhandari PD. Comparative study of peripheral blood smear, quantitative buffy coat and modified centrifuged blood smear in malaria diagnosis. Indian J Pathol Microbiol. 2008;51(1):108–12.

48. Steketee RW, Wirima JJ, Slutsker L, Khoromana CO, Heymann DL, et al. Malaria treatment and prevention in pregnancy: Indications for use and adverse events associated with use of chloroquine or mefloquine. Am J Trop Med Hyg. 1996;55(1):50–56.

49. Uneke CJ. Diagnosis of Plasmodium falciparum malaria in pregnancy in sub-Saharan Africa: the challenges and public health implications. Parasitol Res. 2008;102(3):333–42.

50. Murray CK, Gasser RAJ, Magill AJ, Miller RS. Update on rapid diagnostic testing for malaria. Clin Microbiol Rev. 2008;21(1):97–110.

51. Murphy SC, Shott JP, Parikh S, Etter P, Prescott WR, et al. Malaria diagnostics in clinical

trials. Am J Trop Med Hyg. 2013;89(5):824–39.

52. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). Am J Trop Med Hyg. 2007;77(6 Suppl):119–27.

53. Rogerson SJ, Mkundika P, Kanjala MK. Diagnosis of Plasmodium falciparum malaria at delivery: comparison of blood film preparation methods and of blood films with histology. J Clin Microbiol. 2003;41(4):1370–74.

54. Bulmer JN, Rasheed FN, Francis N, Morrison L, Greenwood BM. Placental malaria. I. Pathological classification. Histopathology. 1993;22(3):211–18.

55. McGready R, Davison BB, Stepniewska K, Cho T, Shee H, et al. The effects of Plasmodium falciparum and P. vivax infections on placental histopathology in an area of low malaria transmission. Am J Trop Med Hyg. 2004;70(4):398–407.

56. Muehlenbachs A, Fried M, McGready R, Harrington WE, Mutabingwa TK, et al. A novel histological grading scheme for placental malaria applied in areas of high and low malaria transmission. J Infect Dis. 2010;202(10):1608–16.

57. Fried M, Muehlenbachs A, Duffy PE. Diagnosing malaria in pregnancy: an update. Expert Rev Anti Infect Ther. 2012;10(10):1177–87.

58. Payne D. Use and limitations of light microscopy for diagnosing malaria at the primary health care level. Bull World Health Organ. 1988;66(5):621–26.

59. Cottrell G, Mary JY, Barro D, Cot M. Is malarial placental infection related to peripheral infection at any time of pregnancy? Am J Trop Med Hyg. 2005;73(6):1112–18.

60. Kattenberg JH, Ochodo EA, Boer KR, Schallig HD, Mens PF, et al. Systematic review and meta-analysis: rapid diagnostic tests versus placental histology, microscopy and PCR for malaria in pregnant women. Malar J. 2011;10(1):321.

61. Gillet P, Scheirlinck A, Stokx J, De Weggheleire A, et al. Prozone in malaria rapid diagnostics tests: how many cases are missed? Malar J. 2011;10(1):166.

62. Kattenberg JH, Tahita CM, Versteeg IAJ, Tinto H, Traoré-Coulibaly M, et al. Antigen persistence of rapid diagnostic tests in pregnant women in Nanoro, Burkina Faso, and the implications for the diagnosis of malaria in pregnancy. Trop Med Int Heal. 2012;17(5):550–57.

63. Oduola AM, Omitowoju GO, Sowunmi A, Makler MT, Falade CO, et al. Plasmodium

falciparum: evaluation of lactate dehydrogenase in monitoring therapeutic responses to standard antimalarial drugs in Nigeria. Exp Parasitol. 1997;87(3):283–89.

64. Mueller I, Betuela I, Ginny M, Reeder JC, Genton B. The sensitivity of the OptiMAL rapid diagnostic test to the presence of Plasmodium falciparum gametocytes compromises its ability to monitor treatment outcomes in an area of Papua New Guinea in which malaria is endemic. J Clin Microbiol. 2007 ;45(2):627–30.

65. Brahmbhatt H, Sullivan D, Kigozi G, Askin F, Wabwire-Mangenm F, et al. Association of HIV and malaria with mother-to-child transmission, birth outcomes, and child mortality. J Acquir Immune Defic Syndr. 2008;47(4):472–76.

66. Morassin B, Fabre R, Berry A, Magnaval JF. One year's experience with the polymerase chain reaction as a routine method for the diagnosis of imported malaria. Am J Trop Med Hyg. 2002;66(5):503–08.

67. Chotivanich K, Silamut K DN. Laboratory diagnosis of malaria infection-a short review of methods. Aust J Med Sci. 2006;27:11–15.

68. MAIERA consultative Group. A research agenda for malaria eradication: diagnoses and diagnostics. PLoS Med. 2011;8(1:e1000396.).

69. Rougemont M, Van Saanen M, Sahli R, Hinrikson HP, Bille J, et al. Detection of four Plasmodium species in blood from humans by 18S rRNA gene subunit-based and species-specific real-time PCR assays. J Clin Microbiol. 2004;42(12):5636–43.

70. Mens PF, van Amerongen A, Sawa P, Kager PA, Schallig HDFH. Molecular diagnosis of malaria in the field: development of a novel 1-step nucleic acid lateral flow immunoassay for the detection of all 4 human Plasmodium spp. and its evaluation in Mbita, Kenya. Diagn Microbiol Infect Dis. 2008 ;61(4):421–27.

71. JM, Korevaar DA, Leeflang MMG, Mens PF. Molecular malaria diagnostics: A systematic review and meta-analysis. Crit Rev Clin Lab Sci. 2016;53(2):87–105.

72. Hanscheid T, Grobusch MP. How useful is PCR in the diagnosis of malaria? Trends Parasitol. 2002;18(9):395–58.

73. Kast K, Berens-Riha N, Zeynudin A, Abduselam N, Eshetu T, et al. Evaluation of Plasmodium falciparum gametocyte detection in different patient material. Malar J. 2013;12:438.

74. Polley SD, Mori Y, Watson J, Perkins MD, Gonzalez IJ, et al. Mitochondrial DNA targets increase sensitivity of malaria detection using loop-mediated isothermal amplification. J Clin

Microbiol. 2010;48(8):2866–71.

75. Paris DH, Imwong M, Faiz AM, Hasan M, Yunus E Bin, et al. Loop-mediated isothermal PCR (LAMP) for the diagnosis of falciparum malaria. Am J Trop Med Hyg. 2007;77(5):972–76.

76. Han ET. Loop-mediated isothermal amplification test for the molecular diagnosis of malaria. Expert Rev Mol Diagn. 2013;13(2):205–18.

77. Landry ML, Garner R, Ferguson D. Comparison of the NucliSens Basic kit (Nucleic Acid Sequence-Based Amplification) and the Argene Biosoft Enterovirus Consensus Reverse Transcription-PCR assays for rapid detection of enterovirus RNA in clinical specimens. J Clin Microbiol. 2003;41(11):5006–10.

78. Swan H, Sloan L, Muyombwe A, Chavalitshewinkoon-Petmitr P, Krudsood S, et al. Evaluation of a real-time polymerase chain reaction assay for the diagnosis of malaria in patients from Thailand. Am J Trop Med Hyg. 2005;73(5):850–54.

79. Mens PF, de Bes HM, Sondo P, Laochan N, Keereecharoen L, et al. Direct blood PCR in combination with nucleic acid lateral flow immunoassay for detection of Plasmodium species in settings where malaria is endemic. J Clin Microbiol. 2012;50(11):3520–25.

80. WHO 2008a. Global malaria control and elimination:report of a technical review. In: World Health Organization. 2008.

81. WHO. World Malaria report. 2014.

82. Eisele TP, Larsen D, Steketee RW. Protective efficacy of interventions for preventing malaria mortality in children in Plasmodium falciparum endemic areas. Int J Epidemiol. 2010;39 Suppl 1:i88–101.

83. Lengeler C. Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database Syst Rev. 2004;2:CD000363.

84. Global fund. Global Fund proposal development: WHO policy brief on malaria. 2011.

85. WHO. Malaria vector control and personal protection: report of a WHO study group (WHO Technical Report Series, No. 936). 2006.

86. White NJ. Intermittent presumptive treatment for malaria. PLoS Med. 2005 ;2(1):e3.

87. Malaria Policy Advisory Committee to the WHO: conclusions and recommendations. Malar J. 2013;12:213.

88. Radeva-Petrova D, Kayentao K, ter Kuile FO, Sinclair D, Garner P. Drugs for preventing malaria in pregnant women in endemic areas: any drug regimen versus placebo or no treatment. Cochrane database Syst Rev. 2014;10:CD000169.

89. Peters W. Chemotherapy and drug resistance in malaria. Acad Press. 1987;

90. ter Kuile FO, van Eijk AM, Filler SJ. Effect of sulfadoxine-pyrimethamine resistance on the efficacy of intermittent preventive therapy for malaria control during pregnancy: a systematic review. JAMA. 2007;297(23):2603–16.

91. Coulibaly SO, Kayentao K, Taylor S, Guirou E a, Khairallah C, et al. Parasite clearance following treatment with sulphadoxine-pyrimethamine for intermittent preventive treatment in Burkina-Faso and Mali: 42-day in vivo follow-up study. Malar J. 2014;13(1):41.

92. Desai M, Gutman J, Taylor SM, Wiegand RE, Khairallah C, et al. Impact of Sulfadoxine-Pyrimethamine Resistance on Effectiveness of Intermittent Preventive Therapy for Malaria in Pregnancy at Clearing Infections and Preventing Low Birth Weight. Clin Infect Dis. 2016;62(3):323–33.

93. Capan M, Mombo-Ngoma G, Makristathis A, Ramharter M. Anti-bacterial activity of intermittent preventive treatment of malaria in pregnancy: comparative in vitro study of sulphadoxine-pyrimethamine, mefloquine, and azithromycin. Malar J. 2010;9:303.

94. WHO. Technical Expert Group meeting on intermittent preventive treatment in pregnancy (IPTp). WHO Headquarters, Geneva, Switzerland; 2007.

95. Hien TT, White NJ. Qinghaosu. Lancet. 1993;341(8845):603–8.

96. White NJ. Qinghaosu (artemisinin): the price of success. Science. 2008 ;320(5874):330–34.

97. Ashley EA, White N. Artemisinin-based combinations. Curr Opin Infect Dis. 2005;18:531–36.

98. Nosten F, White NJ. Artemisinin-Based Combination Treatment of Falciparum Malaria. Am J Trop Med Hyg. 2007;77(6_Suppl):181–92.

99. WHO. Guidelines for the treatment of malaria.2nd ed,World Health Organization. 2010.

100. Clark RL, Lerman SA, Cox EM, et al. Developmental toxicity of artesunate in the rat: comparison to other artemisinins, comparison of embryotoxicity and kinetics by oral and intravenous routes, and relationship to maternal reticulocyte count. Birth Defects Res B Dev Reprod Toxicol. 2008;83:397–406.

101. Longo M, Zanoncelli S, Torre PD, et al. In vivo and in vitro investigations of the effects of the antimalarial drug dihydroartemisinin (DHA) on rat embryos. Reprod Toxicol. 2006;22:797–810.

102. Longo M, Zanoncelli S, Manera D, et al. Effects of the antimalarial drug dihydroartemisinin (DHA) on rat embryos in vitro. Reprod Toxicol. 2006;21:83–93.

103. Manyando C, Kayentao K, D'Alessandro U, Okafor HU, Juma E, et al. A systematic review of the safety and efficacy of artemether-lumefantrine against uncomplicated Plasmodium falciparum malaria during pregnancy. Malar J. 2012;11(1):141.

104. McGready R, Lee SJ, Wiladphaingern J, Ashley EA, Rijken MJ, et al. Adverse effects of falciparum and vivax malaria and the safety of antimalarial treatment in early pregnancy: A population-based study. Lancet Infect Dis. 2012;12(5):388–96.

105. McGready R, Phyo AP, Rijken MJ, Tarning J, Lindegardh N, et al. Artesunate/dihydroartemisinin pharmacokinetics in acute falciparum malaria in pregnancy: absorption, bioavailability, disposition and disease effects. Br J Clin Pharmacol. 2012;73(3):467–77.

106. Moore KA, Simpson JA, Paw MK, Pimanpanarak M, Wiladphaingern J, et al. Safety of artemisinins in first trimester of prospectively followed pregnancies: an observational study. Lancet Infect Dis. 2016;16(5):576-83.

107. McGready R, Cho T, Keo NK, Thwai KL, Villegas L, et al. Artemisinin antimalarials in pregnancy: a prospective treatment study of 539 episodes of multidrug-resistant Plasmodium falciparum. Clin Infect Dis. 2001 ;33(12):2009–16.

108. Adam I, Elhassan EM, Omer EM, Abdulla MA, Mahgoub HM, et al. Safety of artemisinins during early pregnancy, assessed in 62 Sudanese women. Ann Trop Med Parasitol. 2009;103(3):205–10.

109. Deen JL, von Seidlein L, Pinder M, Walraven GE, Greenwood BM. The safety of the combination artesunate and pyrimethamine-sulfadoxine given during pregnancy. Trans R Soc Trop Med Hyg. 2001;95(4):424–28.

110. WHO. Malaria Policy Advisory Committee to the WHO: conclusions and recommendations of eighth biannual meeting (September 2015). Malar J. 2016;15(1):117.

111. World Health Organization. Guidelines for the treatment of malaria, 3rd edition.

112. Coartem. package insert. Novartis. 2013

113. Djimde A, Lefevre G. Understanding the pharmacokinetics of Coartem. Malar J. 2009;8:S4.

114. Mutabingwa TK, Adam I. Use of artemether-lumefantrine to treat malaria during pregnancy: what do we know and need to know? Expert Rev Anti Infect Ther. 2013;11(2):125–35.

115. Lawford H, Zurovac D, O'Reilly L, Hoibak S, Cowley A, et al. Adherence to prescribed artemisinin-based combination therapy in Garissa and Bunyala districts, Kenya. Malar J. 2011;10:281.

116. Mace KE, Mwandama D, Jafali J, Luka M, Filler SJ, et al. Adherence to treatment with artemether-lumefantrine for uncomplicated malaria in rural Malawi. Clin Infect Dis. 2011;53(8):772–79.

117. Cohen JL, Yavuz E, Morris A, Arkedis J, Sabot O. Do patients adhere to over-the-counter artemisinin combination therapy for malaria? evidence from an intervention study in Uganda. Malar J. 2012;11:83.

118. Kaye DK, Nshemerirwe R, Mutyaba TS, Ndeezi G. A randomized clinical trial comparing safety, clinical and parasitological response to artemether-lumefantrine and chlorproguanil-dapsone in treatment of uncomplicated malaria in pregnancy in Mulago hospital, Uganda. J Infect Dev Ctries. 2008 ;2(2):135–39.

119. Piola P, Nabasumba C, Turyakira E, Dhorda M, Lindegardh N, et al. Efficacy and safety of artemether-lumefantrine compared with quinine in pregnant women with uncomplicated Plasmodium falciparum malaria: an open-label, randomised, non-inferiority trial. Lancet Infect Dis. 2010;10(11):762–69.

120. McGready R, Tan SO, Ashley EA, Pimanpanarak M, Viladpai-nguen J, et al. A randomised controlled trial of artemether-lumefantrine versus artesunate for uncomplicated plasmodium falciparum treatment in pregnancy. PLoS Med. 2008;5(12):1699–715.

121. Sevene E, Gonzalez R, Menendez C. Current knowledge and challenges of antimalarial drugs for treatment and prevention in pregnancy. Expert Opin Pharmacother. 2010;11(8):1277–93.

122. Manyando C, Mkandawire R, Puma L, Sinkala M, Mpabalwani E, et al. Safety of artemether-lumefantrine in pregnant women with malaria: results of a prospective cohort study in Zambia. Malar J. 2010;9:249.

123. Nosten F, McGready R, d'Alessandro U, Bonell A, Verhoeff F, et al. Antimalarial

drugs in pregnancy: a review. Curr Drug Saf. 2006;1(1):1–15.

124. Ward SA, Sevene EJP, Hastings IM, Nosten F, McGready R. Antimalarial drugs and pregnancy: safety, pharmacokinetics, and pharmacovigilance. Lancet Infect Dis. 2007;7(2):136–44.

125. Mutabingwa TK, Muze K, Ord R, Briceño M, Greenwood BM, et al. Randomized trial of artesunate+Amodiaquine, sulfadoxine-pyrimethamine+amodiaquine, chlorproguanal-dapsone and SP for malaria in pregnancy in Tanzania. PLoS One. 2009;4(4):1–10.

126. Ukah M, Badejoko O, Ogunniyi S, Loto O, Aboderin O, et al. A randomized trial of artesunate-amodiaquine versus artemether-lumefantrine for the treatment of acute uncomplicated malaria in pregnancy. Int J Gynaecol Obstet. 2015 Oct;131(1):41–44.

127. McGready R, Brockman A, Cho T, Cho D, van Vugt M, et al. Randomized comparison of mefloquine-artesunate versus quinine in the treatment of multidrug-resistant falciparum malaria in pregnancy. Trans R Soc Trop Med Hyg. 2000;94(6):689–93.

128. Valea I, Tinto H, Coulibaly MT, Toe LC, Lindegardh N, et al. Pharmacokinetics of co-formulated mefloquine and artesunate in pregnant and non-pregnant women with uncomplicated Plasmodium falciparum infection in Burkina Faso. J Antimicrob Chemother. 2014;69(9):2499–507.

129. Nosten F, van Vugt M, Price R, Luxemburger C, Thway KL, et al. Effects of artesunate-mefloquine combination on incidence of Plasmodium falciparum malaria and mefloquine resistance in western Thailand: a prospective study. Lancet. 2000;356:297–302.

130. Tangpukdee N, Krudsood S, Thanachartwet W, Chalermrut K, Pengruksa C, et al. An open randomized clinical trial of Artekin vs artesunate-mefloquine in the treatment of acute uncomplicated falciparum malaria. Southeast Asian J Trop Med Public Health. 2005;36(5):1085–91.

131. Hutagalung R, Paiphun L, Ashley EA, McGready R, Brockman A, et al. A randomized trial of artemether-lumefantrine versus mefloquine-artesunate for the treatment of uncomplicated multi-drug resistant Plasmodium falciparum on the western border of Thailand. Malar J. 2005;4:46.

132. Smithuis F, Kyaw MK, Phe O, Aye KZ, Htet L, Barends M, et al. Efficacy and effectiveness of dihydroartemisinin-piperaquine versus artesunate-mefloquine in falciparum malaria: an open-label randomised comparison. Lancet 2006;367(9528):2075–85.

133. Gargano N, Ubben D, Tommasini S, Bacchieri A, Corsi M, et al. Therapeutic efficacy and safety of dihydroartemisinin-piperaquine versus artesunate-mefloquine in uncomplicated

Plasmodium falciparum malaria in India. Malar J. 2012;11:233.

134. European Medicines Agency (EMA). Eurartesim. EU summary of product characteristics. 2011.

135. Rijken MJ, McGready R, Phyo AP, Lindegardh N, Tarning J, et al. Pharmacokinetics of dihydroartemisinin and piperaquine in pregnant and nonpregnant women with uncomplicated falciparum malaria. Antimicrob Agents Chemother. 2011;55(12):5500–06.

136. Benjamin JM, Moore BR, Salman S, Page-Sharp M, Tawat S, et al. Population pharmacokinetics, tolerability, and safety of dihydroartemisinin-piperaquine and sulfadoxine-pyrimethamine-piperaquine in pregnant and nonpregnant Papua New Guinean women. Antimicrob Agents Chemother. 2015 ;59(7):4260–71.

137. Tarning J, Rijken MJ, McGready R, Phyo AP, Hanpithakpong W, et al. Population pharmacokinetics of dihydroartemisinin and piperaquine in pregnant and nonpregnant women with uncomplicated malaria. Antimicrob Agents Chemother. 2012;56(4):1997–2007.

138. Poespoprodjo JR, Fobia W, Kenangalem E, Lampah DA, Sugiarto P, et al. Dihydroartemisinin-piperaquine treatment of multidrug resistant *falciparum* and *vivax* malaria in pregnancy. PLoS One. 2014;9(1).

139. Poespoprodjo JR, Fobia W, Kenangalem E, Lampah DA, Sugiarto P, et al. Treatment policy change to dihydroartemisinin-piperaquine contributes to the reduction of adverse maternal and pregnancy outcomes. Malar J. 2015;14:272.

140. Madanitsa M, Kalilani L, Mwapasa V, van Eijk AM, Khairallah C, et al. Scheduled Intermittent Screening with Rapid Diagnostic Tests and Treatment with Dihydroartemisinin-Piperaquine versus Intermittent Preventive Therapy with Sulfadoxine-Pyrimethamine for Malaria in Pregnancy in Malawi: An Open-Label Randomized Controlled Tr. PLoS Med. 2016;13(9):e1002124.

141. Hill J, Kuile FO Ter. Dihydroartemisinin-piperaquine holds promise as an option for malaria prevention in pregnancy. Evid Based Med. 2016;21(4):146–47.

142. Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, et al. Dihydroartemisinin-Piperaquine for the Prevention of Malaria in Pregnancy. N Engl J Med. 2016;374(10):928–39.

143. Abdul-Ghani R, Al-Maktari MT, Al-Shibani LA, Allam AF. A better resolution for integrating methods for monitoring *Plasmodium falciparum* resistance to antimalarial drugs. Acta Trop. 2014;137:44–57.

144. WHO. Methods for Surveillance of Antimalarial Drug Efficacy. World Health Organization, 2009.

145. Basco LK. Field application of in vitro assays for the sensitivity of human malaria parasites to antimalarial drugs. Geneva World Heal Organ. 2007.

146. Wendler JP, Okombo J, Amato R, Miotto O, Kiara SM, et al. A genome wide association study of Plasmodium falciparum susceptibility to 22 antimalarial drugs in Kenya. PLoS One. 2014;9(5):e96486.

147. Bacon DJ, Latour C, Lucas C, Colina O, Ringwald P, et al. Comparison of a SYBR green I-based assay with a histidine-rich protein II enzyme-linked immunosorbent assay for in vitro antimalarial drug efficacy testing and application to clinical isolates. Antimicrob Agents Chemother. 2007;51(4):1172–78.

148. Quashie NB, Duah NO, Abuaku B, Quaye L, Ayanful-Torgby R, et al. A SYBR Green 1-based in vitro test of susceptibility of Ghanaian Plasmodium falciparum clinical isolates to a panel of anti-malarial drugs. Malar J. 2013;12:450.

149. Wellems TE, Plowe C V. Chloroquine-resistant malaria. J Infect Dis. 2001; 184(6):770–76.

150. Vossen MG, Pferschy S, Chiba P, Noedl H. The SYBR Green I malaria drug sensitivity assay: performance in low parasitemia samples. Am J Trop Med Hyg. 2010;82(3):398–401.

151. Triglia T, Cowman AF. Primary structure and expression of the dihydropteroate synthetase gene of Plasmodium falciparum. Proc Natl Acad Sci. 1994;91(15):7149–53.

152. Peterson DS, Milhous WK, Wellems TE. Molecular basis of differential resistance to cycloguanil and pyrimethamine in *Plasmodium falciparum* malaria. Proc Natl Acad Sci.1990;87(8):3018–22.

153. Fidock DA, Nomura T, Talley AK, Cooper RA, Dzekunov SM, et al. Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. Mol Cell. 2000 ;6(4):861–71.

154. Foote SJ, Galatis D, Cowman AF. Amino acids in the dihydrofolate reductase-thymidylate synthase gene of Plasmodium falciparum involved in cycloguanil resistance differ from those involved in pyrimethamine resistance. Proc Natl Acad Sci. 1990;87(8):3014–17.

155. Nair S, Nash D, Sudimack D, Jaidee A, Barends M, et al. Recurrent gene

amplification and soft selective sweeps during evolution of multidrug resistance in malaria parasites. Mol Biol Evol. 2007;24(2):562–73.

156. Gregson A, Plowe CV. Mechanisms of resistance of malaria parasites to antifolates. Pharmacol Rev. 2005;57(1):117–45.

157. Maiga OM, Kayentao K, Traoré BT, Djimde A, Traoré B, et al. Superiority of 3 over 2 doses of intermittent preventive treatment with sulfadoxine-pyrimethamine for the prevention of malaria during pregnancy in mali: A randomized controlled trial. Clin Infect Dis. 2011;53(3):215–23.

158. Sibley CH, Hyde JE, Sims PF, Plowe C V, Kublin JG, et al. Pyrimethamine-sulfadoxine resistance in Plasmodium falciparum: what next? Trends Parasitol. 2001;17(12):582–88.

159. Aziken ME, Akubuo KK, Gharoro EP. Efficacy of intermittent preventive treatment with sulfadoxine- pyrimethamine on placental parasitemia in pregnant women in midwestern Nigeria. Int J Gynecol Obstet. 2011;112(1):30–33.

160. Kayentao K, Kodio M, Newman RD, Maiga H, Doumtabe D, et al. Comparison of intermittent preventive treatment with chemoprophylaxis for the prevention of malaria during pregnancy in Mali. J Infect Dis. 2005;191(1):109–16.

Chapter 2: Burden of Malaria and malaria control in Burkina Faso

2.1 Country characteristics

Burkina Faso (formerly Upper Volta; French: Haute Volta), the land of the upright/honest people, is a landlocked country located in the middle of West Africa's "hump." It is geographically in the Sahel zone, the transition zone between the Sahara Desert in the North and the tropical savanna in the south. The country is bordered by Mali in the North- West, by Niger in the East, by Benin in the South-East and by Ivory Coast, Ghana and Togo in the South. With an area of 274,222 km², Burkina Faso is about three times the size of Austria, or slightly larger than the U.S. state of Colorado. Burkina Faso has a population of 17.4 million people (est. 2013).

With an estimated gross national income of US$1,215 per inhabitant and per year, Burkina Faso is among the world's least developed countries. Approximately 77% of the population lives in rural areas, and 44% of the population lives below the poverty line. In 2011, the life expectancy at birth was 54 years for men and 57 years for women (1).

Burkina Faso has two very distinct seasons, rainy season and dry season. During the rainy season the country receives between 600 and 1300 millimeters (mm) of rainfall; in the dry season, the harmattan, a hot dry wind from the Sahara, blows. The rainy season lasts approximately four months, from May / June to September, and is shorter in the north of the country (2).

The climate is generally sunny, hot, and dry and Burkina Faso can be divided into 3 different climatic regions:

- The North belongs to the Sahel zone. This area typically sees less than 600 mm of rainfall and the dry season can last between 8 and 10 months.
- The largest climatic zone is the Sudan-Sahel zone, which covers central Burkina Faso. Rainfall is superior to that in the North but rarely goes over 1000 mm.
- Southern Burkina Faso is covered by the Sudan zone, which is by far the most humid of the regions. Its rainy season lasts 6 months with rainfall up to 1300 mm.

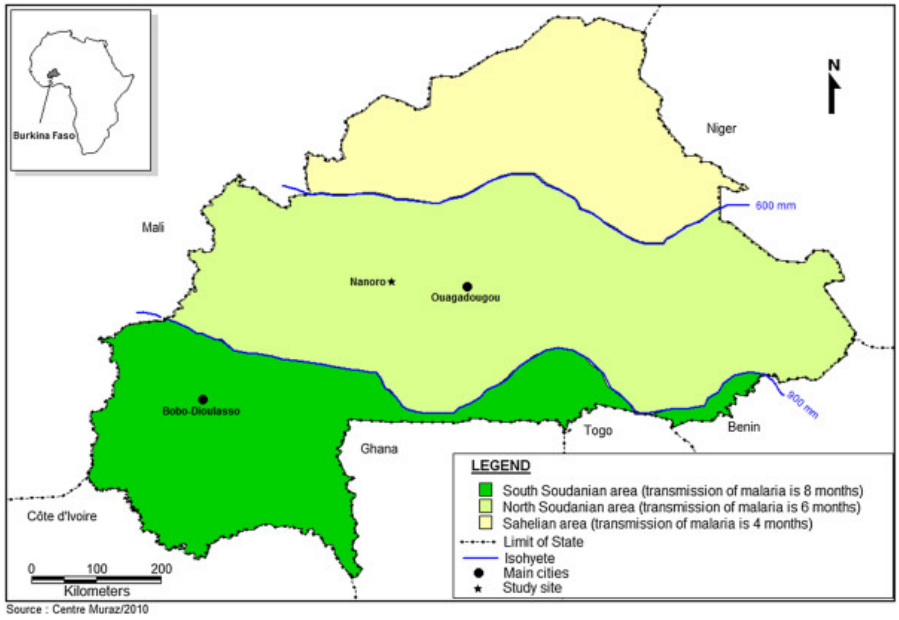


Figure 1: Map of Burkina Faso with the climatic zones

The three main rivers in Burkina Faso, namely Black Volta, White Volta, and Red Volta, lent the country its original name, Upper Volta. These rivers are also known as Mouhoun, Nakambe, and Nazinon, respectively. There are also several tributaries to the Niger that flow

through Burkina Faso, including the Gorouol and the Goudebo. Significant lakes in Burkina Faso are Tingrela, Bam, and Dem (cf figure 2).

Figure 2: Burkina Faso showing rivers and lakes

The humidity, which increases as one moves south, ranges from winter lows of 12% to 45% to rainy season highs of 68% to 96%. The harmattan, a dry east wind, brings considerable heat from March to May, when maximum temperatures range from 40° C to 48° C (104° to 119° F); from May to October, the climate is hot and wet, and from November to March, comfortable and dry (3).

2.2 Health system organization

Burkina Faso has one physician per 25,696 inhabitants, which is low compared to the recommended WHO standard of one doctor per 10,000 inhabitants. The country has one nurse for every 2,817 inhabitants. The share of the state budget allocated to the Department of Health increased from 9.1% in 2011 to 12.5% in 2012. Burkina Faso is divided into 13 administrative regions, 45 provinces, 63 health districts and 351 urban and rural municipalities.

In accordance with the recommendations of the Bamako Initiative, the public health system is organized into a three-level pyramid: central, intermediary, and peripheral.

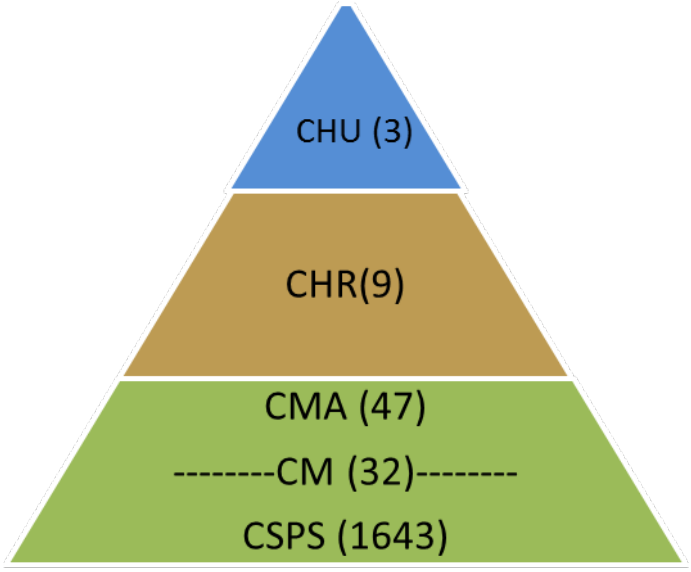
The central level is composed of the Health Minister's office, its central services, different programs and projects, affiliated services, and public administrative establishments (2 national hospitals, the National Blood Transfusion Centre, etc.).

The intermediary level is composed of the 13 Regional Health Directorates (DRSs), in charge of coordination and support to districts.

The peripheral level is currently composed of health districts, which constitute the operational level of the MoH and are administrated by district executive teams (ECDs) headed by district chief medical officers.

To meet the country's needs, there are three university hospitals and a national hospital, nine regional hospitals, 47 district hospitals and 1,643 health centers (4). Health services for the rural population are limited to primary care centers that typically employ two nurses and a midwife.

At the operational level, public health structures are also organized into three levels.



CHUs : University hospitals

CHRs : Regional Hospitals

CMAs: Medical Centers with Surgical Services

CMs: Medical Centers

CSPSs: Health and Social Promotion Centers

Figure 3: Structure of the Health System in Burkina Faso

According to the WHO, however, we note that "hospitals and health districts located in urban areas or around a CHR are poorly functional". In addition to this, district teams and regional directorates lack management capacity, the Support Unit for Health System Decentralization lacks operational capacity, there is no coordination of the healthcare system at the communal level, and the mechanism to address disasters' health components is inappropriate (5).

The private sector includes about 450 for-profit institutions, 45 non-governmental organizations (NGOs) and faith-based health centers and 140 biomedical laboratories.

Malaria Control in Burkina Faso is managed through the National Malaria Control Programme (NMCP). The NMCP is part of the Disease Control department, with three doctors, a pharmacist and 17 employees, and is within the MoH (5).

The Family and Health Direction (FHD) is a technical branch of the MoH, responsible for the design, planning and coordination of all activities relating to family members health (the mother, wife, man, children, adolescents and the elderly). Maternal and child health is managed through the family and health direction.

NMCP and FHD are both housed departments within the MoH. In the past this separation impeded collaboration and resulted in policy duplication or confusion. From now, the NMCP working in synergy with the FHD is responsible of the control and management of malaria in Burkina Faso. The NMCP is also involved in international initiatives such as Roll Back Malaria (RBM), Abuja initiative and the Millennium Development Goals (MDG), specifically the target number 8 and the objective 6.

Four (4) research centres are engaged in malaria research: the Research Institute of Health Science, the National Centre for Research and Training on Malaria, the Muraz Centre and the Nouna Centre for Health Research. These research centres are guiding and giving evidence to the NMCP trough their studies (interventional studies, clinical studies…). They are also monitoring the efficacy of the antimalarial drugs proposed for the two lines treatment.

2.3 Epidemiological stratification of malaria in Burkina Faso

Malaria transmission is holoendemic, with perennial transmission and a very strong seasonal component (6–8). Three malaria transmission zones which largely overlap with the climate zones are defined: the northern-most Sahelian region, with short seasonal transmission (2 – 3 months), the central Sudano-Sahelian region with a long seasonal transmissions period (4-6 months), and the Sudan-Guinea region with permanent transmission (1).

Malaria in Burkina Faso is mainly caused by *P. falciparum* infection (>90%). *P. malariae* and *P. ovale* are observed in 3-8% and 0.5-2% of malaria cases, respectively, most of them co-infections with *P. falciparum* (6). Malaria transmission is high with the entomological inoculation rate (EIR) in 2009 estimated at 50–60 infective bites/person/year in Nanoro (Diabate, pers comm). The more common vectors are *Anopheles gambiae sensu stricto*, *Anopheles funestus* and *Anopheles arabiensis*.

The epidemiology of MiP in Burkina Faso is governed by a large number of factors:

2.3.1 Socio-demographic factors (age, parity, education level and ethnicity)

Malaria infection is strongly associated with age, with women <20 years old more at risk than those ≥35 years old (7,9). Parity is also a risk factor as demonstrated by several studies. Indeed, primigravidae and to a lesser extent secundigravidae, are at greater risk of peripheral and placental parasitemia, and LBW (9,10).

One of the most important determinants of human behavior and knowledge is the formal educational level. It has been recognized that the education level is playing an important role in malaria transmission (8).

In the same area some differences in malaria clinical episode were less marked in the Fulani group compared to the Mossi one (11). These differences could be explained by genetic factors between these groups.

2.3.2 Cultural and socio-economic factors

Cultural and socio-economic factors are different between populations. These differences contribute to marked differences in malaria risk, e.g. through differences in exposure or through differences in health seeking behavior (12).

2.3.3 Climatic and geographic parameters

The climatic parameters affect malaria distribution, seasonality and the transmission intensity (13). Malaria transmission is seasonal, during the months of August-December, and overlaps with the rainy season (July-October). The peak for malaria cases usually occurs in September-October. A longitudinal study on all causes and malaria mortality concluded to a seasonal pattern of mortality (14). Other studies have confirmed this seasonality (12,15).

Geographic parameters are also playing an important role in MiP epidemiology. Malaria prevalence among pregnant women is higher in remote rural areas than urban areas, regardless of climatic zones. In rural areas, prevalence by microscopy varies between 19.4% to 50.8% (13, 23-27) while in urban areas it is around 25% (16).

2.4 Malaria trends in Burkina Faso

In 2013, **6,900,203 cases** of malaria were recorded in Burkina Faso, including 406,103 cases of severe malaria. The cumulative incidence was 398 cases per 1,000 inhabitants, a decrease from the 2012 level of 415 cases per 1,000 inhabitants. This slight decrease has to be interpreted with caution as there is insufficiently consistent data to assess malaria trends in Burkina Faso (17). Nevertheless, malaria remains the primary reason for medical consultation and hospitalization with respectively 46.5% and 61.5% of the hospitalizations and for 30.5% of deaths (18).

2.5 Evolution of malaria control and intervention in Burkina Faso

Malaria infection during pregnancy poses substantial risk to the mother, her fetus, and the neonate. In areas of stable malaria transmission such as Burkina Faso, where adult women have considerable acquired immunity, *P. falciparum* infection during pregnancy typically does not cause symptomatic malaria, but may lead to maternal anemia and placental malaria infection, especially among primigravidae and secundigravidae (19,20). Historically, chloroquine (CQ) has been used as the first line drug for treatment of the general population, including pregnant women, and SP was used as second-line treatment. CQ was used for weekly chemoprophylaxis in pregnancy (21,22).

According to the WHO guidelines available at that time, a policy change was recommended when clinical failures were above 25% (23). After three (3) consecutive year of CQ resistance monitoring, the results were above the recommended threshold and CQ was removed as first line treatment and weekly chemoprophylaxis regimen (21,24). A new malaria treatment policy was formulated in February 2005, whereby CQ was replaced by artemether-lumefantrine (AL) and amodiaquine-artesunate (ASAQ) as first-line treatments for uncomplicated malaria. In the meantime, IPTp with SP was introduced to replace CQ prophylaxis in pregnancy. The main recommended strategies of the NMCP to control malaria are:

2.5.1 Earlier detection and treatment of all malaria cases in health services and in the community

According to the NMCP, malaria is defined at the health service level as: an axillary temperature ≥ 37.5˚C or history of fever during the last 72 hours, and diagnosis of *P. falciparum* by microscopy or RDT. At the community level, malaria is defined: as fever or history of fever during the last 72 hours with a positive RDT (18). In all hospitals (university,

national and regional) where electricity is available, malaria must be confirmed by microscopy and for the dispensaries at the peripheral level by RDT.

At the community level, the case management of malaria is provided by community-based health workers (CBHW). These are individuals reside in villages more than five kilometers from the local health centre. To enable them to properly treat uncomplicated malaria, the CBHW have been trained, endowed with recommended drugs and monitored regularly by health workers in dispensaries to which they belong. This strategy was tested in only three districts: Kaya, Nouna and Saponé. The first line ACTs are used and sold at highly subsidized prices. In 2013, a census showed that 62% of the treatments needed have been distributed and received at the community level (25). It was then decided to generalize the community case management to the whole country.

In health facilities, the management of uncomplicated or severe malaria cases occurs at all levels of the health pyramid. However, it is recommended that the peripheral level (health centres and dispensaries) remains the place for treating uncomplicated cases and referral centres (medical centre with surgical unit, regional hospital / university hospital) for severe cases, after confirmation by microscopy or RDT.

2.5.2 Intermittent preventive treatment during pregnancy with SP

Since 2005, SP is reserved for IPTp, with two recommended doses during pregnancy. Antenatal Care (ANC) sessions are national platforms for malaria in pregnancy prevention and control. Despite a good initial ANC registration rate (95%), the 2010 Demographic and Health Survey showed that over 56% of pregnant women in rural areas did not register until their second or third trimester (26,27). Consequently, they may have missed the full regimen of ANC services, including LLIN and IPTp. Despite significant progress, the coverage of IPTp in Burkina Faso (10-19% in 2009-2011) was still under the Roll Back Malaria (RBM)

target of 80% (28,29). Nowadays, the WHO recommends more doses of IPTp, one each time the woman attend the ANC if at least one month apart. The new guideline was incorporated into the update of Burkina Faso's malaria strategy and has been disseminated since September 2014 (18). In Burkina Faso, the malaria control program recommends at least three doses of SP during pregnancy and the uptake under supervision of a qualified agent. This new strategy poses challenges and needs greater investment as the Ministry of Health (MoH) also experienced SP stock-outs. Many projects jointly with the NMCP are examining new ways for investing in stronger antenatal malaria prevention, capacity building of ANC staff and provision of IPTp using the existing network of volunteer community health workers.

2.5.3 The use of long lasting insecticide treated nets (LLIN), indoor residual spraying (IRS)

The initial strategy of targeting high risks groups (children under five years and pregnant women) was shifted to universal coverage. It is defined as: 'every household should own one ITN for two people living in the household' (30). Therefore, a national distribution campaign was carried out between July 2010 and January 2011. More than 8 million LLINs were made available for the campaign (Ministry of Health, Republic of Burkina Faso, unpublished report). Studies on the coverage of ITNs are rare. In Burkina Faso, Zollner et al (31), investigated the coverage of ITNs before and after the massive distribution campaign. They concluded that the campaign was a success when considering 'ownership of at least one ITN' as indicator, but failed to achieve its own set target of securing one ITN for every two people per household (31).

2.5.4 For treatment, artemether-lumefantrine (AL) and artesunate-Amodiquine (ASAQ) both as first line

For pregnant women, malaria is treated with oral quinine in the first trimester or ACT the second and third trimester. Quinine is also used for the management of complicated and

severe malaria in both children and adults, including pregnant women. The new treatment of choice is IV artesunate (AS), which reduced mortality compared with quinine (32). Free treatment kits are made available to vulnerable groups (children under five and pregnant women).

2.6 Study sites profile

The studies in the framework of this thesis have been carried out in the rural district of Nanoro. Two sites were involved in the recruitment of the patients: Nanoro and Nazoanga. The health district of Nanoro covers an area with 150,000 inhabitants and comprises 18 dispensaries, all with maternity, general clinic and immunization programmes (EPI) (33). All the trials described in this thesis were conducted at the Clinical Research Unit of Nanoro (CRUN), which is located within the district hospital. The CRUN has setup the Nanoro Health and Demographic Surveillance System (HDSS) in 2009. The demographic surveillance area (DSA) covers 24 villages belonging to two departments (Nanoro and Soaw), located in the Boulkiemde province. In this DSA, health care is provided by seven peripheral health centres and one referral hospital (CMA). The clinical research facility is located in this hospital, which is run by an Italian religious order (Camillian). It consists of a medical centre with eight departments: surgery, maternity, pediatrics, dentistry, laboratory services, nutrition, pharmacy and internal medicine, and an outpatient service. The staff consists of 45 personnel and includes three physicians. A total of 54,781 inhabitants were recorded at the initial census, of which 56.1% were female.

Nanoro population is characterized by a high birth rate, typical of rural low and middle-income countries. The total fertility rate is 5.6. Therefore, the population is growing rapidly and is young, with a low proportion of older residents. Migration is especially high for adult men who move to urban areas (Ouagadougou, Bobo-Dioulasso, Koudougou) or abroad (Ivory

Coast and Ghana) looking for a job. The main ethnic groups are the Mossi (90%), the Gourounsi (7.9%) and the Fulani (1.7%). The majority of them are Animists (37.4%), Muslims (29.5%) and Catholics (26.6%), with a few Protestants (6.3%). More than half are married, with polygamy predominating. The majority (75%) of the population has little education. The DSA is populated mostly by subsistence farmers and cattle-keepers (50.6%), housewives (31.2%), scholars (9.6%) and civil servants (2.5%). Nanoro belongs to the Sudan-Sahel zone, with an estimate of 58,868 recorded malaria cases in 2015. The number of pregnancies was estimated at 8,696 per year (34).

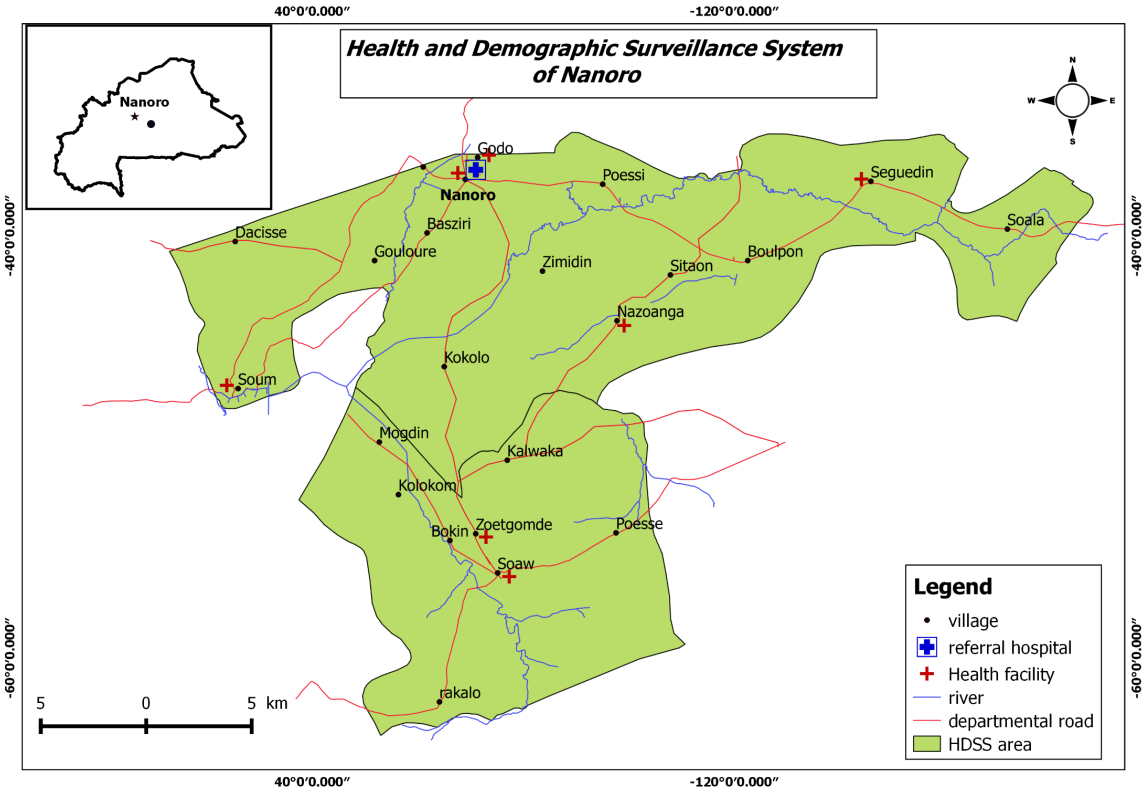


Figure 4: Nanoro HDSS area

2.7 References

1. INSD. Enquête démographique et de santé. 2010.

2. Weather and climate. Burkina Faso. 2016.

3. Nation encyclopedia. Carte climat Burkina Faso. 2015.

4. Ministère de la Santé. Plan d'action du district sanitaire de Nanoro. 2010.

5. Sanofi. The fight against malaria: A comprehensive commitment. 2014.

6. Muller O, Becher H, van Zweeden AB, Ye Y, Diallo DA, et al. Effect of zinc supplementation on malaria and other causes of morbidity in west African children: randomised double blind placebo controlled trial. BMJ. 2001;322(7302):1567.

7. Tahita MC, Tinto H, Menten J, Ouedraogo JB, Guiguemde RT, van Geertruyden JP, et al. Clinical signs and symptoms cannot reliably predict Plasmodium falciparum malaria infection in pregnant women living in an area of high seasonal transmission. Malar J. 2013;12:464.

8. Muller O, Traore C, Becher H, Kouyate B. Malaria morbidity, treatment-seeking behaviour, and mortality in a cohort of young children in rural Burkina Faso. Trop Med Int Health. 2003;8(4):290–96.

9. Cisse M, Sangare I, Lougue G, Bamba S, Bayane D, et al. Prevalence and risk factors for Plasmodium falciparum malaria in pregnant women attending antenatal clinic in Bobo-Dioulasso (Burkina Faso). BMC Infect Dis. 2014;14(1):631.

10. Brabin BJ. An analysis of malaria in pregnancy in Africa. Bull World Health Organ. 1983;61(6):1005–16.

11. Modiano D, Petrarca V, Sirima BS, Nebie I, Diallo D, et al. Different response to Plasmodium falciparum malaria in West African sympatric ethnic groups. Proc Natl Acad Sci. 1996;93(23):13206–11.

12. Brinkmann U, Brinkmann A. Malaria and health in Africa: the present situation and epidemiological trends. Trop Med Parasitol. 1991;42(3):204–13.

13. White NJ. Antimalarial drug resistance and combination chemotherapy. Philos Trans R Soc L B Biol Sci. 1999;354:739–49.

14. Otte im Kampe E, Müller O, Sie A, Becher H, Muller O, et al. Seasonal and temporal trends in all-cause and malaria mortality in rural Burkina Faso, 1998-2007. Malar J. 2015;14:300.

15. Jaffar S, Leach A, Greenwood AM, Jepson A, Muller O, et al. Changes in the pattern of infant and childhood mortality in upper river division, The Gambia, from 1989 to 1993. Trop Med Int Health. 1997;2(1):28–37.

16. Douamba Z, Bisseye C, Djigma FW, Compaore TR, Bazie VJT, et al. Asymptomatic malaria correlates with anaemia in pregnant women at Ouagadougou, Burkina Faso. J Biomed Biotechnol. 2012;198317.

17. WHO. World Malaria Report. 2013.

18. PNLP. Directives nationales pour la prise en charge du paludisme dans les formations sanitaires du Burkina Faso. 2014.

19. Newman RD, Hailemariam A, Jimma D, Degifie A, Kebede D, et al. Burden of malaria during pregnancy in areas of stable and unstable transmission in Ethiopia during a nonepidemic year. J Infect Dis. 2003;187(11):1765–72.

20. Steketee RW, Nahlen BL, Parise ME, Menendez C. The burden of malaria in pregnancy in malaria-endemic areas. American Journal of Tropical Medicine and Hygiene. 2001;28–35.

21. Tinto H, Zoungrana EB, Coulibaly SO, Ouedraogo JB, Traore M, et al. Chloroquine and sulphadoxine-pyrimethamine efficacy for uncomplicated malaria treatment and haematological recovery in children in Bobo-Dioulasso, Burkina Faso during a 3-year period 1998-2000. Trop Med Int Health. 2002;7(11):925–30.

22. Ministère de la santé. Directives nationales pour une prise en charge rationnelle des cas de paludisme au Burkina faso (traitement curative et chimioprophylaxie). 2000.

23. WHO. Regional Office for Africa, Brazzaville, “Framework for Developing, Implementing, and Updating Antimalaria Treatment Policy: A Guide for Country Malaria Control Programmes.” 2003.

24. Sirima SB, Cotte AH, Konate A, Moran AC, Asamoa K, et al. Malaria prevention during pregnancy: Assessing the disease burden one year after implementing a program of intermittent preventive treatment in Koupéla District, Burkina Faso. Am J Trop Med Hyg. 2006;75(2):205–11.

25. Ministère de la santé du Burkina Faso. Annuaire statistique de santé. 2014;337.

26. Roll Back Malaria. Burkina Faso: Defeating Malaria by Investing in Expanding Intermittent Preventive Treatment in Pregnancy. 2015.

27. Grietens KP, Gies S, Coulibaly SO, Ky C, Somda J, et al. Bottlenecks for high coverage of intermittent preventive treatment in pregnancy: the case of adolescent pregnancies in rural Burkina Faso. PLoS One. 2010;5(8):e12013.

28. Roll Back Malaria Partnership. Refined/updated global malaria action plan objectives, targets, milestones and priorities beyond. 2011.

29. van Eijk AM, Hill J, Larsen DA, Webster J, Steketee RW, et al. Coverage of intermittent preventive treatment and insecticide-treated nets for the control of malaria during pregnancy in sub-Saharan Africa: a synthesis and meta-analysis of national survey data, 2009-11. Lancet Infect Dis. 2013;13(12):1029–42.

30. Lengeler C. Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database Syst Rev. 2004;2:CD000363.

31. Zöllner C, De Allegri M, Louis VR, Yé M, Sié A, et al. Insecticide-treated mosquito nets in rural Burkina Faso: Assessment of coverage and equity in the wake of a universal distribution campaign. Health Policy Plan. 2015;30(2):171–80.

32. Dondorp AM, Fanello CI, Hendriksen ICE, Gomes E, Seni A, et al. Artesunate versus quinine in the treatment of severe falciparum malaria in African children : An open-label, randomised trial. Lancet. 2010;376(9753):1647–57.

33. Derra K, Rouamba E, Kazienga A, Ouedraogo S, Tahita MC, et al. Profile: Nanoro health and demographic surveillance system. Int J Epidemiol. 2012;41(5):1293–01.

34. Ministère de la santé. Plan d'action District Sanitaire de Nanoro. 2015.

Chapter 3: Clinical signs and symptoms cannot reliably predict *P. falciparum* malaria infection in pregnant women living in an area of high seasonal transmission

Tahita MC, Tinto H, Menten J, Ouedraogo JB, Guiguemde RT, van Geertruyden JP, Erhart A, D'Alessandro U. Clinical signs and symptoms cannot reliably predict *P. falciparum* malaria infection in pregnant women living in an area of high seasonal transmission. *Malaria Journal*. 2013;12:464.

3.1 Abstract

Background

Malaria in pregnancy is a major public health problem in endemic countries. Though the signs and symptoms of malaria among pregnant women have been already described, clinical presentation may vary according to intensity of transmission and local perceptions. Therefore, determining common signs and symptoms among pregnant women with a malaria infection may be extremely useful to identify those in need of further investigation by rapid diagnostic test or microscopy.

Methods

Six hundred pregnant women attending the maternity clinic of Nanoro District Hospital, Burkina Faso were recruited, 200 with suspected clinical malaria and 400 as controls. Cases were matched with controls by gestational age and parity. Signs and symptoms were collected and a blood sample taken for rapid diagnostic test, microscopy and haemoglobin measurement. A multivariate model was used to assess the predictive value of signs and symptoms for malaria infection.

Results

The overall prevalence of malaria was 42.6% (256/600) while anemia was found in 60.8% (365/600) of the women. Nearly half (49%) of the cases and 39.5% of the controls had a malaria infection (p = 0.03). The most common signs and symptoms among the cases were fever (36%,72/200), history of fever (29%,58/200) and headache (52%,104/200). The positive predictive value for fever was 53% (95%CI:41–64), history of fever 58% (95%CI:37–63) and headache 51% (95%CI:41–61).

Conclusion

Signs and symptoms suggestive of malaria are frequent among pregnant women living in areas of intense transmission. Common malaria symptoms are not strong predictors of infection. For a better management of malaria in pregnancy, active screening to detect and treat malaria infection early should be performed on all pregnant women attending a health facility.

Keywords

Malaria, pregnancy, signs, and symptoms.

3.2 Background

Malaria in pregnancy (MiP) is a major public health problem in endemic countries where 31 million pregnancies occur annually, resulting in approximately 23 million live births (1). Where malaria transmission is moderate to high and stable, the most common parasite species is *P. falciparum* and MiP is predominantly asymptomatic and yet a major cause of maternal anemia and low birth weight (LBW), the latter increasing the risk of infant death. Successful control of MiP may prevent 75,000-200,000 infant deaths every year (2). In areas of low transmission, women by the time they become pregnant have acquired little immunity to malaria and so infections are often symptomatic and more likely to become severe, resulting

in maternal and fetal deaths (2,3). To prevent the consequences of MiP, the World Health Organization (WHO) recommends intermittent preventive treatment during pregnancy (IPTp), adequate management of clinical malaria, administering of supplements such as iron and folic acid, and the use of insecticide-treated nets (4).

The current strategy for clinical case management of malaria in pregnant women is based on the result of rapid diagnostic tests (RDT) or microscopy if available. Though the signs and symptoms of malaria among pregnant women have been already described in few settings, clinical presentation may vary according to intensity of transmission and local perceptions. Therefore, determining common signs and symptoms among pregnant women with a malaria infection may be extremely useful to identify those in need of further investigation by rapid diagnostic test or microscopy (5-7). Furthermore, there is little information on the association between peripheral parasitaemia and the presence of signs and symptoms of malaria during pregnancy. This study aimed to document the clinical presentation of malaria among pregnant women and assess their predictive value.

3.3 Methods

Study area

The study was carried out at the Clinical Research Unit of Nanoro (CRUN), situated in the centre of Burkina Faso, 85 km from Ouagadougou, the capital city. Nanoro district is one of the five districts of the Centre-West health region. The main local language is Mooré, though French is the official language. The literacy rate is low for both men and women (about 23%) and there is a high migration flux among the youngsters toward the capital city and/or the border countries.

Malaria transmission is high with the entomological inoculation rate (EIR) in 2009 estimated at 50–60 infective bites/person/year (Diabate, pers comm). The more common vectors are *Anopheles gambiae sensu stricto*, *Anopheles funestus* and *Anopheles arabiensis*, and malaria is one of the most common reasons for attending a health facility (8). *P. falciparum* is the predominant malaria parasite. Malaria transmission is seasonal, during the months of August-December, and overlaps with the rainy season (July-October). The peak for malaria cases usually occurs in September-October.

Since February 2009, the CRUN has implemented a demographic surveillance system (DSS) involving about 54,000 individuals. Some important information such as socio-economic status, e g, characteristics of households, living conditions, health conditions, are collected through the DSS on a regular basis.

The national anti-malarial drug policy was changed in 2005 from chloroquine to either amodiaquine-artesunate (AQ-AS) or artemether-lumefantrine (AL) as first-line treatments. Pregnant women received an insecticide-treated net (ITN) free of charge as soon as they complete two prenatal consultations or after delivery, while children receive theirs at each vaccination campaign. Nevertheless, a campaign for the promotion of insecticide-treated materials (ITM) targeting pregnant women and children under five years has been implemented since 2010 and nowadays an ITN is given to any pregnant woman attending for the first time during pregnancy a health facility.

Study design and procedures

The study protocol was approved by the Institutional Ethic Committee of Centre Muraz (registration no. 005-2010/CE-CM). All pregnant women attending either the routine antenatal care (ANC) or the outpatient clinics were asked to participate in the study. During the visit and after having obtained a written informed consent, women were divided in cases

or controls. Cases had at least one of the following signs and symptoms: temperature ≥37.5°C (measured by electronic thermometer) or history of fever in the previous 48 hours, headache, pallor, arthromyalgia, convulsions, vomiting, dizziness, malaise, fatigue, enlarged liver or enlarged spleen. Controls had none of them. For each case, two controls, matched by parity (0, 1–3, ≥4), gestational age (measured by fundal height) and seasonality (recruited within one month from the corresponding case). In order to capture possible seasonal variations in the clinical presentation, the study was conducted during both the low and high malaria transmission season.

Capillary blood was collected for an HRP-2 RDT, haemoglobin and blood slide. Women with a positive RDT were treated with AQ-AS (standard adult dose) if they were in the second and third trimester of pregnancy while quinine was given for seven days to those in the first trimester (8 mg quinine base/kg every eight hours). Severe malaria cases were hospitalized and treated with parenteral quinine. Anemia was treated according to the national guidelines with oral ferrous sulphate (200 mg) and folic acid (0.25 mg) daily for one month. All patients with malaria parasites and anemia were promptly and adequately treated free of charge. Project staff was available 24 hours a day to identify women and to ensure adequate documentation and clinical management.

Laboratory methods

RDTs were carried out and results read according to the manufacturer's procedures. All slides were read twice by two independent readers. Parasite density was estimated by counting the number of asexual parasites per 200 leukocytes in the thick blood film and assuming white blood cells (WBC) count of 8,000 parasites/µl. In case of discrepancy between the two readers (positive/negative, more than two-fold difference for parasite densities ≥400/µl, or more than the $\log_{10}$ for those <400/µl) (9), a third independent reading was done. The latter

was taken as the final result. Haemoglobin was measured using a portable spectrophotometer (Hemocue®, Angelholm, Sweden) and controls were run daily to ensure the quality of the results.

Sample size calculation

The sample size was calculated using the power calculation module for a two-sample comparison in order to detect with a two-sided significance level of 5% and 90% power important predictors of malaria infection in pregnant women. As there are no data available for the study area, the estimation was based on a study conducted in Mozambique (5). It was assumed that 67% women would report history of fever and that among them 33% would be infected with malaria, while the prevalence of infection among the other women would be 20%. Following these parameters, six hundred pregnant women (200 cases and 400 controls) were needed. With an expected malaria prevalence of 27%, i e, 162 infected women, 16 possible predictors could be assessed, on the assumption that each of them would need at least ten events.

Statistical methods and definitions

Double data entry, validation and cleaning were done using Epidata 3.1, and statistical analysis was performed with STATA.10 (STATA Corporation, College Station, TX, USA). Participants were categorized first as (i) cases and (ii) controls. The frequencies of signs and symptoms were compared between these groups. Odds ratio (OR) estimates and their confidence intervals (CI) were estimated in log binomial regression analyses. A P-value of ≤ 0.05 was considered statistically significant. The anemia was classified as mild (Hb <11 g/dl), moderate (Hb 7–9 g/dl) and severe (Hb <7 g/dl). Parasite density was log transformed for the comparisons of mean parasite densities between cases and controls.

3.4 Results

Most recruited women were either in the second (34.5%, 207/600) or third (60.5%, 363/600) trimester of pregnancy, and the mean age was 27.6 years (95% CI:27.1-28.0). More than half of them had already had one to three pregnancies and most of them were married (Table 1). The large majority of them worked at home (99%, 593/600) and could not read (92.1%, 553/600). Most women slept under a bed net (86.5%%). Baseline characteristics were similar between cases and controls (Table 1).

Table 1: Baseline characteristics of the recruited pregnant women by study group (%)

		Cases	**Controls**	**Total**
		N = 200	**N = 400**	**N = 600**
Age group	<20 years	18 (9.0)	23 (5.8)	41 (6.8)
	20-34 years	160 (80.0)	328 (82.0)	488 (81.3)
	≥35 years	22 (11.0)	49 (12.2)	71 (11.8)
Trimester ǂ	1st	10 (5.00	20 (5.0)	30 (5.0)
	2nd	84 (42.0)	123 (30.8)	207 (34.5)
	3rd	106 (53.00	257 (64.2)	363 (60.5)
Parity	0	17 (8.5)	34 (8.5)	51 (8.5)
	1-3	118 (59.0)	237 (59.3)	355 (59.2)
	≥4	65 (32.5)	129 (32.2)	194 (32.3)
Transmission season Φ	No	47 (23.5)	8 (2.0)	55 (9.2)
	Yes	153 (76.5)	392 (98.0)	545 (90.8)
Sleep under bed net		155 (77.5)	364 (91.0)	519 (86.5)
Malaria infection		98 (49.0)	158 (39.5)	256 (42. 7)

ǂ 1st trimester: 0–12 weeks; 2nd trimester: 13–24 weeks; 3rd trimester: ≥25.

Φ Transmission season: i) Yes (high transmission season): August to December; ii) No (low transmission season): June to July.

The prevalence of malaria infection using the RDT was 60% among the cases and 47.8% among the controls. When using microscopy, the prevalence of malaria infection was 49.0% among cases and 39.5% among controls (p = 0.03). The latter would not have been treated in case of clinical diagnosis. The mean (geometric) parasite density among infected women was not significantly different among cases (3.0/µl, 95% CI: 2.9-3.2) and controls (2.8/µl, 95% CI: 2.7-3.0) (p = 0.06). This is clearly illustrated by the Receiver Operating Characteristics (ROC) curve, with only 56% under the curve and no clear-cut off point distinguishing a case from a control (Figure 1). More than half of infected women (56.3%, 144/256) had a parasite density <1,000/µl. Malaria infection was strongly associated with anemia (OR:1.96, 95% CI:1.40-2.74; p < 0.01), with more than half of all malaria-infected women (68.7%,176/256) being anemic. Among symptomatic cases, age was significantly related to malaria infection, with women <20 years more at risk than those ≥35 years old (Table 2). Conversely, neither season gestational age or bed net use were significantly associated with malaria, though there was the tendency of having a lower risk of malaria with bed nets, in multigravidae and before the transmission season (Table 2).

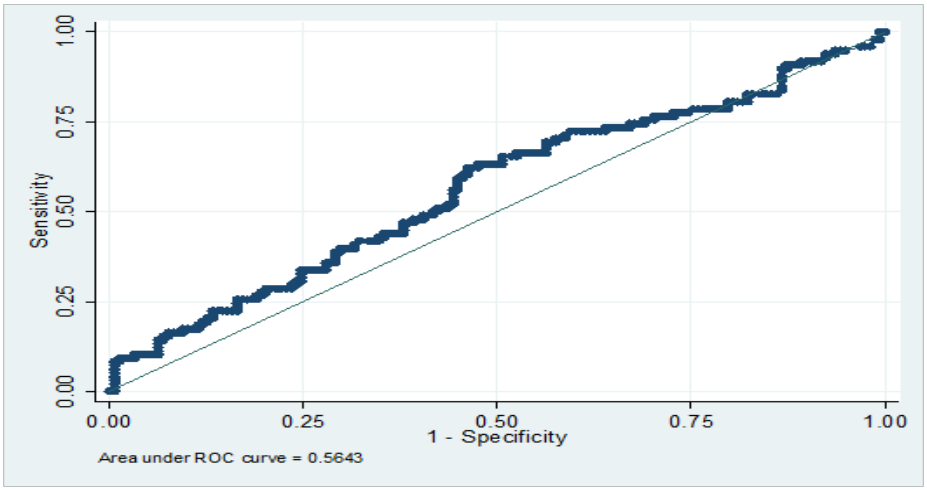


Figure 1: ROC curve assessing the parasite density discriminating a case from a control (case threshold).

Table 2: ***P. falciparum*** **infection by risk factors among symptomatic pregnant women**

	Risk factors	n/N (%)	OR (95%CI)	P-value
Age	<20 years	11/16 (69.0)	Ref	--
	20-34 years	79/158 (50.0)	0.45 (0.15-1.37)	0.16
	≥35 years	8/26 (31.0)	0.20 (0.05-0.78)	0.02
Transmission season	No	16/47 (34.0)	Ref	---
	Yes	82/153 (53.0)	1.59 (0.88-2.86)	0.12
Parity	Nulliparous	10/17 (59.0)	Ref	--
	1-3	59/100 (50.0)	0.70 (0.25-1.95)	0.49
	≥4	29/65 (45.0)	0.56 (0.19-1.66)	0.30
Gestational age	1st	3/10 (30.0)	Ref	---
	2nd	48/84 (57.1)	3.31 (0.75-12.86)	0.11
	3rd	47/106 (44.3)	1.85 (0.45-7.58)	0.38
Bed net use	Yes	20/50 (40.0)	Ref	---
	No	76/147(51.7)	1.60 (0.83-3.08)	0.15

Fever, history of fever, headache, and dizziness each had a positive predictive value (PPV) around 50%, while for all the others the PPV was well below (Table 3). The highest PPV were found when combining fever and dizziness (61.5%, 95%CI:35–82), and fever and vomiting (66.7%, 95%CI:20–93). If fever had been used to diagnose malaria, 47.2% of the febrile women would have been unnecessarily treated. Also 46.8% of the non-febrile women would have missed treatment for their malaria infection. When considering only fever as symptom, the ROC curve was flat, indicating that no fever "threshold" could be determined (Figure 2).

Table 3: **Positive predictive value (PPV) of clinical signs and symptom for *P. falciparum* infection among the 200 pregnant women recruited as cases**

Signs/Symptoms	n	%	PPV (%)	(95%CI)
Fever	38	19.0	53	(41–64)
History of fever	29	14.5	58	(37–63)
Pain in the joints	6	3.0	25	(6 – 44)
Headache	57	28.5	51	(41 – 61)
Dizziness	3	1.5	47	(41–64)
Vomiting	3	1.5	38	(37–63)
Convulsions	1	0.5	33	(6–79)
Malaise	7	3.5	30	(41 –61)

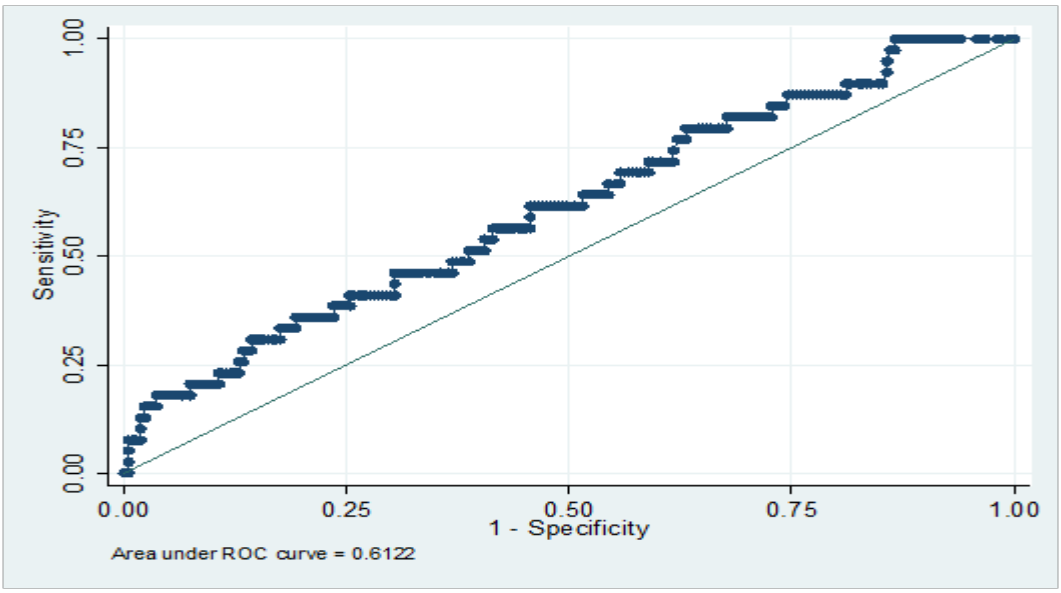


Figure 2: ROC curve assessing the parasite density discriminating women with or without fever (fever threshold).

The occurrence of signs and symptoms was associated with higher parasite densities; in women complaining of headache, parasite density was significantly higher than in those without (6.4/µl *vs* 1.8/µl) (p = 0.003). Moreover, the mean (geometric) parasite density tended to be higher in women with vomiting (14,617.8/µl *vs* 2.7/µl; p = 0.05), fever (5.7/µl *vs* 2.2/µl) (p = 0.07), and complaining of malaise (53.5 *vs* 2.7; p = 0.07).

In the univariate analysis, presence of symptoms (OR = 1.47, 95%CI:1.05-2.07; p = 0.03), fever (OR = 1.76, 95%CI:0.99-3.11; p = 0.05) and headache (OR = 1.53, 95% CI: 0.96-2.46; p = 0.07) were or tended to be associated with malaria infection. In the multivariate model, fever and history of fever (OR = 1.83, 95%CI:1.16-2.88; p = 0.009) also fever and history of fever and headache infection (OR = 1.63, 95%CI:1.13-2.37; p = 0.01) were significantly associated with malaria infection (Table 4).

Table 4: Comparison of the association between signs/symptoms and malaria infection in symptomatic pregnant women and their matched controls

		(Number of Malaria infection /Total number with the symptoms) (%)	OR (95% CI)	P value
Any symptom	Controls	158/400 (40)	Ref.	---
	Cases	95/200 (49)	1.47 (1.05-2.07)	0.03
Fever	Controls	56/144 (39)	Ref.	---
	Cases	38/72 (53)	1.76 (0.99-3.11)	0.05
History of fever	Controls	39/110 (35)	Ref.	
	Cases	27/55 (49)	1.76 (0.91-3.39)	0.09
Joint pain	Controls	14/48 (29)	1.23 (0.40 - 3.76)	0.71
	Cases	6/24 (25)	ref	---
Headache	Controls	84/208 (40)	Ref.	
	Cases	53/104 (51)	1.53 (0.96-2.46)	0.08
Dizziness	Controls	39/76 (51)	1.17 (0.53-2.55)	0.69
	Cases	18/38 (47)	Ref	---
Vomiting	Controls	9/16 (56)	2.14 (0.37-12.19)	0.39
	Cases	3/8(37)	ref	
Convulsions	Controls	0 (0)	-	
	Cases	1/3 (33)	-	
Malaise	Controls	17/48 (35)	1.33 (0.46-3.84)	0.60
	Cases	7/24 (29)	ref	
Fever and	Controls	84/230 (37)	Ref.	
history of fever	Cases	59/115 (51)	1.83 (1.16, 2.88)	0.01
Fever and	Controls	131/340 (39)	Ref.	
history of fever and headache	Cases	86/170 (51)	1.63 (1.13, 2.37)	0.01

3.5 Discussion

In this study, malaria infection was extremely common among pregnant women, regardless of signs and symptoms. Signs and symptoms were not discriminative for malaria infections. In peripheral health facilities without access to parasitological diagnosis by either RDT or microscopy, health staff may have unnecessarily treated half of the women with signs and symptoms suggestive of malaria, while a substantial proportion of infected pregnant women would have gone undetected. Assuming diagnostic tests were available, the recommendation of performing them only on suspected cases would have still missed several infected women. This is particularly worrying for those in the first trimester of pregnancy as they do not receive the sulphadoxine-pyrimethamine (SP) for IPTp. Moreover, it has been repeatedly reported that coverage of IPTp with at least two administered doses of SP is still relatively low, despite women attending antenatal clinics several times during their pregnancy (10,11).

In endemic countries, assuming that any pregnant woman attending an antenatal clinic is infected with malaria, regardless of symptoms, seems to be the right approach. This means that pregnant women should be either systematically screened for malaria infection and treated if positive or given SP at any time they visit a health centre, provided this is done at least one month apart. The latter has been recently recommended by the WHO Malaria Policy Advisory Committee and Secretariat (12) though it has not been implemented widely yet. For women in the first trimester of pregnancy systematic screening is probably the best approach as they cannot be given SP as IPTp. Active detection of malaria infection among pregnant women can have a major impact on miscarriage and pre-term delivery (13,14) and maternal mortality. Indeed, at the north-western border of Thailand this strategy was associated with a six-fold decline in the overall maternal mortality ratio (MMR), with *P. falciparum* malaria

related MMR falling from an estimated 1,000/100,000 live births prior to weekly screening to zero in 2005 (15).

The prevalence of malaria infection was significantly higher in women with symptoms suggestive of malaria but none of them or no combination of any of them had a very high PPV. This is confirmed by the weak association between malaria infection and any of the signs and symptoms, underlying the importance of parasitological diagnosis for any suspected case. The parasite density in pregnancy is not related to being a symptomatic patient and does not increase the probability that fever is related to malaria.

In places where transmission is high, malaria infection during pregnancy is often asymptomatic (16,17). In Mozambique, signs and symptoms suggestive of malaria were extremely common in pregnant women but malaria infection was confirmed in only a minority of them (5). The large majority of infections detected in pregnant women was asymptomatic in Benin (6). Nevertheless, in Ghana malaria-infected pregnant women were often symptomatic, with history of fever, headache, vomiting, malaise, and dizziness occurring significantly more frequently than in uninfected women (7). This is surprising when considering that the study was carried out in an area of stable and intense transmission. Indeed, in most of the previous reports from areas of intense transmission, malaria infection in pregnancy was often asymptomatic and this is also confirmed by this study. All common signs and symptoms, taken alone or in combination, had low PPV, with the only exception of two combinations, i e, fever and dizziness, and fever and vomiting. Nevertheless, even though the PPV for these two combinations was above 60%, about 30-40% of women with these complaints would have been unnecessarily treated for malaria. Pregnant women displaying signs and symptoms suggestive of malaria but without confirmed malaria should also be screened for others infectious diseases.

Several known risk factors such as maternal age (18,19) and parity (20) were confirmed to be associated with malaria infection during pregnancy, although in some instances the difference was not statistically significant, probably due to the need for a larger sample size. A limited number of patients had symptoms like vomiting, joint pain or malaise. Therefore, these results should be interpreted with caution as the power to find true differences is lacking or significant differences may have been found by chance. In addition, the risk of malaria infection did not vary significantly by gestational age, though the prevalence was the highest in the second trimester, an observation reported in other studies in which the peak prevalence during pregnancy was at 13–16 weeks of gestation (21,22).

3.6 Conclusion

Malaria-related signs and symptoms, e.g. fever or headache, although more frequent in pregnant women with a malaria infection, cannot be used to identify those in need of treatment as a large proportion of asymptomatic women can be infected. Any pregnant women attending a health facility should be tested regardless of symptoms for malaria infection, either by microscopy or RDT. This would be particularly useful for women in the first trimester of pregnancy as they cannot be administered SP as IPTp.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

The study was conceived by UDA and this paper drafted by MCT and UDA. It was conducted by MCT and HT with substantial contributions from JM. Data analyses were conducted by MCT, and supervised by AE and JM. AE, JBO, RTG, JPVG, HT and UDA participated in the overall running of the study, contributed to the interpretation of data, and gave critical review of the final draft. All authors read and approved the final version.

Acknowledgements

We are grateful to all the women who participated in this study. We thank the entire medical laboratory and administrative staff of the Clinical Research Unit of Nanoro, the medical staff at the CSPSs of Nanoro and Nazoanga for their valuable contribution. We would particularly like to thank Dr. Sabine Gies for her advice and comments, Dr. Kabore William for his commitment to the implementation of the study in the field, Dr. Valea Innocent and Dr. Sorgho Hermann for their useful advice; Ouedraogo Sayouba and Kazienga Adama for the double entry and cleaning of the data and Lompo Palpouguini for coordinating the slide reading.

3.7 References

1. Dellicour S, Tatem AJ, Guerra CA, Snow RW, ter Kuile FO. Quantifying the number of pregnancies at risk of malaria in 2007: a demographic study. PLoS Med. 2010;7:1.

2. Rodriguez-Morales AJ, Sanchez E, Vargas M, Piccolo C, Colina R, et al. Pregnancy outcomes associated with Plasmodium vivax malaria in Northeastern Venezuela. Am J Trop Med Hyg. 2006;74:755–57.

3. Singh N, Mehra RK, Srivastava N. Malaria during pregnancy and infancy, in an area of intense malaria transmission in central India. Ann Trop Med Parasitol. 2001;95:15–29.

4. WHO/AFRO. A strategic framework for malaria prevention and control during pregnancy in the African region. 2004.

5. Bardaji A, Sigauque B, Bruni L, Romagosa C, Sanz S, et al. Clinical malaria in African pregnant women. Malar J. 2008;7:27

6. Huynh BT, Fievet N, Gbaguidi G, Borgella S, Mevo BG, et al. Malaria associated symptoms in pregnant women followed-up in Benin. Malar J. 2011;10:72.

7. Tagbor H, Bruce J, Browne E, Greenwood B, Chandramohan D. Malaria in pregnancy in an area of stable and intense transmission: is it asymptomatic? Trop Med Int Health. 2008;13:1016–21.

8. Ministère de la Santé (Burkina Faso): Statistiques sanitaires. 1994.

9. Greenwood BM, Armstrong JRM. Comparison of two simple methods for determining malaria parasite density. Trans R Soc Trop Med Hyg. 1991;85:186–88.

10. Koen P, Gies S, Coulibaly SO, Ky C, Somda J et al. Bottlenecks for High coverage of intermittent preventive treatment in pregnancy: the case of adolescent pregnancies in rural Burkina Faso. PLoS One. 2010;5:8.

11. Chima AO, Kara H, Obinna EO. Low coverage of intermittent preventive treatment for malaria in pregnancy in Nigeria: demand-side influences. Malar J. 2012;11:82.

12. WHO. Malaria Policy Advisory Committee and Secretariat Malaria Policy Advisory Committee to the WHO: conclusions and recommendations of September 2012 meeting. Malar J. 2012;11:424.

13. Bardaji A, Sigauque B, Sanz S, Maixenchs M, Ordi J et al. Impact of malaria at the end of pregnancy on infant mortality and morbidity. J Infect Dis. 2011;203:691–99.

14. Luxemburger C, McGready R, Kham A, Morison L, Cho T, et al. Effects of malaria during pregnancy on infant mortality in an area of low malaria transmission. Am J Epidemiol. 2001,154:459–65.

15. McGready R, Boel M, Rijken MJ, Ashley EA, Cho T, et al. Effect of early detection and treatment on malaria related maternal mortality on the north-western border of Thailand 1986–2010. PLoS One. 2012;7:7.

16. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoa K, et al. Epidemiology and burden of malaria in pregnancy. Lancet Infect Dis. 2007;7:93–104.

17. Nosten F, Rogerson SJ, Beeson JG, McGready R, Mutabingwa TK, et al. Malaria in pregnancy and the endemicity spectrum: what can we learn? Trends Parasitol. 2004;20:425–32.

18. Rogerson SJ, van den Broek NR, Chaluluka E, Qongwane C, Mhango CG, et al. Malaria and anemia in antenatal women in Blantyre, Malawi: a twelve-month survey. Am J Trop Med Hyg. 2000;62:335–40.

19. Dicko A, Mantel C, Aly Thera M, Doumbia S, Diallo M, et al. Risk factors for malaria infection and anemia for pregnant women in the Sahel area of Bandiagara, Mali. Afr J Med Med Sci. 2003;89:17–23.

20. Kyabayinze DJ, Tibenderana JK, Nassali M, Tumwine LK, Riches C, et al. Placental P. falciparum malaria infection: operational accuracy of HRP2 rapid diagnostic tests in a malaria endemic setting. Malar J. 2011;10:306.

21. Brabin BJ. An analysis of malaria in pregnancy in Africa. Bull World Health Organ. 1983;61:1005–16.

22. Diagne N, Rogier C, Sokhna CS, Tall A, Fontenille D, et al. Increased susceptibility to malaria during the early postpartum period. New Microbiol. 2000;343:598–03.

Chapter 4: Prevalence of the *dhfr* and *dhps* mutations among pregnant women in rural Burkina Faso five years after the introduction of Intermittent Preventive Treatment with Sulfadoxine-Pyrimethamine

Tahita MC, Tinto H, Erhart A, Kazienga A, Fitzhenry R, et al. Prevalence of the *dhfr* and *dhps* Mutations among Pregnant Women in Rural Burkina Faso Five Years after the Introduction of Intermittent Preventive Treatment with Sulfadoxine-Pyrimethamine. *PLoS ONE*. 2015;10:e0137440.

4.1 Abstract

Background

The emergence and spread of drug resistance represents one of the biggest challenges for malaria control in endemic regions. Sulfadoxine-pyrimethamine (SP) is currently deployed as intermittent preventive treatment in pregnancy (IPTp) to prevent the adverse effects of malaria on the mother and her offspring. Nevertheless, its efficacy is threatened by SP resistance which can be estimated by the prevalence of dihydropteroate synthase (*dhps*) and dihydrofolate reductase (*dhfr*) mutations. This was measured among pregnant women in the health district of Nanoro, Burkina Faso.

Methods

From June to December 2010, two hundred and fifty six pregnant women in the second and third trimester, attending antenatal care with microscopically confirmed malaria infection were invited to participate, regardless of malaria symptoms. A blood sample was collected on filter paper and analyzed by PCR-RFLP for the alleles 51, 59, 108, 164 in the *pfdhfr* gene and 437, 540 in the *pfdhps* gene.

Results

The genes were successfully genotyped in all but one sample (99.6%; 255/256) for *dhfr* and in 90.2% (231/256) for *dhps*. The *dhfr* C59R and S108N mutations were the most common, with a prevalence of 61.2% (156/255) and 55.7% (142/255), respectively; 12.2% (31/255) samples had also the *dhfr* N51I mutation while the I164L mutation was absent. The *dhps* A437G mutation was found in 34.2% (79/231) isolates, but none of them carried the codon K540E. The prevalence of the *dhfr* double mutations N**RN**I and the triple mutations **IRN**I was 35.7% (91/255) and 11.4% (29/255), respectively.

Conclusion

Though the mutations in the *pfdhfr* and *pfdhps* genes were relatively common, the prevalence of the triple *pfdhfr* mutation was very low, indicating that SP as IPTp is still efficacious in Burkina Faso.

Keywords

Malaria, pregnancy, IPTp, dhfr, dhps, mutations, resistance, sulfadoxine pyrimethamine, and Burkina Faso.

4.2 Background

In Burkina Faso, malaria remains a major cause of morbidity and mortality as it accounts for 60.6% of all hospitalizations and 40.4% of all deaths (1). *P. falciparum* is the dominant species, whose transmission is intense and highly seasonal, due mainly to the vector *Anopheles gambiae* (2). Until 2005, chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) were the first and second line treatment for uncomplicated malaria, respectively. Following reports of CQ and SP treatment failures, the first line treatment was changed to artemether-lumefantrine (AL), with amodiaquine-artesunate (ASAQ) as an alternative (3). Nevertheless,

SP is still recommended for intermittent preventive treatment during pregnancy (IPTp) in Burkina Faso.

IPTp reduces the prevalence of placental malaria, severe anemia among primigravidae, pre-term delivery (4;5), low birth-weight and improves neonatal survival (4-6). It is considered to be safe, efficacious and easy to administer at antenatal clinics (ANC) (7). However, in East Africa, resistance to SP administered as IPTp has been associated with an increased risk of fetal anemia and severe malaria in the offspring (8).

Pyrimethamine selectively inhibits dihydrofolate reductase (*dhfr*), a part of the folate pathway in the malaria parasites, and *P. falciparum* resistance, both in vivo and in vitro, has been associated with specific point mutations (A16V, N51I, C59R, S108N/T and I164L) in the *dhfr* gene (9;10). Sulfadoxine selectively inhibits dihydropteroate synthetase (*dhps*) earlier in the parasite folate pathway; five point mutations (S436A/F, A437G, K540E, A581G and A613S/T) have been associated with in vitro resistance under low or no folate conditions (11;12). Increasing SP resistance is associated with an increasing number of mutations in both the *pfdhfr* and *pfdhps* genes; in Africa, the combined *pfdhfr* triple mutant (51I-59R-108N) and the *pfdhps* double mutant (437G-540E), the so called *dhfr/dhps* quintuple mutation, are predictive of SP treatment failure (13). Therefore, determining the prevalence of these mutations and their evolution over time can provide reasonably good information on temporal trends in SP efficacy. The prevalence of molecular SP resistance markers was determined in asymptomatic and symptomatic malaria-infected pregnant women five years after the introduction of IPTp with SP in Burkina Faso.

4.3 Methods

Study area, subjects and sample collection

The study was carried out at the Clinical Research Unit in Nanoro (CRUN), situated about 85 km north-west of Ouagadougou. The literacy rate in this area is low for both men and women (about 23%) and there is a high migration flow towards the capital city and/or the neighboring countries (14). Malaria transmission is high and seasonal, mainly occurring during the months of August-December, with an entomological inoculation rate (EIR) estimated at 50–60 infective bites/person/year in 2009. The most common vectors are *Anopheles gambiae sensu stricto*, *Anopheles funestus* and *Anopheles arabiensis* (Diabate A., personal communication). Malaria is one of the most common reasons for attending health facilities while *P. falciparum* is the predominant malaria species (15).

Blood samples were collected as part of a study on the clinical presentation of malaria during pregnancy whose results have been reported elsewhere (16). Briefly, all pregnant women attending the routine ANC at Nanoro district (Nanoro and Nazoanga health centers) were invited to participate in the study. A finger prick blood sample for slide and dried spots on filter paper (Whatmann grade 3) were collected. Molecular genotyping was performed only on samples from women with a positive blood slide (Fig. 1). All molecular tests were performed at the Institute of Tropical Medicine (ITM), Antwerp, Belgium.

Laboratory procedures

DNA extraction

DNA extraction from bloodspots was carried out according to the manufacturer's instructions using a QIAamp® DNA Micro Kit 50 (Qiagen, Hilden, Germany). Eluted DNA was immediately ready for use in amplification reactions or was stored at –20°C until further processing.

Nested PCR

The desired molecular products within the *dhfr* and *dhps* genes were amplified by nested PCR following a standardized protocol described elsewhere (17;18). The primary *dhfr* amplicon was produced by amplifying sample DNA with primer pairs AMP1 and AMP2 (18). This PCR product was used in a mutation-specific second PCR reaction to determine the presence of mutations at sites 51, 59, 108 and 164 in the *dhfr* gene. Two separate sets of PCRs were carried out for each codon, one for the wild-type allele and one for the mutant allele.

The HotStarTaq DNA polymerase (Qiagen, Hilden, Germany) was used with the manufacturer's buffer containing 1.5 mM MgCl2. The primers were used at a concentration of 0.2 mM, and other reaction conditions were as described previously [18] with the following cycling parameters: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 92°C for 1 minute, annealing at 52°C for 45 seconds, extension at 72°C for 45 seconds, and a final extension at 72°C for 10 minutes. Screening for *dhps* mutations was carried out as for *dhfr* screening with the following modifications. DNA was amplified using primers DPHS-R1 and DHPS-R2. This primary amplification product was subjected to a second round diagnostic PCR as part of a nested PCR, with the primers DHPS-K and DHPS-K1, followed by restriction enzyme digestion (17). Digestion products were analyzed on a 2% agarose gel with ethidium bromide.

Mutation-specific restriction enzyme digests analysis. For all *dhfr* codons, the *P. falciparum* strain 3D7 was used as wild-type control and V1S as mutant control. For the detection of *dhps* mutations, PS-Mali-clone DNA and PS-Peru-clone DNA were used as wild-type and mutant control for positions 437 and 540, respectively. The presence of *dhps* mutations A437G was evaluated by digestion with AvaII, and of *dhps* K540E mutation by digestion with FokI, both enzymes cleaving the mutated sequences.

Statistical methods

Data were entered in Excel version 2007 and analyzed using STATA v10 (STATA Corporation, College Station, TX, USA). In this analysis, mixed genotypes were considered as mutants, and the prevalence of each type of allele (wild or mutant) were calculated together with their respective confidence intervals. Participants were categorized as symptomatic and asymptomatic. Symptomatic women were defined as women having at least one of the following signs and symptoms: temperature ≥37.5°C (measured by electronic thermometer) and/or history of fever in the previous 48 hours, headache, pallor, arthro-myalgia, convulsions, vomiting, dizziness, malaise, fatigue, enlarged liver or enlarged spleen. Asymptomatic women had none of the above mentioned symptoms.

The frequencies of the mutations were compared between these groups, parasite density, and age using chi-square test, and a p-value <0.05 was considered as statistically significant.

Ethical considerations

After reviewing the study protocol, the Institutional Ethics Committee of the Centre Muraz, Bobo-Dioulasso, Burkina Faso (registration no. 005-2010/CE-CM) approved the study. Participants were only included after obtaining their written informed consent.

4.4 Results

Six hundred pregnant women were included in the study, of whom two hundred and fifty six (42.7%) had a microscopically-confirmed malaria infection (Fig. 1). Most of them (81%) were aged 20-34 years old and had already one to three children (85%). Parasite density was not associated with the occurrence of symptoms (Table 1).

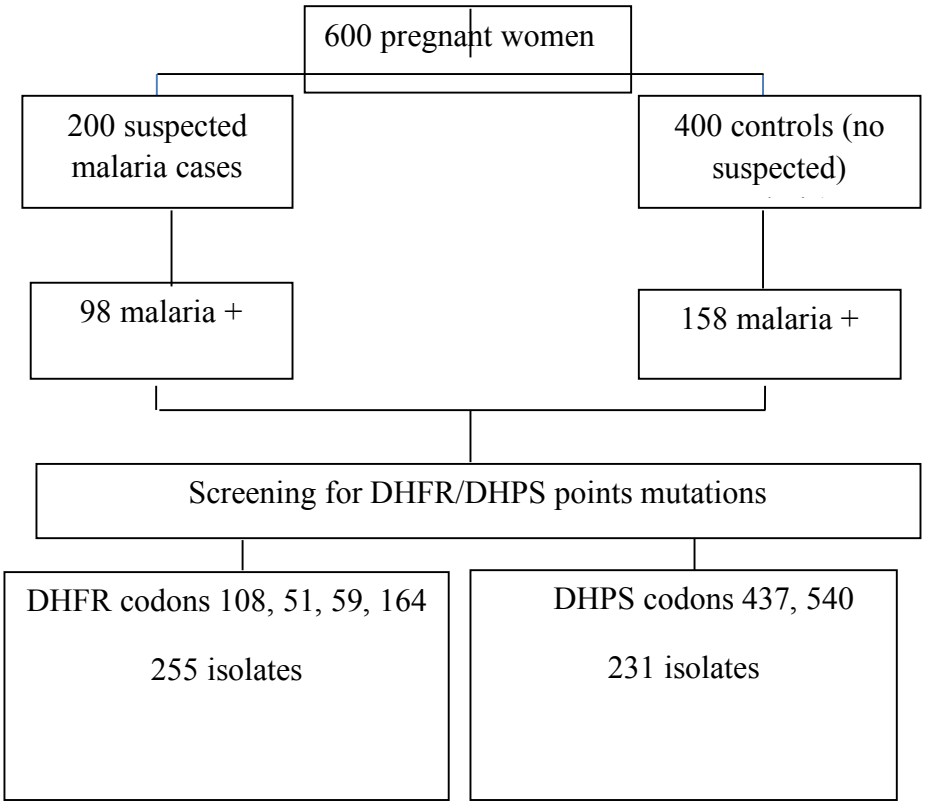


Figure 1: Study profile

Table 1: Baseline characteristics by presence or not of symptoms

	Asymptomatic	**Symptomatic**	**p-value**
	n=157	n=198	
Age group (years)			
<20	13(8.3%)	11(11.2%)	0.66
20-34	128(81.5%	79(80.6%)	
≥35	16(10.2%)	8(8.2%)	
Parasite density	710.61(541.45 - 932.62)	1107.46(747.48 - 640.82)	0.08
Parity			
Nulliparous	13(8.3%)	10(10.2%)	0.77
1 to 3	101(64.3%)	59(60.2%)	
≥4	43(27.3%)	29(29.6%)	
Anemia	95(60.5%)	75(76.5%)	0.01
IPTp(doses received)			
0	47(30%)	28(28.6%)	077
1	90(57.3%)	60(61.2%)	
2	20(12.7%)	10(10.2%)	

Most samples could be successfully genotyped, 99.6% (255/256) for the *dhfr* gene and 90.2% (231/256) for the *dhps* gene (Fig. 8). More than half of the samples had the *dhfr* C59R (61.2%, 156/255) and/or the S108N (55.7%, 142/255) mutations while only 12.2% (31/255) had the N51I mutation, and no I164L mutation was found (Table 2).

Table 2: Prevalence of the *dhfr* and *dhps* point mutations associated with SP resistance.

	***dhfr* (N=255)**				***dhps* (N=231)**	
Codon	**51**	**59**	**108**	**164**	**437**	**540**
Mutant, n(%)	31(12.2)	156(61.2)	142(55.7)	0	79(34.2)	0
[95% CI]	[8.7 - 16.7]	[55.1 - 67.0]	[49.5 - 61.7]	-	28.4 - 40.5]	-

There were 6 different *dhfr* alleles; the prevalence of the sequence NCSI (wild type) was 30.2% (77/255). Among the mutant alleles, the double mutation N**RN**I was the most frequent (35.7%, 91/255), followed by the triple mutation **IRN**I (11.4%, 29/255) (Table 3).

Table 3: *Dhfr* mutations among isolates

	NCSI	**ICSI**	**NRSI**	**NCNI**	**NRNI**	**IRSI**	**ICNI**	**IRNI**
51								
59								
108								
164								
n	77	0	34	22	91	2	0	29
%(x/255)	30.2	0	13.3	8.6	35.7	0.8	0	11.4
95%CI	24.8 - 36.2	-	9.7 - 18.1	5.7 - 12.7	30.1 - 41.7	0.2 - 2.8	-	8.1 - 15.9

More than a third of the samples (34.2%, 79/231) carried the *dhps* mutations A437G but none of them had the mutation K540E.

The occurrence of the mutations N51I and A437G were significantly associated with higher parasite density (Table 4). No other factor (age, parity, and number of SP doses taken) was found to be associated with the risk of double or triple mutation.

Table 4: Trends of the molecular markers according to the parasite density

	Parasite density(geometric mean)		P-value
	Symptomatic	**Asymptomatic**	
***dhfr* 51**	12558.38	3970.26	0.02
	(5078.04 - 31057.85)	(2391.12 - 6592.31)	
***dhfr* 59**	1419.81	1076.25	0.37
	(841.74 - 2394.88)	(754.03 - 1536.17)	
***dhfr* 108**	1576.97	1454.58	0.80
	(890.82 - 2791.60)	(1024.57 - 2065.06)	
***dhps* 437**	1873.07	708.17	0.01
	(989.73 - 3544.78)	(443.41 - 1131.01)	

4.5 Discussion

Despite several studies on the association between genetic polymorphisms and response to SP treatment, the role of certain *dhfr* and *dhps* mutations in treatment outcome is still poorly understood. SP resistance increases with the increasing number of point mutations in the *dhfr* and *dhps* genes (19). In Nanoro, among pregnant women, the most prevalent *dhfr* allele's mutations were 59R and the 108N, as more than half of the isolates carried one of them. However, the prevalence of the double mutation 59R and 108N was much lower, about 36%, and the triple mutation 108N-51I-59R had an even lower prevalence, around 11%. Though single or double mutations in the *dhfr* gene have been associated with pyrimethamine resistance (20-23), the *dhfr* triple mutation is known to confer intense pyrimethamine resistance in vitro (24) and is associated with an approximate 1,000-fold reduction in pyrimethamine susceptibility (25). Nevertheless, SP is systematically administered to all pregnant women in the second and third trimester attending ANC, while only a proportion of them would carry a malaria infection, often of low density. For other non-infected pregnant women at the time of treatment, SP would have a prophylactic effect as it would clear emerging malaria infections for a given period of time. The low prevalence of the triple *dhfr* mutations indicates that in Nanoro SP should be able to clear malaria infections present at the time of its administration, particularly when considering that their density would be generally

low. The effect of the *dhfr* double or triple mutation on the duration of the protection period is unknown but its duration may be shorter and parasites carrying the double or triple mutation may be able to emerge earlier than the wild ones.

No isolate had the *dhps* double mutation, at position 437 and 540, commonly associates with sulfadoxine resistance (26;27). Indeed, in more than a third of all isolates it was possible to identify only the 437 mutation, which usually occurs first in the progressive selection of resistant parasites (28). Such mutation, alone or combined with the K540E, has been associated with treatment failure with SP (27;29;30). Nevertheless, in Nanoro, the A437G prevalence was low compared to that found in north western Burkina Faso where in the year 2000 the prevalence was already above 50% and had increased to almost 80% in 2009 (31).

The low prevalence of the triple *dhfr* mutation is surprising when considering that SP was extensively used as first line treatment in Burkina Faso and that in other areas of Burkina Faso it was significantly higher (32), with suggestion of its increase over time (31). Such discrepancy may be due to differences in drug pressure and differing access to ACT treatment. Indeed, before 2005, SP was used as second line treatment and after 2005 as first line treatment, before the large scale implementation of ACT. The remaining high prevalence of *dhfr* triple mutants could indicate a less than optimal access to ACT in Ziniare (32) and Nouna (31), while in Nanoro the ready access to ACT could have resulted in a decreased prevalence of the triple mutants. Such phenomenon has already been observed in South America where a significant decline of the prevalence of *dhfr* triple mutants coincided with the change of the treatment policy from SP to ACT (33;34), suggesting a lower fitness of SP-resistant parasites in the absence of a significant drug pressure. Nevertheless, in Malawi the prevalence of mutant parasites did not decrease after five years without SP as first line

treatment, indicating no fitness cost of the *dhfr* triple and *dhps* double mutant haplotypes in the absence of strong SP pressure (35).

None of the isolates carried the *dhps* K540E mutation while more than one third had the A437G mutation. This is not surprising when considering that none of the recent studies carried out in Burkina Faso found isolates with the K540E mutation (31;32). Nevertheless, these same studies reported a much higher prevalence of the A437G mutation in other parts of the country, over 70%, possibly indicating a much higher drug pressure compared to Nanoro.

Infections carrying one or more of the *dhfr* mutations were associated to higher parasite density together with the presence of symptoms. This was true for the *dhfr* 51 and *dhps* 437 mutations. This could have major implications for the severity of the disease and for selection pressure as these mutations do not seem to confer a survival disadvantage for the parasite.

4.6 Conclusion

Mutations in the *Pfdhfr* and *Pfdhps* genes associated with SP resistance were relatively common among pregnant women in the study area. Nevertheless, the prevalence of the triple *dhfr* mutations was very low suggesting that SP may still be efficacious when used as IPTp. Nevertheless, molecular markers linked to SP resistance should continue to be monitored.

Acknowledgements

We are grateful to all the women who participated in this study. We thank the entire medical laboratory and administrative staff of the Clinical Research Unit of Nanoro, the medical staff at the CSPSs of Nanoro and Nazoanga for their valuable contribution. We would particularly like to thank Prof Harald Noedl and Prof Christopher Plowe for their support.

Author Contributions

Conceived and designed the experiments: UDA, MCT, JPVg and HT. Performed the experiments: MCT, RF, CVO and ARU. Analyzed the data: AK, AE, JBO, RTG, HT. Contributed reagents/materials/analysis tools: UDA, wrote the manuscript: MCT, AE, JPVg, UDA.

4.7 References

1. Programme National de Lutte contre le Paludisme (PNLP). Directives nationales pour la prise en charge du paludisme dans les formations sanitaires du Burkina faso. Ministère de la santé. 2010.

2. Cuzin-Ouattara N, Van den Broek AH, Habluetzel A, Diabate A, Sanogo Ilboudo E, et al. Wide-scale installation of insecticide-treated curtains confers high levels of protection against malaria transmission in a hyperendemic area of Burkina Faso. Trans R Soc Trop Med Hyg. 1999;93:473-79.

3. Tinto H, Ouédraogo JB, Zongo I, Van Overmeir C, Van Marck E, et al. Sulfadoxine-pyrimethamine efficacy and selection of P. falciparum DHFR mutations in Burkina Faso before its introduction as intermittent preventive treztment for pregnant women. Am J Trop Med Hyg. 2007;76:608-13.

4. Maiga OM, Kayentao K, Traore BT, Djimde A, Traore B, et al: Superiority of 3 over 2 doses of intermittent preventive treatment with sulphadoxine-pyrimethamine for the prevention of malaria during pregnancy in mali: a randomized controlled trial. Clin Infect Dis. 2011; 53:215-23.

5. Aziken ME, Akubuo KK, Gharoro EP. Efficacy of intermittent preventive treatment with sulphadoxine-pyrimethamine on placental parasitemia in pregnant women in Midwestern Nigeria. Int J Gynaecol Obstet. 2011;112:30-33.

6. Kayentao K, Kodio M, Newman RD, Maiga H, Doumtabe D, et al. Comparison of intermittent preventive treatment with chemoprophylaxis for the prevention of malaria during pregnancy in Mali. Int J Infect Dis. 2005;191:109-16.

7. Sicuri E, Bardaji A, Nhampossa T, Maixenchs M, Nhacolo A, et al. Cost-effectiveness of intermittent preventive treatment of malaria in pregnancy in southern Mozambique. PLoS One. 2010;5:e13407.

8. Harrington WE, Mutabingwa TK, Kabyemela E, Fried M, Duffy PE. Intermittent Treatment to Prevent Pregnancy malaria does not confer benefit in an area of widespread drug resistance. Clin Infect Dis. 2011;54:224-30.

9. Foote SJ, Galatis D, Cowman AF. Amino acids in the dihydrofolate reductase-thymidylated synthase gene of P. falciparum involved in cycloguanil resistance differ from those involved in pyrimethamine resistance. Proc Natl Acad Sci. 1990;87:3014-17.

10. Peterson DS, Milhous WK, Wellems TE. Molecular basis of differential resistance to cycloguanil and pyrimethamine in P. falciparum malaria. Proc Natl Acad Sci. 1990;87:3018-22.

11. Brooks DR, Wang P, Read M, Watkins WM, Sims PFG, et al. Sequence variation of the hydroxymethyldihydropterin pyrophosphokinase: dihydropteroate synthase gene in lines of the human malaria parasite, P. falciparum, with differing resistance to sulfadoxine. Eur J Biochem. 1994;224:397-05.

12. Triglia T, Cowman AF. Primary structure and expression of the dihydropteroate synthase gene of P. falciparum. Proc Natl Acad Sci. 1994;91:7149-53.

13. Vinayak S, Alam MT, Mixson-Hayden T, McCollum AM, Sem R, et al. Origin and evolution of sulphadoxine resistant P. falciparum. PLos Pathog. 2010.

14. Tinto H, Valea I, Sorgho H, Tahita MC, Traore M, et al. The impact of clinical research activities on communities in rural Africa: the development of the Clinical Research Unit of Nanoro (CRUN) in Burkina Faso. Malar J. 2014;13:113.

15. Pfeiffer K, Some F, Müller O, Sie A, Kouyaté B, Haefeli WE, et al. Clinical diagnosis of malaria and the risk of chloroquine self-medication in rural health centres in Burkina Faso. Trop Med Int Health. 2008;13:418-26.

16. Tahita MC, Tinto H, Menten J,Ouedraogo JB,Guiguemde RT, et al. Clinical signs and symptoms cannot reliably predict P. falciparum malaria infection in pregnant women living in an area of high seasonal transmission. Malaria Journal. 2013;12:464.

17. Duraisingh MT, Curtis J, Warhurst DC. P. falciparum detection of polymorphisms in the dihydrofolate reductase and dihydropteroate synthetase genes by PCR and restriction digestion. Exp Parasitol. 1998;89:1-8.

18. Plowe CV, Djimde A, Bouare M, Doumbo O, Wellems TE. Pyrimethamine and proguanil resistance-conferring mutations in P. falciparum dihydrofolate reductase: polymerase chain reaction methods for surveillance in Africa. Am J Trop Med Hyg. 1995;52:565-68.

19. Sibley CH, Hyde JE, Sims PF, Plowe CV, Kublin JG, et al. Pyrimethamine-sulfadoxine resistance in P. falciparum: what next? Trends Parasitol. 2001;17:582-88.

20. Nzila AM, Mberu EK, Sulo J, Dayo H, Winstanley PA, et al. Towards an understanding of the mechanism of pyrimethamine-sulfadoxine resistance in P. falciparum: genotyping of dihydrofolate reductase and dihydropteroate synthase of Kenyan parasites. Antimicrob Agents Chemother. 2000;44:991-96.

21. Basco LK, Ringwald P. Molecular epidemiology of malaria in Yaoundé, Cameroon.II. Baseline frequency of point mutations in the dihydropteroate synthase gene of P. falciparum. Am J Trop Med Hyg. 1998;58:374-77.

22. Cowman AF, Morry MJ, Biggs BA, Cross GA, Foote SJ. Amino acid changes linked to pyrimethamine resistance in the dihydrofolate reductase-thymidylate synthase gene of P. falciparum. Proc Natl Acad Sci. 1988;85:9109-13.

23. Wang P, Lee CS, Bayoumi R, Djimde A, Doumbo O, et al. Resistance to antifolate in P. falciparum monitored by sequence analysis of dihydropteroate synthetase and dihydrofolate reductase alleles in a larger number of field samples of diverse origin. Mol Biochem Parasitol. 1997;89:161-77.

24. Gregson A, Plowe CV. Mechanisms of resistance of malaria parasites to antifolates. Pharmacol Rev. 2005;57:117-45.

25. White NJ. Intermittent presumptive treatment for malaria. PLoS Med. 2005;2:e3.

26. Plowe CV, Kublin JG, Dzinjalamala FK, Kamwendo DS, Mukadam RA, et al. Sustained clinical efficacy of sulfadoxine-pyrimethamine for uncomplicated falciparum malaria in Malawi after 10 years as first line treatment: five year prospective study. BMJ. 2004;328:45.

27. Kublin JG, Dzinjalamala FK, Kamwendo DD, Malkin EM, Cortese JF, et al. Molecular markers for failure of sulfadoxine-pyrimethamine and chlorproguanil-dapsone treatment of P. falciparum malaria. J Infect Dis. 2002;185:380-88.

28. Plowe CV, Cortese JF, Djimde A, Nwanyanwu OC, Watkins WM, et al. Mutations in P. falciparum dihydrofolate reductase and dihydropteroate synthase and epidemiologic patterns of pyrimethamine-sulfadoxine use and resistance. J Infect Dis. 1997;176:1590-96.

29. Omar SA, Adagu IS, Warhurst DC. Can pretreatment screening for dhps and dhfr point mutations in P. falciparum infections be used to predict sulfadoxine-pyrimethamine treatment failure?Trans R Soc Trop Med Hyg. 2001;95:315-19.

30. Pearce RJ, Pota H, Evehe MS, Ba E, Mombo-Ngoma G, Malisa AL, et al. Multiple origins and regional dispersal of resistant dhps in African P. falciparum malaria. PLoS Med. 2009;6:e1000055.

31. Geiger C, Compaore G, Coulibaly B, Sie A, Dittmer M, et al. Substantial increase in mutations in the genes pfdhfr and pfdhps puts sulphadoxine-pyrimethamine-based intermittent preventive treatment for malaria at risk in Burkina Faso. Trop Med Int Health. 2014;19:690-97.

32. Coulibaly SO, Kayentao K, Taylor S, Guirou EA, Khairallah C, et al. Parasite clearance following treatment with sulphadoxine-pyrimethamine for intermittent preventive treatment in Burkina-Faso and Mali: 42-day in vivo follow-up study. Malar J. 2014;13:41.

33. Bacon DJ, McCollum AM, Griffing SM, Salas C, Soberon V, et al. Dynamics of malaria drug resistance patterns in the Amazon basin region following changes in Peruvian national treatment policy for uncomplicated malaria. Antimicrob Agents Chemother. 2009;53:2042-51.

34. Zhou Z, Griffing SM, de Oliveira AM, McCollum AM, Quezada WM, et al.: Decline in sulfadoxine-pyrimethamine-resistant alleles after change in drug policy in the Amazon region of Peru. Antimicrob Agents Chemother. 2008;52:739-41.

35. Artimovich E, Schneider K, Taylor TE, Kublin JG, Dzinjalamala FK, et al. Persistence of sulfadoxine-pyrimethamine resistance despite reduction of drug pressure in Malawi. J Infect Dis. 2015;212:694-01.

Chapter 5: Ex vivo anti-malarial drug susceptibility of *P. falciparum* isolates from pregnant women in an area of highly seasonal transmission in Burkina Faso

Tahita MC, Tinto H, Yarga S, Kazienga A, Traore Coulibaly M, Valea I, Van Overmeir C, Rosanas-Urgell A, Ouedraogo JB, Guiguemde RT, van Geertruyden JP, Erhart A, D'Alessandro U. Ex vivo anti-malarial drug susceptibility of P. falciparum isolates from pregnant women in an area of highly seasonal transmission in Burkina Faso. Malaria J. 2015;14:251.

5.1 Abstract

Background

Ex vivo assays are usually carried out on parasite isolates collected from patients with uncomplicated *P. falciparum* malaria, from which pregnant women are usually excluded as they are often asymptomatic and with relatively low parasite densities. Nevertheless, *P. falciparum* parasites infecting pregnant women selectively sequester in the placenta and may have a different drug sensitivity profile compared to those infecting other patients. The drug sensitivity profile of *P. falciparum* isolates from infected pregnant women recruited in a treatment efficacy trial conducted in Burkina Faso was determined in an ex vivo study.

Methods

The study was conducted between October 2010 and December 2012. *P. falciparum* isolates were collected before treatment and at the time of any recurrent infection whose parasite density was at least 100/µl. A histidine-rich protein-2 assay was used to assess their susceptibility to a panel of seven anti-malarial drugs. The concentration of anti-malarial drug inhibiting 50% of the parasite maturation to schizonts (IC_{50}) for each drug was determined with the IC Estimator version 1.2.

Results

The prevalence of resistant isolates was 23.5% for chloroquine, 9.2% for mefloquine, 8.0% for monodesethylamodiaquine, and 4.4% for quinine. Dihydroartemisinin, mefloquine, lumefantrine, and monodesethylamodiaquine had the lowest mean IC_{50} ranging between 1.1 nM and 1.5 nM respectively. The geometric mean IC_{50} of the tested drugs did not differ between chloroquine- sensitive and resistant parasites, with the exception of quinine, for which the IC_{50} was higher for chloroquine-resistant isolates. The pairwise comparison between the IC_{50} of the tested drugs showed a positive and significant correlation between dihydroartemisinin and both mefloquine and chloroquine, between chloroquine and lumefantrine and between monodesethylamodiaquine and mefloquine.

Conclusion

These ex vivo results suggest that the currently available artemisinin-based combination treatments are efficacious for the treatment of malaria in pregnancy in Burkina Faso.

ClinicalTrials.gov ID: NCT00852423

5.2 Background

Artemisinin-based combination treatment (ACT) is recommended by the World Health Organization (WHO) for the treatment of uncomplicated falciparum malaria (1). They comprise a fast-acting artemisinin derivative that rapidly reduces the parasite biomass and gametocyte carriage (2;3), and slower-acting partner drugs that clear the remaining parasites and provide post-treatment prophylaxis whose duration depends on its pharmacokinetic properties (4-6). The rationale for combining antimalarials with different mechanisms is to prevent the development of resistance or at least slow down its onset (1). Nevertheless, artemisinin-resistant *P. falciparum* malaria has emerged in western Cambodia and the bordering regions with Thailand, the hotspot of multidrug-resistant parasites (7-10), and is

now reported in five countries of the Greater Mekong Subregion, i.e., Cambodia, Thailand, Myanmar, Vietnam, and Laos (11). Artemisinin-resistant parasites could either spread to other regions and continents or emerge independently in areas of extensive artemisinin use (12). Therefore, it is important to monitor ACT efficacy and P. falciparum sensitivity to different antimalarials in endemic countries in order to timely update treatment guidelines (13). This can be done by using different methods, including ex vivo assays that can provide useful information on the susceptibility of the local parasite population to different anti-malarial drugs.

Burkina Faso changed its treatment policy in 2005, from chloroquine monotherapy to ACT with either artemether-lumefantrine (AL) or amodiaquine-artesunate (ASAQ). Since the implementation of the new policy in 2006, several trials have shown high efficacy of the recommended treatments (14-16), while only one ex vivo assay has been carried out (17) . Ex vivo assays are usually done on parasite isolates from patients with uncomplicated falciparum malaria, mostly children in sub-Saharan Africa. Malaria-infected pregnant women, who represent an important group at risk, are usually not included in these assays as they are often asymptomatic and with relatively low parasite densities (18;19). However, *P. falciparum* parasites infecting pregnant women can selectively accumulate in the placenta and represent a distinct sub-set of parasites expressing a unique *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) that binds to the host-receptor chondroitin sulphate A (CSA). These PfEMP1s are structurally, antigenically and functionally distinct from those expressed by parasites that infect children and non-pregnant women (20). In addition, some studies concluded that parasites seen on the blood smear of a pregnant woman may be CSA-binding parasites derived from the placenta, may be CD36-binding parasites circulating outside the placenta, or may be a mixture of the two subpopulations (21). It was then hypothesized that such parasites harvested intravenously could have a different sensitivity profile compared to those from

children and non-pregnant women. Therefore, ex vivo assays were carried out on isolates collected from pregnant women attending antenatal clinics and diagnosed with malaria in the Burkina Faso study site of a larger multi-centre trial.

5.3 Methods

Study area

The study was carried out at the Clinical Research Unit of Nanoro (CRUN), located at about 85 km from the capital city of Ouagadougou. Malaria is hyperendemic and highly seasonal, occurring between July and December, corresponding to the rainy season. The entomological inoculation rate (EIR) is estimated at 50-60 infective bites/person/year (Diabate A, pers comm). Malaria is the major reason for attending health facilities, with *P. falciparum* being responsible for more than 90% of the infections (22).

Study design

This study was part of a multi-centre (Burkina Faso, Ghana, Malawi, and Zambia) trial (ClinicalTrials.gov ID: NCT00852423) (23) investigating the efficacy and safety of four anti-malarial treatments, namely dihydroartemisinin-piperaquine (DHAPQ), mefloquine-artesunate (MQAS), ASAQ and AL in falciparum malaria-infected pregnant women. In Burkina Faso, an ex vivo study on the drug sensitivity of isolates from pregnant women was nested into the trial. Pregnant women were included in the trial if they fulfilled the following inclusion criteria: gestation ≥16 weeks, *P. falciparum* mono-infection at any density with or without symptoms, haemoglobin ≥7 g/dL, residence within the health facility catchment's area, and willingness to deliver at the health facility. For the ex vivo study the inclusion was limited to women with a parasite density of at least 100/µl. The study protocol was approved by the respective ethical review boards (the Ethics Committee of the University Hospital of

Antwerp, Institutional Ethic Committee of Centre Muraz and the National Ethic Committee) and written informed consent was obtained from all study participants.

Collection of field isolates

P. falciparum field isolates were collected at day 0 (before treatment) and at any day of recurrent infection during a 63-day in vivo drug efficacy follow-up. A volume of 3-4 ml of venous blood was collected in heparinized tubes and transferred within 24 hrs to the laboratory for testing. Blood was centrifuged, plasma and buffy coat were removed, and parasitized erythrocytes were washed three times with RPMI 1640 medium (Sigma-Aldrich, St Louis, USA) at 37°C. Field parasite samples with more than 1% parasitemia were diluted with uninfected erythrocytes (human blood type O+) to avoid any influence of an inoculum effect on assay results. Prior to the study start, successful experiments were conducted with initial parasite densities as low as 0.002%.

Drug sensitivity testing

Monodesethylamodiaquine (MDAQ), chloroquine diphosphate (CQ), quinine hydrochloride (QN) and mefloquine (MQ) were purchased from Sigma-Aldrich (St Louis, USA). Dihydroartemisinin (DHA) and piperaquine phosphate (PQ) were donated by Sigma-Tau (Rome, Italy) and lumefantrine (LM) by Novartis Pharma (Basel, Switzerland).

The drugs stock solutions were prepared at a concentration of 1 mg/ml in the following solvents: MDAQ and CQ in distilled water, MQ and PQ in lactic acid, LM in ethanol, QN and DHA in methanol. Multiple wells of a 96-well culture plate were predosed with two-fold serial dilutions of each drug at final concentrations that ranged from 12.5 nM to 3.200 nM for CQ, 6.25 nM to 400 nM for MDAQ, 13 nM to 3.333 nM for QN, 0.2 nM to 64 nM for DHA, 1.2 nM to 300.2 nM for LM, 1.6 nM to 100 nM for PQ and 3.2 nM to 206.3 nM for MQ. For each sample, 200µl aliquots of cultured parasites, prepared as described above, were added to

each well and incubated at 37°C at 5% CO2 for 72 hrs. The culture plates were then frozen and stored at -20°C for up to four weeks.

Parasite growth inhibition was quantified using an enzyme-linked immunosorbent assay (ELISA) that quantifies parasite histidine-rich protein-2 (HRP-2) (24). Two commercially available monoclonal antibodies (Immunology Consultants Laboratory, Inc., Newberg, OR, USA) directed against *P. falciparum*-specific HRP2, i.e., MPFM-55A and MPFG-55P, were used for the ELISA. Plates were pre-coated with the first monoclonal antibody MPFM-55A (original concentration from the manufacturer was 7,200 µg/ml) diluted at a concentration of 1.0 µg/ml. In order to obtain complete haemolysis before starting the ELISA, cultured samples were diluted 1:2 in distilled water. One-hundred µl of each haemolysed samples were added to the ELISA plate for 1 hr. then washed three times with the washing solution (PBS + 0.05% Tween 20, Sigma-Aldrich). The second antibody (MPFG-55P) was diluted at a concentration of 0.05 µg/ml and then added (100 µl) for 1 hr. at room temperature, washed again three times, and incubated with 100 µl of TMB (3,3'_,5,5'_- tetramethylbenzidine) chromogen (Sigma-Aldrich, St Louis, USA) in the dark for 5-10 minutes. After incubation the reaction was stopped with 50 µl of 1 M sulphuric acid. Spectrophotometric analysis was performed with an ELISA plate reader (Multiskan FC, plate reader) at 450 nm.

Statistical analysis

Inhibitory concentrations (IC_{50s}) for antimalarials were calculated using IC50 Estimator Version1.2 available online (25).

Data were entered in Excel version 97 and analyzed with Stata version 10.0. Results were expressed as geometric mean IC_{50} (concentration at which 50% of parasite growth was inhibited) with 95% confidence intervals. Pairwise comparisons were done and the Spearman rank-order correlation test used to determine the correlation between IC_{50} values. For all statistical tests, the significance level was set at $p \leq 0.05$. The threshold IC_{50} for ex vivo

resistance was defined as ≥100 nM for CQ, ≥60 nM for MDAQ, ≥800 nM for QN (26), and ≥30 nM for MQ (27;28).

5.4 Results

A total of 108 *P. falciparum* isolates were tested and 90 (83.3%) had interpretable results for at least one of the study drugs (Table 1). The overall culture success rate was around 80% and varied little between study drugs (from 76.8 and 83.3%). Missing results were due to poor ex vivo growth or failure to achieve adequate fit on a log dose-response curve.

CQ (geometric mean IC_{50} = 40.7 nM; 95% CI: 28.6-57.9) had the highest rate of resistant isolates (23.5%), followed by MQ (geometric mean IC_{50} = 1.1 nM; 95% CI: 0.8-1.7) with 9.2%, MDAQ (geometric mean IC_{50} =1.5; 95% CI: 1.0-2.2) with 8.0%, and then QN (geometric mean IC_{50} =34.2; 95% CI: 24.1-48.5) with 4.4%. DHA, LM and MDAQ had a low IC_{50} that ranged between 1.1 nM and 1.5 nM (Table 1).

Table 1: Ex vivo susceptibility of *P. falciparum* isolates by drug tested

	Culture success rate % (n/N)	**Geometric Mean IC50 (nM) [95%CI]**	**Range**		**Resistant isolates n (%)**
MDAQ	80.6 (87/108)	1.5 [1.0-2.2]	0.1	158.5	7 (8.0)
MQ	80.6 (87/108)	1.1 [0.8-1.7]	0.02	103.7	8 (9.2)
DHA	79.6 (86/108)	1.2 [0.9-1.6]	0.1	10.2	NA*
CQ	78.7 (85/108)	40.1[28.6-58]	1.2	1060.1	20 (23.5)
LM	78.7 (85/108)	1.4 [1.0-2.1]	0.1	97.7	NA
QN	83.3 (90/108)	34.2 [24.1-48.5]	1.9	995.5	4 (4.4)
PQ	76.8 (83/108)	5.0 [4.1-6.3]	0.5	47.9	NA

Resistance threshold defined for CQ at 100 nM, QN at 800 nM, MQ at 30 nM and MDAQ at 60 nM;

NA*: not applicable as the threshold is not *defined yet.*

The geometric mean and the resistance cutoff (where available) are shown in Additional file 1: Figure S1. Most drugs were equally active against the CQ-sensitive and CQ-resistant isolates except for QN where the IC_{50} for CQ-resistant isolates was higher than that for CQ-sensitive ones, although the difference did not reach statistical significance (Table 2).

Table 2: Geometric mean IC_{50} of the different drug tested by chloroquine sensitivity

Drug	**Chloroquine-resistant isolates IC_{50} (95% CI) N=20**	**Chloroquine-sensitive isolates IC_{50} (95% CI) N=65**	***p*-value°**
MDAQ	1.2 (0.6-2.7)	1.4 (0.9-2.2)	0.98
MQ	0.9 (0.4-1.9)	1.2 (0.8-2.0)	0.317
DHA	1.8 (0.9-3.4)	1.1 (0.8-1.5)	0.23
CQ	487.2 (334.9-708.9)	18.9 (15.2-23.7)	<0.001
LM	2.1 (0.7-5.9)	1.3 (0.8-2.0)	0.18
QN	64.4 (29.1-142.1)	28.8 (19.4-42.7)	0.06
PQ	6.6 (4.3-10.1)	4.7 (3.6-6.1)	0.29

There was a positive significant correlation between the sensitivity of DHA and both MQ and CQ, between CQ and LM and between MDAQ and MQ (Table 3).

Table 3: Pairwise comparison between the IC_{50} of tested drugs

Drug pairs	r[§]	p value
MDAQ-MQ	0.36	<0.001*
MDAQ-DHA	0.13	0.22
MDAQ-CQ	0.13	0.21
MDAQ-LM	0.14	0.20
MDAQ-QN	0.13	0.20
MDAQ-PQ	-0.037	0.74
MQ-DHA	0.23	0.02*
MQ-CQ	-0.03	0.78
MQ-LM	0.10	0.35
MQ-QN	0.08	0.43
MQ-PQ	-0.05	0.60
DHA-CQ	0.21	0.04*
DHA-LM	0.19	0.07
DHA-QN	0.07	0.48
DHAPQ	0.14	0.20
CQ-LM	0.21	0.05
CQ-QN	0.27	0.001*
CQ-PQ	0.21	0.05
LM-QN	0.01	0.90
LM-PQ	0.17	0.11
QN -PQ	-0.09	0.41

[§] Spearman's rank-order correlation coefficient (r)

5.5 Discussion

As expected, a substantial proportion of isolates were resistant to CQ while for the other drugs whose sensitivity threshold is known, the prevalence of resistant isolates was <10%. The prevalence of CQ-resistant (CQ-R) isolates in Bobo Dioulasso, a town situated at several hundreds of kilometers to the southwest of Nanoro, was 50% in 2006 (Lea Bonkian, pers comm) and 42.1% in 2008-2010 (17), a slight decrease that coincided with the change in the national treatment policy. As the latter was implemented in 2006, it may be too early to detect a substantial decrease of CQ-R isolates, although this may happen. Indeed, such a decrease has been observed in other African countries after withdrawing CQ as first-line treatment and hence decreasing the selective pressure on the local parasite population (29-31). In Nanoro, the prevalence of CQ-R isolates as well as the CQ mean IC_{50} were lower than in Bobo Dioulasso. However, these may have already been lower in the past and probably reflect the higher drug pressure usually found in urban (Bobo Dioulasso) compared to rural (Nanoro) areas. To make any conclusion on the trend of CQ resistance, it is necessary to carry out serial ex vivo studies at regular intervals and in the same sites.

The persistence of CQ-R *P. falciparum* isolates may be due to the continued use of CQ by the local population (32), maintaining the drug selective pressure, and/or the cross-resistance to other drugs with similar chemical structure. Indeed, the use of ASAQ as one of the first-line treatments may contribute to maintain the drug pressure on CQ-R parasites and could explain the high prevalence found in this study. Nevertheless, MDAQ seems to have a good activity on the local parasites, with only seven (8.0%) isolates with IC_{50} above the accepted threshold of resistance, indicating that ASAQ should still have a reasonable efficacy in pregnant women with malaria. LM, MQ and PQ are partner drugs in ACT and all of them had a relatively low mean IC_{50}, the first two around 1 nM and the latter at 5.0 nM. In addition, DHA's mean IC_{50}

was also low, confirming earlier reports from other parts of Burkina Faso as well as other sub-Saharan African countries (17;33-35). This suggests that the efficacy of both AL and ASAQ should be good as most parasite isolates were sensitive to both components of the two ACT. The prevalence of MQ-resistant isolates was slightly higher than that of MDAQ, indicating that MQAS efficacy in vivo would probably be similar to that of ASAQ. It is important to point out that the prevalence of *P. falciparum* isolates resistant to MQ was higher in other West African countries where ex vivo studies were carried out (27;36).

PQ had a higher mean IC_{50} than the other partner drugs in currently available ACT, namely MDAQ, MQ and LM. Nevertheless, the upper value of its range was lower and the PQ IC_{50} in CQ-R and CQ-S isolates was not significantly different, indicating that although belonging to the same class as CQ and AQ, PQ may be much more efficacious. Therefore, the combination DHAPQ, although not recommended yet for the treatment of malaria in pregnancy, may be the most promising among currently available ACT. In addition, given PQ's long elimination half-life, it would have the advantage of providing a long post-treatment prophylaxis period in which the patient could be protected from emerging infections.

QN, together with CQ, had one of the highest mean IC_{50}. Oral QN was recommended until recently (2014) in Burkina Faso for the management of uncomplicated malaria during pregnancy, including during the first trimester (37), and it is used as rescue treatment in case of ACT failure. Although just a few isolates were above the resistance threshold, QN IC_{50} was strongly associated to that of CQ and was much higher in CQ-R than CQ sensitive (CQ-S) isolates, indicating some cross-resistance. A similar association was observed in a previous ex vivo study carried out in Burkina Faso in an urban area, although the mean IC_{50} was higher, possibly indicating a higher drug selective pressure (17). The relative good efficacy of most of the drugs tested in our study is encouraging, as these drugs will rapidly clear the circulating parasites. This is particularly important as peripheral infection confers a five-fold increased

risk of placental malaria (38) and can therefore have a markedly negative impact on mothers and babies (39;40).

5.6 Conclusion

P. falciparum parasites isolated from pregnant women show a drug sensitivity profile comparable to that recently reported from Bobo Dioulasso, Burkina Faso (17). The mean IC_{50} values and the prevalence of isolates resistant to drugs for which the threshold is known were generally lower in Nanoro than in Bobo Dioulasso, possibly due to the lower drug pressure in this rural area. Indeed, it is not possible to ascribe the observed differences to the type of patients from which the isolates were collected, namely pregnant women in Nanoro and children in Bobo Dioulasso. Ex vivo results indicate that all currently available ACT would probably have good efficacy among pregnant women with malaria. Nonetheless, the therapeutic response is not only dependent on the parasite susceptibility but also on the dose given, the drug disposition and metabolism. Pregnancy is associated with physiological changes that may alter the pharmacokinetic of treatments administered and hence influence the therapeutic response (41). In vivo treatment efficacy will be provided by the trial in which this ex vivo study is nested. The drug sensitivity profile of the parasite population circulating in Nanoro represents the baseline on which to monitor the evolution of the drug sensitivity profile of the local parasite population.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

The study was conceived by UDA and this paper drafted by MCT and UDA. It was conducted by MCT and HT with substantial contributions from SY, IV and MT. Data analysis was conducted by MCT, and supervised by AK and AE. AE, CVO, AR, JBO, RTG, JPVG, HT,

and UDA participated in the overall running of the study, contributed to the interpretation of data, and gave critical review of the final draft. All authors read and approved the final version.

Acknowledgements

We are grateful to all the women who participated in this study. We thank the entire medical laboratory and administrative staff of the Clinical Research Unit of Nanoro, the medical staff at the CSPSs of Nanoro and Nazoanga for their valuable contribution.

We would particularly like to thank Prof Harald Noedl for his tremendous help, Dr Petra Mens and Dr Henk Schallig for the training received at KIT, Amsterdam and Dr Chansuda Wongsrichanalai for his advice.

This publication is supported by the Malaria in Pregnancy (MiP) Consortium, which is funded through a grant from EDCTP and co-funding from Bill & Melinda Gates Foundation (BMGF).

5.7 References

1. WHO. Guidelines for the treatment of malaria. 2010;2nd ed, 13-47.
2. Hien TT, White NJ. Qinghaosu. Lancet 1993;341:603-08.
3. White NJ. Qinghaosu (artemisinin): the price of success. Science 2008;320:330-34.
4. Ashley EA, White NJ. Artemisinin-based combinations. Curr Opin Infect Dis. 2005; 18:531-36.
5. Nosten F, White NJ. Artemisinin-based combination treatment of falciparum malaria. Am J Trop Med Hyg. 2007;77:181-92.
6. White NJ. The role of anti-malarial drugs in eliminating malaria. Malar J. 2008;7:S8
7. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, et al. Artemisinin resistance in P. falciparum malaria. N Engl J Med. 2009;361:455-67.
8. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, et al. Evidence of artemisinin-

resistant malaria in western Cambodia. N Engl J Med. 2008;359:2619-20.

9. Noedl H, Socheat D, Satimai W. Artemisinin-resistant malaria in Asia. N Engl J Med. 2009;361:540-41.

10. Wongsrichanalai C, Meshnick SR. Declining artesunate-mefloquine efficacy against falciparum malaria on the Cambodia-Thailand border. Emerg Infect Dis. 2008; 14:716-19.

11. WHO. Malaria status report on artemisinin resistance. 2014.

12. Takala-Harrison S, Jacob CG, Arze C, Cummings MP, Silva JC, et al. Independent Emergence of Artemisinin Resistance Mutations Among P. falciparum in Southeast Asia. J Infect Dis. 2015;211(5):670-79

13. WHO. Containment of Malaria Multi-Drug Resistance on the Cambodia-Thailand Border. Report No.: Report of an Informal Consultation Phnom Penh. 2011.

14. Zongo I, Dorsey G, Rouamba N, Dokomajilar C, Séré Y, Rosenthal PJ, et al. Randomized comparison of amodiaquine plus sulfadoxine-pyrimethamine, artemether-lumefantrine, and dihydroartemisinin-piperaquine for the treatment of uncomplicated P. falciparum malaria in Burkina Faso. Clin Infect Dis. 2007;45:1453-61.

15. Somé AF, Séré YY, Dokomajilar C, Zongo I, Rouamba N, et al. Selection of known P. falciparum resistance-mediating polymorphisms by artemether-lumefantrine and amodiaquine-sulfadoxine-pyrimethamine but not dihydroartemisinin-piperaquine in Burkina Faso. Antimicrob Agents Chemother. 2010;54:1949-54.

16. Bassat Q, Mulenga M, Tinto H, Piola P, Borrmann S, et al. Dihydroartemisinin-piperaquine and artemether-lumefantrine for treating uncomplicated malaria in African children: a randomised, non-inferiority trial. PLoS ONE. 2009;4:e7871.

17. Tinto H, Bonkian LN, Nana LA, Yerbanga I, Lingani M, et al. Ex vivo anti-malarial drugs sensitivity profile of P. falciparum field isolates from Burkina Faso five years after the national policy change. Malar J. 2014;13:207.

18. Bardají A, Sigauque B, Bruni L, Romagosa C, Sanz S, et al. Clinical malaria in African pregnant women. Malar J. 2008;7:27.

19. Tahita MC, Tinto H, Menten J,Ouedraogo JB, Guiguemde RT, et al. Clinical signs and symptoms cannot reliably predict P. falciparum malaria infection in pregnant women living in an area of high seasonal transmission. Malar J. 2013;12:464.

20. Nunes MC, Scherf AP. falciparum during pregnancy: a puzzling parasite tissue adhesion

tropism. Parasitology. 2007;134:1863-69.

21. Fried M, Duffy PE. Adherence of P. falciparum to chondroitin sulfate A in the human placenta. Science. 1996,272:1502-04.

22. Pfeiffer K, Some F, Müller O, Sie A, Kouyaté B, et al. Clinical diagnosis of malaria and the risk of chloroquine self-medication in rural health centres in Burkina Faso. Trop Med Int Health. 2008;13:418-26.

23. Nambozi M, Mulenga M, Tinto H, Tagbor H, Mwapasa V, et al. Safe and efficacious artemisinin-based combination treatments for African pregnant women with malaria: a multicentre randomized control trial. Reproductive Health. 2015;12:5.

24. Noedl H, Krudsood S, Leowattana W, Tangpukdee N, Thanachartwet W, et al. In vitro antimalarial activity of azithromycin, artesunate, and quinine in combination and correlation with clinical outcome. Antimicrob Agents Chemother. 2007;51:651-56.

25. ICEstimator software: http://www.antimalarial-icestimator.net, Version 1.

26. Ringwald P, Bickii J, Basco KL. In vitro activity of dihydroartemisinin against clinical isolates of P. falciparum in Yaounde, Cameroon. Am J TropMed Hyg. 1999,61:187-92.

27. Fall B, Diawara S, Sow K, Baret E, Diatta B, et al. Ex vivo susceptibility of P. falciparum isolates from Dakar, Senegal, to seven standard anti-malarial drugs. Malar J. 2011,10:310.

28. Basco LK, LeBras J. In vitro susceptibility of Cambodian isolates of P. falciparum to halofantrine, pyronaridine and artemisinin derivatives. Ann Trop Med Parasitol. 1994; 88:131-6.

29. Laufer MK, Thesing PC, Eddington ND, Masonga R, Dzinjalamala FK, et al. Return of Chloroquine Antimalarial Efficacy in Malawi. N Engl J Med. 2006;355:1959-66.

30. Kublin JG, Cortese JF, Njunju EM, Mukadam RAG, Wirima JJ, et al. Reemergence of chloroquine-sensitive P. falciparum malaria after cessation of chloroquine use in Malawi. J Infect Dis. 2003;187:1870-75.

31. Parzy D, Doerig C, Pradines B, Rico A, Fusai T, et al. Proguanil resistance in P. falciparum African isolates: assessment by mutation-specific polymerase chain reaction and in vitro susceptibility testing. Am J Trop Med Hyg. 1997;57:646-50.

32. Frosch AEP, Venkatesan M, Laufer MK. Patterns of chloroquine use and resistance in sub-Saharan Africa: a systematic review of household survey and molecular data. Malar J. 2011;10:116.

33. Basco LK and Ringwald P. Molecular epidemiology of malaria in Cameroun. XXIV. Trends of in vitro antimalarial drug responses in Yaounde, Cameroon. Am J Trop Med Hyg. 2007;76:20-26.

34. Mwai L, Kiara SM, Abdirahman A, Pole L, Rippert A, et al. In vitro activities of piperaquine, lumefantrine, and dihydroartemisinin in Kenyan P. falciparum isolates and polymorphisms in pfcrt and pfmdr1. Antimicrob Agents Chemother. 2009; 53:5069-73.

35. Pascual A, Fall B, Wurtz N, Fall M, Camara C, et al. In vitro susceptibility to quinine and microsatellite variations of the P. falciparum Na+/H+ exchanger transporter (Pfnhe-1) gene in 393 isolates from Dakar, Senegal. Malar J. 2013;12:189.

36. Quashie NB, Duah NO, Abuaku B, Quaye L, Ayanful-Torgby R, et al. A SYBR Green 1-based in vitro test of susceptibility of Ghanaian P. falciparum clinical isolates to a panel of anti-malarial drugs. Malar J. 2013;12:450.

37. Gansané A, Nébié I, Soulama I, Tiono A, Diarra A, et al. Change of antimalarial first-line treatment in Burkina Faso in 2005. Bull Soc Pathol Exot. 2009;102:31–35.

38. Steketee RW. Malaria prevention in pregnancy: the effects of treatment and chemoprophylaxis on placental malaria infection, low birth weight, and fetal, infant, and child survival. Mangochi Malaria Research Project: United States Agency for International Development. 1996.

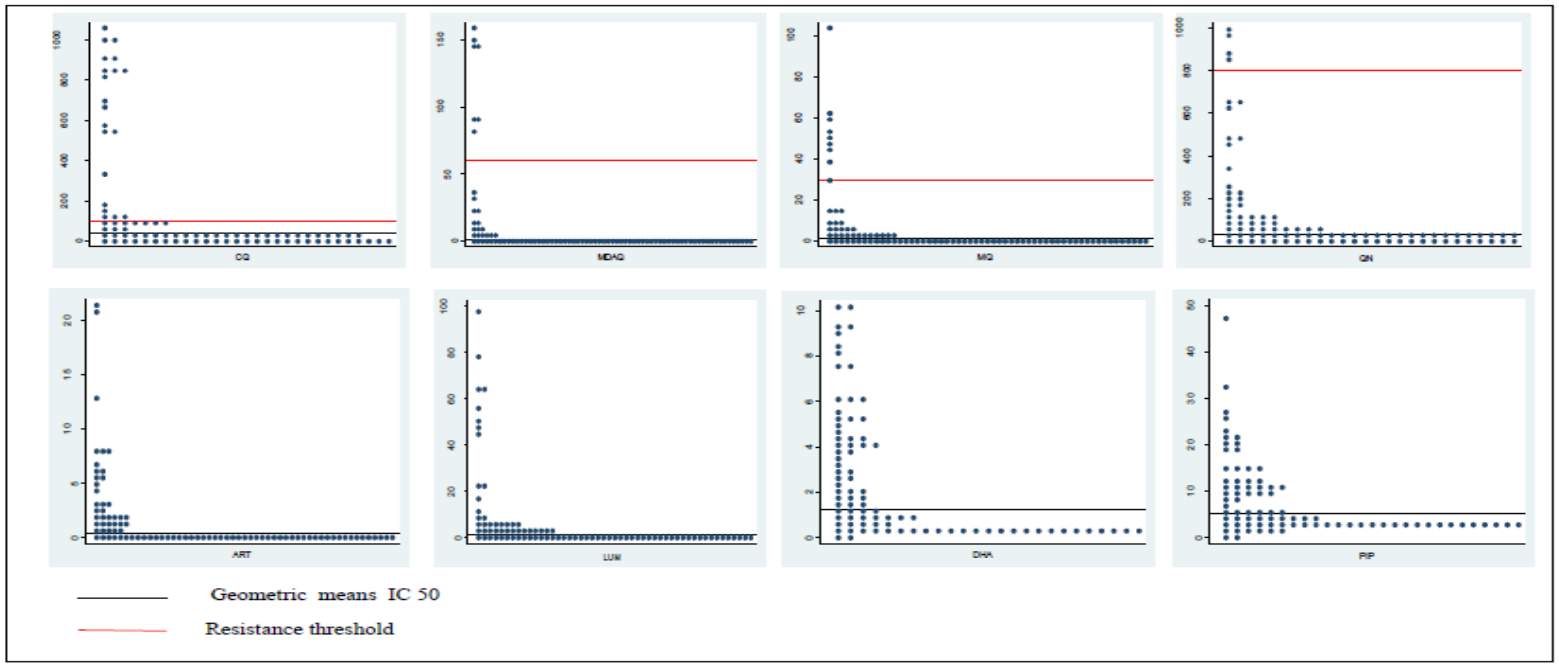
39. Brabin BJ, Romagosa C, Abdelgalil S, Menendez C, Verhoeff FH, et al. The sick placenta-the role of malaria. Placenta. 2004;25:359-78.

40. Dellicour S, Tatem AJ, Guerra CA, Snow RW, ter Kuile FO. Quantifying the number of pregnancies at risk of malaria in 2007: a demographic study. PLoS Med. 2010; 7:e1000221.

41. Ward SA, Sevene EJP, Hastings IM, Nosten F, McGready R. Antimalarial drugs and pregnancy: safety, pharmacokinetics, and pharmacovigilance. Lancet Infect Dis. 2007; 7:136-44.

Additional file

Figure S1 : Scatter of the IC50 values with geometric mean and the resistance cutoff (where available) of each drug



In order to better visualize and understand the data, a figure showing the scatter of the IC50 values with geometric mean and the resistance cutoff (where available) of each drug is proposed.

Chapter 6: The safety and efficacy of four artemisinin-based combination treatments in African pregnant women with malaria

The Pregnancy and Artemisinin-based Combination Treatments (PREGACT) study group. Four Artemisinin-Based Treatments in African Pregnant Women with Malaria. *N Engl J Med* 2016;374:913-27.

6.1 Abstract

Background

There is limited information on the safety and efficacy of artemisinin-combination treatments in pregnancy, particularly in sub-Saharan Africa.

Methods

A non-inferiority multicenter, randomized, open-label trial on the treatment of malaria in pregnancy was carried out in four African countries. A total of 3,428 pregnant women in the second and third trimester with falciparum malaria (any density and regardless of symptoms) were treated with either artemether-lumefantrine (AL), amodiaquine-artesunate (ASAQ), mefloquine-artesunate (MQAS) or dihydroartemisinin-piperaquine (DHAPQ). The primary endpoints of the study were the PCR-adjusted cure rates at day 63 and the safety outcomes.

Results

The PCR adjusted cure rates were 94.8% for AL, 98.5% for ASAQ, 99.2% for DHAPQ and 96.8% for MQAS. There was no significant difference between ASAQ, DHAPQ and MQAS. The cure rate for AL was significantly lower, although the difference was within the 5% non-inferiority margin. The unadjusted cure rates were significantly lower for AL (52.5%) than for ASAQ (82.3%), DHAPQ (86.9%) and MQAS (73.8%). No significant difference in serious adverse events and birth outcomes was found between treatment arms. Drug-related adverse

events such as asthenia, poor appetite, dizziness, nausea, and vomiting were significantly more frequent in the MQAS (50.6%) and ASAQ (48.5%) than in the DHAPQ (20.6%) and AL (11.5%) arms (P<0.001).

Conclusions

AL had the best tolerability profile, acceptable cure rates but the shortest post-treatment prophylaxis. Based on safety and efficacy, DHAPQ seems the most suitable treatment for uncomplicated malaria in pregnancy.

6.2 Introduction

Malaria in pregnancy is a major public health problem in endemic countries (1). Where transmission is stable, most malaria infections during pregnancy remain asymptomatic but increase the risk of maternal anemia and low birth weight, the latter associated with increased infant mortality (2). In areas with unstable malaria transmission, pregnant women can develop symptomatic malaria and severe disease, with an increased risk of fetal loss and maternal death (2). Considering its harmful effects during pregnancy, it is extremely important to adequately treat malaria with efficacious medicines. However, often little information is available of the pharmacokinetics, safety and efficacy of new antimalarials in pregnancy (3-5) as pregnant women are systematically excluded from regulatory trials because of concerns of harming the unborn baby.

In the second and third trimester of pregnancy, WHO guidelines recommend a 3-day course with artemisinin-based combination therapy (ACT) known to be effective in the country/region, or clindamycin with either 7 days of artesunate or quinine (6). Though the experience on the use of ACTs in pregnancy is increasing (6), this information is still limited, particularly in sub-Saharan Africa (sSA).

6.3 Material and methods

The trial was conducted as a randomized, open-label study between June 2010 and August 2013 in seven sites in four SSA countries, namely Burkina Faso, Ghana, Malawi and Zambia. The study protocol has been described in detail elsewhere (7). Briefly, pregnant women in the second and third trimester with a *P. falciparum* mono-infection of any density, regardless of symptoms, an Hb≥7g/dl and without other serious illnesses were recruited into the trial and randomized to one of the following treatments: artemether-lumefantrine (AL), amodiaquine-artesunate (ASAQ), mefloquine-artesunate (MQAS) and dihydroartemisinin-piperaquine (DHAPQ). The trial was set up in a pragmatic approach with 3 arms per country using a balanced incomplete block design (Table 1). All doses of the study drugs were given under direct observation at day 0, 1 and 2, and according to manufacturer's recommendations (Table 2). At recruitment, gestational age was estimated by symphysio-fundal height and the fetal viability assessed by pocket ultrasonography. After completing the 3-day treatment, patients were asked to return to the clinic (visited at home in Ghana) for follow-up visits on day 3, 7 and then once every week until day 63. At each visit, a medical history, and current signs and symptoms were collected, including information on any adverse events (AE), a blood sample taken for malaria smears and dried blood spots for later genotyping, for full blood counts (days 7, 14, 28 and 63 only) and for total bilirubin, ALAT and creatinine (days 7 and 14 only). Rescue treatment for recurrent infections was given according to local national guidelines. At the end of the active follow-up period, women were asked to attend the antenatal clinic monthly or when they felt unhealthy until delivery. The new-born was examined for congenital malformations, weighed and the gestational age estimated by the total Ballard score (8).

Giemsa-stained thick and thin blood films were read independently by two readers, followed by a third reader in case of discrepancy. Parasite density was estimated by counting the number of asexual parasites per 200 white blood cells (WBCs) assuming a WBC counts of 8000/µl. Total bilirubin, ALAT and creatinine were measured using Flexor Junior biochemistry analyzer. Full blood count was obtained using the Sysmex XT-2000i haematology analyzer. Haemoglobin was measured using Haemocue (Angelholm, Sweden). For PCR analysis, blood samples were collected on filter paper (Whatman 3MM) that were subsequently transported to the Institute of Tropical Medicine, Antwerp, Belgium, where centralized genotyping (GluRP, MSP2 and MSP1) was conducted (9). Samples that failed to produce a result were classified as indeterminate.

The primary endpoints of the study were the PCR-adjusted cure rates at day 63 and the safety outcomes (7) (Supplemental Table S1). AEs and serious adverse events (SAEs) were recorded and monitored throughout the study by an independent Data and Safety Monitoring Board (DSMB). The relationship between treatment and AE/SAE was done by the local investigator on the basis of clinical judgment, existence of alternative causes, e.g. concomitant therapy, time of occurrence relative to and available information on the study treatment. The DSMB reviewed SAE listings regularly. Secondary endpoints were PCR-unadjusted cure rates at day 63, time to treatment failure (PCR adjusted and unadjusted), asexual parasite clearance (10), gametocytemia (prevalence and density) and hemoglobin changes during follow-up. The study was designed to show that all 4 treatments have similar (PCR adjusted) cure rates (within 5%), with 95% power for each of the 6 pair-wise comparisons and 80% power for the combined hypothesis that all treatments were non-inferior (7).

Data were captured into an electronic CRF developed in MACRO (Infermed©). A statistical analysis plan was produced before the database lock. For the primary outcome, three analysis populations were used: (1) per-protocol (PP), (2) intention-to-treat (ITT) that excluded

LTFU/withdrawals and missing/indeterminate PCR results, and (3) ITT with multiple imputations of LTFU/withdrawals and missing/indeterminate PCR results. The PP analysis was considered as primary analysis approach. Major protocol violators, defined as violation of the inclusion or exclusion criteria, having received a treatment different to the randomly allocated one, missing at least a full day of treatment, intake of other drugs with anti-malarial activity and missing day 63 slides, were excluded from the PP analysis. All secondary outcomes were analyzed using an available data approach.

The primary hypothesis was tested by calculating the 95% confidence interval for the difference in cure rates. If the difference in true (PCR adjusted) cure rates was less than 5%, treatments were considered therapeutically non-inferior. The confidence interval was calculated from a generalized linear model adjusting for differences between the 4 countries. A number of sensitivity analyses were performed and included: (1) multiple imputation of missing outcomes, (2) pairwise comparison limited to study sites where head-to-head comparison of treatments was done, (3) adjusting the analysis for parasite density, gestational age and gravidity at study entry, (4) analysis of time to treatment failure using Cox-regression models. For safety, all individuals having received at least one treatment dose were included. Details for subgroup analyses can be found in Supplemental Material.

Authors' contributions are listed in Supplemental Table S2. The study was approved by the Ethical Committee of the Antwerp University Hospital, by the relevant national/local ethics committee and by the national drug regulatory authority. The trial was registered at clinicaltrials.gov (NCT00852423).

6.4 Results

A total of 3,428 pregnant women with *P. falciparum* infection were randomized: 881 to AL, 843 to ASAQ, 855 to DHAPQ and 849 to MQAS (Figure 1). The study sites in Burkina Faso

recruited 870 women, in Ghana 788, in Malawi 870 and in Zambia 900. Five women were withdrawn immediately after starting the treatment due to protocol deviations (three did not have malaria, one was not pregnant and one had been enrolled previously) though they were included in the safety analysis. The ITT analysis included 3,150 women (830 in the AL, 791 in the ASAQ, 759 in the DHAPQ and 770 in the MQAS arms). The PP analysis included 3,000 women (810 in the AL, 742 in the ASAQ, 720 in the DHAPQ and 728 in the MQAS arms) (Figure 1). About half of the exclusions from the PP analysis (53.6%, 227/423) were due to loss to follow-up, withdrawal or death.

Baseline characteristics were comparable between treatment arms (Table 3). Most women were included in the second trimester of pregnancy and primigravidae represented about half of the study population. Parasite density was >2,000/µl in about a third of women, and only few of them (6.1%) had fever at time of recruitment. Use of malaria preventive measures was low at recruitment (Table 3).

By PP analysis, the overall day 63 PCR adjusted cure rate was 94.8% (748/789)(95% CI: 93.0-96.1) for AL, 98.5% (718/729)(95%CI: 97.3-99.2) for ASAQ, 99.2% (701/707)(95%CI 98.2-99.6) for DHAPQ and 96.8% (693/716)(95%CI: 95.2-97.9) for MQAS (Table 4 and Supplemental Figure S1). There was no significant difference between ASAQ, DHAPQ and MQAS. The cure rate for AL was significantly lower ($p<0.001$), although the difference was within the non-inferiority margin of 5% (Figure 2). The unadjusted cure rates were significantly lower ($p<0.001$) for AL (52.5%, 425/810) than for ASAQ (82.3%, 611/742), DHAPQ (86.9%, 626/720) and MQAS (73.8%, 537/728) (Table 4). Country specific results are in Supplemental Table S3. The ITT analyses and adjustment with covariates further supported the efficacy results. Results from the primary and sensitivity analyses were generally consistent.

At day two after initiation of treatment, nearly all women (>99.5%) had a negative blood slide. However, parasite clearance was slower in those treated with AL as at day one after start of treatment 24.8% (217/875) still had detectable parasitaemia as compared to 6.9% (57/828) for ASAQ, 8.0% (67/837) for DHAPQ , and 13.5% (113/837) for MQAS (P<0.001). Gametocyte prevalence at enrolment was low (Table 3), with a median density between 11 and 40/µl. Gametocyte carriage remained low throughout the follow-up with no difference between study arms. Similarly, haemoglobin changes throughout the follow-up did not differ between treatment arms (Supplemental Figure S2).

Placental malaria infection was similar between the treatment groups (p=0.47). Mean birth weight of the babies, after adjusting by country, was similar between treatment groups [AL: 2854gr (SD 449); ASAQ: 2880gr (SD 452); DHAPQ: 2901gr (SD: 454); MQAS: 2875gr (SD 433), p=0.40)]. Similarly, the proportion of babies with low birth weight did not vary significantly between treatment groups (AL: 17.2%, ASAQ: 15.5%, DHAPQ : 14.1%, MQAS: 15.2%) (p=0.32).

A total of 72 SAEs occurred during the 63-day follow-up, including one death in the MQAS arm, approximately one month after treatment and probably due to meningitis. There were 10 treatment-related SAEs, 5 in the ASAQ arm (2 with anaemia, one with upper abdominal pain and 2 with malaise), 4 in the MQAS arm (one with abdominal pain, 2 with vomiting and one with malaise), and 1 in the DHAPQ arm (a possible adverse drug reaction with headache and general weakness 2 days after the completion of treatment who recovered completely). No significant difference in the occurrence of SAEs was found between treatment arms.

Women treated with MQAS (84.9%, 722/850) or ASAQ (79.0%, 665/842) had a significantly higher incidence of any AE than those in the AL (72.8%, 641/881) and DHAPQ (70.4%, 602/855) arms (P<0.001) (Table 5). Drug-related AEs were significantly more frequent in the MQAS (50.6%, 430/850) and ASAQ (48.5%, 408/842) arms than in the DHAPQ (20.6%,

176/855) and AL (11.5%, 101/881) arms (P<0.001). This was mainly due to the higher occurrence of asthenia, poor appetite, dizziness, nausea, and vomiting among women treated with MQAS or ASAQ (Table 5). Behavioral changes were observed in four women, two (at day 2 and 3 post- treatment) in the ASAQ, one in the MQAS (at day 2 post-treatment) and one in the AL group (day 60 post-treatment); two in the ASAQ group were considered as possibly related to treatment. All of them recovered completely. A woman treated with ASAQ complained of hallucinations at day 3 post-treatment, possibly related to treatment, but she recovered completely. Women treated with ASAQ complained more significantly of insomnia (4.0%, 34/842) than those treated with MQAS (2.5%, 21/850), DHAPQ (1.6%, 14/855) and AL (0.3%, 3/881) (P=0.04). Pulse rate and blood pressure tended to be lower in women treated with ASAQ (Supplemental FigureS3 and S4). Women with a diastolic blood pressure <50mmHg and a systolic blood pressure <90mmHg were more frequent in the ASAQ group (P<0.001). Similarly, the percentage of women with a pulse rate <60BPM appeared to be higher in the ASAQ group, but this difference was not statistically significant (P=0.4). Hypotension/low diastolic blood pressure as an AE was more frequent in the ASAQ group (1.5%) than in the other treatment groups (0.6-0.8%). There were no differences in the laboratory safety values between treatment arms.

There were 13 miscarriages (1 in the AL arm and 4 in each of the other groups) and 78 stillbirths [16 (1.9%) for AL, 17 (2.1%) for ASAQ, 22 (2.7%) for DHAPQ, and 23 (2.8%) for MQAS. The proportion of live births, was not significantly different between treatment groups (p=0.85). The proportion of preterm babies as determined by the total Ballard score was 10.2% in the Al arm, 3.4% in the ASAQ arm, 9.5% in the DHAPQ and 7.7% in the MQAS arm (P=0.64). Forty four congenital malformations were observed, 17 (2.0%) for AL, 8 (1%) for ASAQ, 6 (0.8%) for DHAPQ and 13 (1.7%) for MQAS.

6.5 Discussion

PCR-adjusted cure rates were high (94.8% to 99.2%) for all four ACTs and differences between them were within the pre-defined non-inferiority margin of 5%. The high success rates are remarkable given the long follow-up period, i.e. 3 weeks longer than the 6 weeks recommended by WHO. Nevertheless, AL cure rates were significantly lower than those for the other three ACTs, which had similar high efficacy, with very few true failures detected during the follow-up. In a previous trial in Uganda, AL efficacy (until day 42) during pregnancy was 99.3% (11). The longer follow-up until day 63 in our study cannot explain the lower AL cure rates as most failures occurred between day 28 and day 42 (Supplemental Figure S1). AL efficacy was low (82%) in pregnant women at the Thai-Burmese border (12), a finding attributed to low drug concentrations and low antimalarial immunity (13, 14). Indeed, in Uganda, lumefantrine plasma concentration was 27% lower in pregnant than in non-pregnant women (15), suggesting that the high AL efficacy was probably due to the higher background immunity. In our trial, AL was tested in 3 countries with intense malaria transmission so that background immunity among recruited pregnant women was probably high.

Patients treated with AL had the highest reinfection rates and the shortest time to reinfection, while non-inferiority could not be shown on pairwise comparisons for the other treatments. The duration of post-treatment prophylaxis is an important factor in the choice of antimalarial drugs, especially in high transmission areas. Lumefantrine has the shortest elimination half-life (16), followed by mefloquine (17), amodiaquine (18), and then piperaquine (19).

AL efficacy was relatively low in Burkina Faso (93.2%). This was the same study site where AL efficacy in children with malaria was the lowest (90.2%) compared to the other sSA study sites (20). For the current trial, the Burkinabe site had also the highest intensity of

transmission as suggested by high rates of reinfection observed in all study arms (Supplemental Table S3). This may influence the genotyping, with the risk of misclassifying new infections as recrudescences (21). In our trial, capillary electrophoresis, a technique that can minimize misclassification (22), was used for MSP2. In addition, transmission intensity may influence the individual treatment cure rates but not the risk difference between treatments (22).

There was no difference in birth outcomes between treatment arms; mean birth weight as well as proportion of miscarriages, stillbirths, preterm deliveries and congenital malformations were similar.

AL and DHAPQ were well tolerated (14, 19, 23). About half of the AEs in the ASAQ and MQAS groups were considered treatment-related. Asthenia was the most common in the ASAQ group, followed by dizziness, both possibly related to low blood pressure and pulse rate. Nausea and vomiting were also relatively common. General weakness, vomiting, dizziness, and nausea were the most commonly reported AEs in Ghanaian pregnant women treated with AQ (24, 25). Dizziness was the most frequent treatment-related AE in the MQAS group, followed by vomiting, nausea and asthenia. The association between MQ and dizziness has already been reported; more than 30% of pregnant women treated with 15 mg/kg MQ monotherapy complained of dizziness whose occurrence decreased after subsequent doses (26). As this was an open-label trial, the AE causal allocation may have been influenced by the knowledge of the treatment given (27).

Three women had behavior change within the first few days after onset of treatment, 1 in the MQAS arm and 2 in the ASAQ arm. MQ use has been associated with neuropsychiatric AE (28), a phenomenon described also for 4-aminoquinolines (6). In addition, insomnia occurred more frequently in the ASAQ and MQAS groups than with AL.

In conclusion, AL had the best tolerability profile and acceptable cure rates but provided the shortest post-treatment prophylaxis. Considering the higher occurrence of AEs in both the ASAQ and MQAS groups, DHAPQ seems the most suitable treatment for uncomplicated malaria in pregnancy as its PCR-adjusted cure rate was close to 100% and it prevented new infections for about 35 days. In terms of pregnancy outcomes, no difference was found between study arms. ASAQ, and possibly MQAS, which were not as well tolerated, but highly effective, may be good options for women with treatment failure after AL or DHAPQ.

All members of the **PREGACT study group** (corresponding author Umberto D'Alessandro, udalessandro@mrc.gm) meet ICMJE criteria as authors, and are: by affiliation (affiliations in alphabetical order): **Clinical Trial Unit Nanoro, Burkina Faso**: Halidou Tinto, Maminata Traoré/Coulibaly, Marc C Tahita, Innocent Valéa; **College of Medicine, Blantyre, Malawi**: Victor Mwapasa, Linda Kalilani-Phiri, Gertrude Kalanda, Mwayiwawo Madanitsa; **Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Belgium/Institute of Tropical Medicine, Antwerp, Belgium**: Raffaella Ravinetto; **Kwame Nkrumah University of Science and Technology, Kumasi, Ghana**: Harry Tagbor, Gifty Antwi, Divine Pekyi, Akua Agyemang Ampromfi, Prosper Gbekor; **Hubert Kairuki Memorial University, Dar es Salaam, Tanzania**: Theonest Mutabingwa; **Institute of Tropical Medicine, Antwerp, Belgium**: Joris Menten, , Maaike De Crop, Yves Claeys, Celine Schurmans, Chantal Van Overmeir; **Institute of Tropical Medicine, Antwerp, Belgium/Menzies School of Health Research, Darwin, Australia:** Kamala Thriemer; **International Health Unit, University of Antwerp**: Jean-Pierre Van geertruyden; **Medical Research Council Unit, The Gambia/ London School of Hygiene and Tropical Medicine/Institute of Tropical Medicine, Antwerp, Belgium**: Umberto D'Alessandro;

Tropical Diseases Research Centre, Ndola, Zambia: Michael Nambozi, Modest Mulenga, Sebastian Hachizovu, Jean-Bertin Bukasa Kabuya, Joyce Mulenga.

6.6 Acknowledgements

This study was part of the research activities of the Malaria in Pregnancy Consortium. It was funded by the European Developing Countries Clinical Trials Partnership (EDCTP) and by the Malaria in Pregnancy Consortium, which is funded through a grant from the Bill and Melinda Gates Foundation to the Liverpool School of Tropical Medicine. In addition, it benefited of the support of the following institutions: Belgian Development Cooperation Agency, Liverpool School of Tropical Medicine, Medical Research Council UK, Netherlands Organisation for Scientific Research (NWO). Thanks to the members of the Data Safety and Monitoring Board: Prof Michael Boele van Hensbroek (chairman), Prof Emmanuel Lesaffre, and Drs, Kalifa Bojang and Robert Busingye. We would also like to thank the following people at the Institute of Tropical Medicine, Antwerp, for their important contribution to the success of this trial: Diana Arango, Christophe Burm, Jozefien Buyze, Dr Annette Erhart, Greta Gondol, Pieter Guetens, Tom Koyen, Evi Pockele, Harry van Loen, Danielle Van Melle, and Jef Verellen. Special thanks to Prof Bruno Gryseels, the director of the Institute of Tropical Medicine, Antwerp, for his continuous support throughout the study. Finally, we would like to thank the field teams at each of the sites where the trial was implemented, and the pregnant women who participated in this study.

6.7 References

1. Takem EN & D'Alessandro U. Malaria in Pregnancy. *Mediterr J Hematol Infect Dis.* 2012; 4(1):e2013010.

2. White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, et al. Malaria. *Lancet.* 2014;383(9918):723-35.

3. D'Alessandro U. Existing antimalarial agents and malaria-treatment strategies. Expert Opin Pharmacother. 2009;10(8):1291-06.

4. Lutje V, Gerritsen A, Siegfried N. Randomized controlled trials of malaria intervention trials in Africa, 1948 to 2007: a descriptive analysis. *Malar J.* 2011;10:61.

5. Orton LC, Omari AAA. Drugs for treating uncomplicated malaria in pregnant women. Cochrane Database of Systematic Reviews. 2008;4:CD004912.

6. WHO. Guidelines for the treatment of malaria, Third edition. 2015.

7. Nambozi M, Mulenga M, Halidou T, Tagbor H, Mwapasa V, et al. Safe and efficacious artemisinin-based combination treatments for African pregnant women with malaria: a multicentre randomized control trial Reproductive Health. 2015,12:5.

8. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, et al. New Ballard Score, expanded to include extremely premature infants. *J Pediatrics.* 1991;119:417-23.

9. World Health Organization. Methods and techniques for clinical trials on antimalarial drug efficacy: genotyping to identify parasite populations. 2008.

10. WWARN: Clinical Module, 2012. Data Management and Statistical Analysis Plan.

11. Piola P, Nabasumba C, Turyakira E, Dhorda M, Lindegardh N, et al. Efficacy and safety of artemether–lumefantrine compared with quinine in pregnant women with *uncomplicated P. falciparum* malaria: an open-label, randomised, non-inferiority trial. *Lancet Infect Dis.* 2010;10: 762–69.

12. McGready R, White N, Nosten F. Parasitological efficacy of antimalarials in the treatment and prevention of falciparum malaria in pregnancy 1998 to 2009: a systematic review. *BJOG.* 2011;118:123–35.

13. McGready R, Tan SO, Ashley EA, Pimanpanarak M, Viladpai-Nguen J, et al. A randomised controlled trial of artemether-lumefantrine versus artesunate for uncomplicated *P. falciparum* treatment in pregnancy. *PLoS Med.* 2008;5(12):e253.

14. Manyando C, Kayentao K, D'Alessandro, Okafor HU, Juma E, et al. A systematic review of the safety and efficacy of artemether-lumefantrine against uncomplicated *P. falciparum* malaria during pregnancy. Malar J. 2012;11:141.

15. Kloprogge F1, Piola P, Dhorda M, Muwanga S, Turyakira E, et al. Population pharmacokinetics of lumefantrine in pregnant and non-pregnant women with uncomplicated *P. falciparum* malaria in Uganda. *CPT Pharmacometrics Syst Pharmacol* 2013;2:e83.

16. Douglas NM, Anstey NM, Angus BJ, Nosten F, Price RN. Artemisinin combination therapy for vivax malaria. *Lancet Infect Dis.* 2010;10:405–16.

17. Valea I, Tinto H, Traore-Coulibaly M, Toe LC, Lindegardh N et al. Pharmacokinetics of co-formulated mefloquine and artesunate in pregnant and non-pregnant women with uncomplicated *P. falciparum* infection in Burkina Faso. *Antimicrob Chemother*. 2014;69(9):2499-07.

18. Rijken MJ1, McGready R, Jullien V, Tarning J, Lindegardh N, et al. Pharmacokinetics of amodiaquine and desethylamodiaquine in pregnant and postpartum women with *Plasmodium vivax* malaria. *Antimicrob Agents Chemother.* 2011;55: 4338–42.

19. Hoglund RM1, Adam I, Hanpithakpong W, Ashton M, Lindegardh N, et al. A population pharmacokinetic model of piperaquine in pregnant and non-pregnant women with uncomplicated *P. falciparum* malaria in Sudan. *Malar J*. 2012;11:398.

20. Four Artemisinin-Based Combinations (4ABC) Study Group: A head-to-head comparison of four artemisinin-based combinations for treating uncomplicated malaria in African children: a randomized trial. *PLoS Med*. 2011; 8(11):e1001119.

21. Greenhouse B, Dokomajilar C, Hubbard A, Rosenthal PJ, Dorsey G. Impact of transmission intensity on the accuracy of genotyping to distinguish recrudescence from new infection in antimalarial clinical trials. *Antimicrob Agents Chemother*. 2007,51(9):3096-03.

22. Gupta V, Dorsey G, Hubbard AE, Rosenthal PJ, Greenhouse B. Gel versus capillary electrophoresis genotyping for categorizing treatment outcomes in two anti-malarial trials in Uganda. *Malar J.* 2010;15;9:19.

23. Adam I, Tarning J, Lindegardh N, Mahgoub H, McGready R, et al. Pharmacokinetics of piperaquine in pregnant women in Sudan with uncomplicated *P. falciparum* malaria. *Am J Trop Med Hyg.* 2012;87:35–40.

24. Tagbor H, Bruce J, Browne E, Randal A, Greenwood B, et al. Efficacy, safety, and tolerability of amodiaquine plus sulphadoxine-pyrimethamine used alone or in combination for malaria treatment in pregnancy: a randomised trial. *Lancet.* 2006;368:1349–56.

25. Clerk CA1, Bruce J, Affipunguh PK, Mensah N, Hodgson A, et al. A randomized, controlled trial of intermittent preventive treatment with sulfadoxine-pyrimethamine, amodiaquine, or the combination in pregnant women in Ghana. *JID.* 2008;198:1-10.

26. González R, Mombo-Ngoma G, Ouédraogo S, Kakolwa MA, Abdulla S, et al. Intermittent preventive treatment of malaria in pregnancy with mefloquine in HIV-negative women: A Multicentre Randomized Controlled Trial. *PLoS Med.* 2014;11:e1001733.

27. González R, Hellgren U, Greenwood B, Menéndez C. Mefloquine safety and tolerability in pregnancy: a systematic literature review. *Malar J.* 2014;13:75.

28. van Riemsdijk MM, Sturkenboom MC, Ditters JM, Tulen JH, Ligthelm RJ, et al. Low body mass index is associated with an increased risk of neuropsychiatric adverse events and concentration impairment in women on mefloquine. *Br J Clin Pharmacol*. 2004;57(4):506-12.

Tables and figures:

Table 1: Treatment arms per country/ site (planned number of patients)

West Africa: comparator ASAQ			
Burkina (870)	AL (290)	ASAQ (290)	MQAS (290)
Ghana (870)	ASAQ (290)	MQAS (290)	DHAPQ (290)
Eastern-Southern Africa: comparator AL			
Malawi (870)	DHAPQ (290)	AL (290)	ASAQ (290)
Zambia (870)	MQAS (290)	DHAPQ (290)	AL (290)

ASAQ: artesunate-amodiaquine-; DHAPQ: dihydroartemisinin-piperaquine; AL: artemether-lumefantrine; MQAS: mefloquine-artesunate;

Table 2: Study treatments and daily dose

Treatment	Manufacturer	Tablets per day
Eurartesim®: 40mg of dihydroartemisinin and 320mg of piperaquine phosphate per tablet	Sigma-Tau Industrie Farmaceutiche Riunite S.p.A.	3
Mefloquine-artesunate: 100mg artesunate and 220mg mefloquine per tablet	Far-Manguinhos, Ministério da Saúde - Fundação Oswaldo Cruz	3
Artesunate-amodiaquine: 100mg artesunate and 270mg amodiaquine per tablet	Winthrop, Sanofi Aventis	2
Coartem®: 20mg artemether and 120mg lumefantrine per tablet	Novartis Pharma AG	4 x 2*

*with the second dose given 8 hours after the first dose

Table 3: Baseline Characteristics

	AL (N=880)	**ASAQ** (N=842)	**DHAPQ** (N=853)	**MQAS** (N=848)
Country				
Burkina Faso: N	290	291		288
Ghana: N		261	265	260
Malawi: N	290	290	288	
Zambia: N	300		300	300
Age (years): mean (SD)	22.6 (5.6)	23.4 (5.9)	22.3 (5.4)	23.5 (5.9)
Symptomatic Malaria*: %	37.2	34.9	37.4	43.8
Fever (temperature ≥ 37.5°C) : %	6.5	6.8	3.2	8.0
Parasite density > 2000/µL: %	30.6	25.3	29.1	32.1
At least 3 symptoms†: %	7.2	9.3	11.8	14.3
Gametocytes present: %	2.4	2.9	2.5	0.7
Parasite density (/µL): median (IQR)	800 (213, 2880	569 (165, 2025)	680 (200, 2760)	840 (218, 3040)
Haemoglobin (g/dL): median (IQR)	10.2 (9.2,11.0)	10.1 (9.1,11.0)	10.1 (9.1,11.0)	10.1 (9.1,10.9)
Gravidity:				
1st pregnancy: %	53.4	52.9	65.3	49.2
2nd pregnancy: %	15.7	16.5	15.1	18.4
3rd pregnancy or more: %	30.9	30.6	19.6	32.4
Gestational Age:				
2nd trimester: %	71.8	75.0‡	68.5	65.8
3rd timester: %	28.2	24.9	31.5	34.2
Bednet used before study entry: %	34.4	34.6	27.9	37.5
ITN used before study entry¥: %	24.8	23.9	17.1	27.9
IPT use (before day 0): %	9.9	10.9	13.7	16.4

* Symptomatic malaria is defined as any of the following: (i) fever (temperature >37.5˚C) at baseline with parasitaemia (any density), (ii) parasite count > 2,000/µl, regardless of symptoms; (iii) at least 3 or more of the following symptoms: fever in the past 24h, weakness/fatigue; muscle and/or joint aches, headache, convulsion, with parasitaemia of any density.

† Of the following: fever in the past 24h, weakness/fatigue; muscle and/or joint aches, headache.

‡ One woman was included during the first trimester of pregnancy.

¥ Women were provided with ITN at study start.

ITN= Insecticide treated net

IPT= Intermittent preventive treatment

IQR= Inter quartile range

Table 4: Summary of efficacy outcomes and treatment success rates

	AL (N=880)	**ASAQ** (N=842)	**DHAPQ** (N=853)	**MQAS** (N=848)
Summary of Efficacy Outcomes: n (%)				
Early Treatment Failure	0	2 (0.2)	1 (0.1)	0
Development danger signs/severe malaria	0	0	1 (0.1)	0
Rescue treatment day 0-3	0	1 (0.1)	0	0
Parasitaemia day 3 > day 0	0	1 (0.1)	0	0
Late Clinical Failure	26 (3.0)	13 (1.5)	6 (0.7)	28 (3.3)
Recrudescence	4 (0.5)	2 (0.2)	1 (0.1)	8 (0.9)
New Infection	21 (2.4)	10 (1.2)	5 (0.6)	18 (2.1)
Indeterminate/Sample Unavailable	1 (0.1)	1 (0.1)	0	2 (0.2)
Late Parasitological Failure	362 (41.1)	123 (14.6)	91 (10.7)	176 (20.8)
Recrudescence	37 (4.2)	9 (1.1)	5 (0.6)	17 (2.0)
New Infection	303 (34.4)	100 (11.9)	71 (8.3)	144 (17.0)
Indeterminate/Sample Unavailable	22 (2.5)	14 (1.7)	15 (1.8)	15 (1.8)
Adequate Clinical and Parasitological Response	436 (49.6)	642 (76.3)	653 (76.6)	557 (65.7)
Cannot be Determined	56 (6.4)	62 (7.4)	102 (12.0)	87 (10.3)
Rescue treatment and no infection	6 (0.7)	11 (1.3)	8 (0.9)	9 (1.1)
Died: not related to malaria or treatment	0	0	0	1 (0.1)
LTFU/Withdrawn	50 (5.7)	51 (6.1)	94 (11.0)	77 (9.1)
Treatment Success Rates: % (95% Confidence interval)				
PP-Analysis: PCR-adjusted	94.8 (93.0, 96.1)	98.5 (97.3, 99.2)	99.2 (98.2, 99.6)	96.8 (95.2, 97.9)
PP-Analysis: PCR-unadjusted	52.5 (49.0, 55.9)	82.3 (79.4, 84.9)	86.9 (84.3, 89.2)	73.8 (70.4, 76.8)
ITT-Analysis†: PCR-adjusted	92.9 (90.7, 94.6)	96.1 (94.2, 97.4)	97.3 (95.4, 98.4	94.6 (92.2, 96.3)
ITT-Analysis†: PCR-unadjusted	52.6 (49.1, 56.0)	79.5 (76.5, 82.2)	82.1 (76.2, 86.8)	69.8 (65.8, 73.5)

LTFU: Lost to follow-up; PP: per-protocol; ITT: intention-to-treat.
† Intention to treat analysis using multiple imputations of unavailable outcomes.

Table 5: Summary of safety outcomes

	AL (N=881)	**ASAQ** (N=842)	**DHAPQ** (N=855)	**MQAS** (N=850)
Adverse Events, % of patients with:				
Any Serious AE during 63 day follow-up	0.7	2.6	2.2	2.9
• Blood disorders (anaemia, sickle cell, …)	-	0.7	0.3	0.1
• Abdominal pain	-	0.3	-	0.1
• Diarrhoea	0.1	-	-	-
• Vomiting	-	-	-	0.2
• Malaise	-	0.2	-	0.1
• Adverse drug reaction	-	-	0.1	-
• Infections	0.5	1.0	1.1	1.5
• Complications of pregnancy and delivery	0.2	0.6	0.6	0.8
• Asthma	-	-	-	0.1
Any Adverse Event during 63 day follow-up	72.8	79.0	70.4	84.9
Any Adverse Event during first 7 days	24.3	59.5	34.2	60.7
Any Drug-Related Adverse Event	11.5	48.5	20.6	50.6
Specific Drug-Related Adverse Events (occurring in >5% of patients in any treatment arm)				
Abdominal Pain	2.7	7.1	2.1	5.3
Asthenia	1.8	26.6	6.8	14.2
Decreased Appetite	0.3	8.2	2.1	7.7
Dizziness	1.2	23.5	1.6	30.6
Musculoskeletal Pain	0.8	7.2	2.6	4.4
Nausea	0.9	11.5	4.0	13.9
Vomiting	0.9	15.9	5.7	18.9
Vital Signs Abnormalities during Treatment, % of patients with any day (day 1, 2 or 3):				
Pulse Rate < 60 BPM	1.1	2.1	1.6	1.5
Diastolic Blood Pressure < 50 mmHg	8.4	15.1	7.7	4.7
Systolic Blood Pressure < 90 mmHg	5.1	12.2	6.3	4.1

Chapter 7: Key Results and discussion of the Burkina Faso specific dataset

7.1 Results

7.1.1 Baseline characteristics

Out of the 1178 pregnant women screened, 869 fulfilled initial inclusion criteria and were randomized in the 3 treatment groups. The repartition in the different arm was as follow: 290 in the AL group, 291 in the ASAQ group and 288 in the MQAS one. The intention-to-treat analysis (ITT) included 814 pregnant women distributed as follow: 268 in the AL group, 275 in the ASAQ group and 271 in the MQAS group. At enrolment, there was no significant difference between treatment groups with respect to age, haemoglobin level, and parasite density. Most of the pregnant women were recruited during the third trimester of pregnancy and multigravidae represented half of the study group (Table 1).

7.1.2 Treatment efficacy

Efficacy outcomes were assigned on the basis of time to early treatment failure or recurrent parasitaemia, according to Pregact protocol (1). Outcomes were assessed after day-28, day-42 and day-63 considered with and without genotyping to distinguish recrudescence from new infection.

There were no ETFs. Almost 90% of the clinical failures were late parasitological failure. Considering outcomes adjusted by genotyping both in ITT and PPA, ASAQ was superior to both AL and MQAS regimens at day-63 (Table 2). According to per-protocol analysis, the overall PCR-adjusted cure rate at day 63 was 96.7% (93.8-98.2) in ASAQ group, 93.2% (89.5-95.6) in AL group, and 92.5% (88.8-95.1) for MQAS group. Similar differences were seen when the day-63 risk of treatment failure was 8.1% for MQAS, compared with 7.7% for AL and 3.6% for ASAQ.

Risk difference 95% CIs were 1.1%–9.2% for AL *vs* ASAQ, -6.4%-5.5% for AL *vs* MQAS and -9.2%-0.3% for ASAQ *vs* MQAS. Most treatment failures were seen on or after day-27 in AL group, day-35 in the ASAQ and MQAQ group (Figure 2). Almost 50% of the LTFs randomized to AL were classified as reinfections.

7.1.3 Secondary outcomes

Haemoglobin level did not differ significantly among the treatment groups throughout the follow-up period. All participants in both cohorts cleared their infection by day 2 following treatment (Figure 3).

7.1.4 Adverse events

AL was generally associated with fewer adverse events than ASAQ and MQAS. The most frequent adverse events in the ASAQ and MQAS groups were dizziness, followed by nausea and abdominal pain.

7.2 Discussion of Burkina Faso set of data

This study describes the day 63 in vivo efficacy of 3 antimalarial regimens tested in Burkina Faso. Although the therapeutic efficacy remains good (over 90%) giving this long follow up, the combination ASAQ performed better than the other two (AL and MQAS). Our study is in line with previous results that found ASAQ to be more effective compared to AL and MQAS (2–4). We observed higher PCR-uncorrected cure rate for AS-AQ (65.6%) than for AL (43.7%) and MQAS (58.4%). Similar patterns were observed in the same site (5) and in others regions of the country (6–8). This is explained by the longer half-life of AQ (6-18 days) as compared to LUM (3-6 days) and the low level of AQ resistance in West Africa, which maintain the post-treatment prophylactic effect of ASAQ (9–11). In area of high transmission like Burkina Faso, the duration of the length of the post-treatment prophylactic period should

be used to guide the National Malaria Control Program (NMCP) for the choice of first line treatments.

Abuaku et al, recently demonstrated high cure rate of ASAQ and AL in Ghana (12). Owing to the fact that the two studies were conducted in high transmission area, such difference can be explained by the length of the follow up period. In the Ghanaian study, children were followed up for 28 days compared to 63 days in our study. Nevertheless, an effectiveness study conducted in 2014 by Tinto et al (2) in the same site found low cure rate for both ASAQ and AL. This difference could be explained by to the low compliance to the treatment. Indeed in this effectiveness study, medical staff did not directly observe the treatment and thus difference in term of treatment adherence may have happened.

In our study, all pregnant women received an ITN. Therefore, the substantial proportion of women who experience a new infection could be explained by either the low use of ITN and/or by an extremely intense transmission.

The therapeutic efficacy of the two first line treatments of malaria in Burkina, AL and ASAQ remains over 90%. This is encouraging when considering the long follow up in our study (3 weeks more than the recommended). The tree regimens (AL, ASAQ and MQ) were effective in clearing parasites by day-2, with AL associated with few adverse events. Studies like this should be conducted at regular interval in order to monitor the efficacy of the recommended drugs and to timely detect any sign of declining efficacy.

7.3 References

1. Nambozi M, Mulenga M, Halidou T, Tagbor H, Mwapasa V, et al. Safe and efficacious artemisinin-based combination treatments for African pregnant women with malaria: a multicentre randomized control trial. Reprod Health. 2015;12:5.

2. Tinto H, Diallo S, Zongo I, Guiraud I, Valea I, et al. Effectiveness of artesunate-amodiaquine vs. artemether-lumefantrine for the treatment of uncomplicated falciparum malaria in Nanoro, Burkina Faso: a non-inferiority randomised trial. Trop Med Int Health. 2014;19(4):469–75.

3. The Four Artemisinin-Based Combinations Study group. A head-to-head comparison of four artemisinin-based combinations for treating uncomplicated malaria in african children: A randomized trial. PLoS Med. 2011;8(11):e1001119.

4. Owusu-Agyei S, Asante KP, Owusu R, Adjuik M, Amenga-Etego S, et al. An open label, randomised trial of artesunate+amodiaquine, artesunate+chlorproguanil-dapsone and artemether-lumefantrine for the treatment of uncomplicated malaria. PLoS One. 2008;3(6):e2530.

5. Bassat Q, Mulenga M, Tinto H, Piola P, Borrmann S, et al. Dihydroartemisinin-piperaquine and artemether-lumefantrine for treating uncomplicated malaria in African children: A randomised, non-inferiority trial. PLoS One. 2009;4(11).

6. Zongo I, Dorsey G, Rouamba N, Dokomajilar C, Sere Y, et al. Randomized comparison of amodiaquine plus sulfadoxine-pyrimethamine, artemether-lumefantrine, and dihydroartemisinin-piperaquine for the treatment of uncomplicated Plasmodium falciparum malaria in Burkina Faso. Clin Infect Dis. 2007;45(11):1453–61.

7. Zongo I, Dorsey G, Rouamba N, Tinto H, Dokomajilar C, et al. Artemether-lumefantrine versus amodiaquine plus sulfadoxine-pyrimethamine for uncomplicated falciparum malaria in Burkina Faso: a randomised non-inferiority trial. Lancet. 2007;369(9560):491–98.

8. Sirima SB, Tiono AB, Gansané A, Diarra A, Ouédraogo A, et al. The efficacy and safety of a new fixed-dose combination of amodiaquine and artesunate in young African children with acute uncomplicated Plasmodium falciparum. Malar J. 2009;8:48.

9. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, et al. Spread of Artemisinin Resistance in Plasmodium falciparum Malaria. N Engl J Med. 2014;371(5):411–23.

10. Yeka A, Lameyre V, Afizi K, Fredrick M, Lukwago R, et al. Efficacy and safety of fixed-

dose artesunate-amodiaquine vs. artemether-lumefantrine for repeated treatment of uncomplicated malaria in Ugandan children. PLoS One. 2014;9(12):e113311.

11. Yeka A, Kigozi R, Conrad MD, Lugemwa M, Okui P, et al.

12. Artesunate/Amodiaquine Versus Artemether/Lumefantrine for the Treatment of Uncomplicated Malaria in Uganda: A Randomized Trial. J Infect Dis. 2016; 213(7):1134–42.

13. Abuaku B, Duah N, Quaye L, Quashie N, Malm K, et al. Therapeutic efficacy of artesunate-amodiaquine and artemether-lumefantrine combinations in the treatment of uncomplicated malaria in two ecological zones in Ghana. Malar J. 2016;15:6.

Tables and Figures

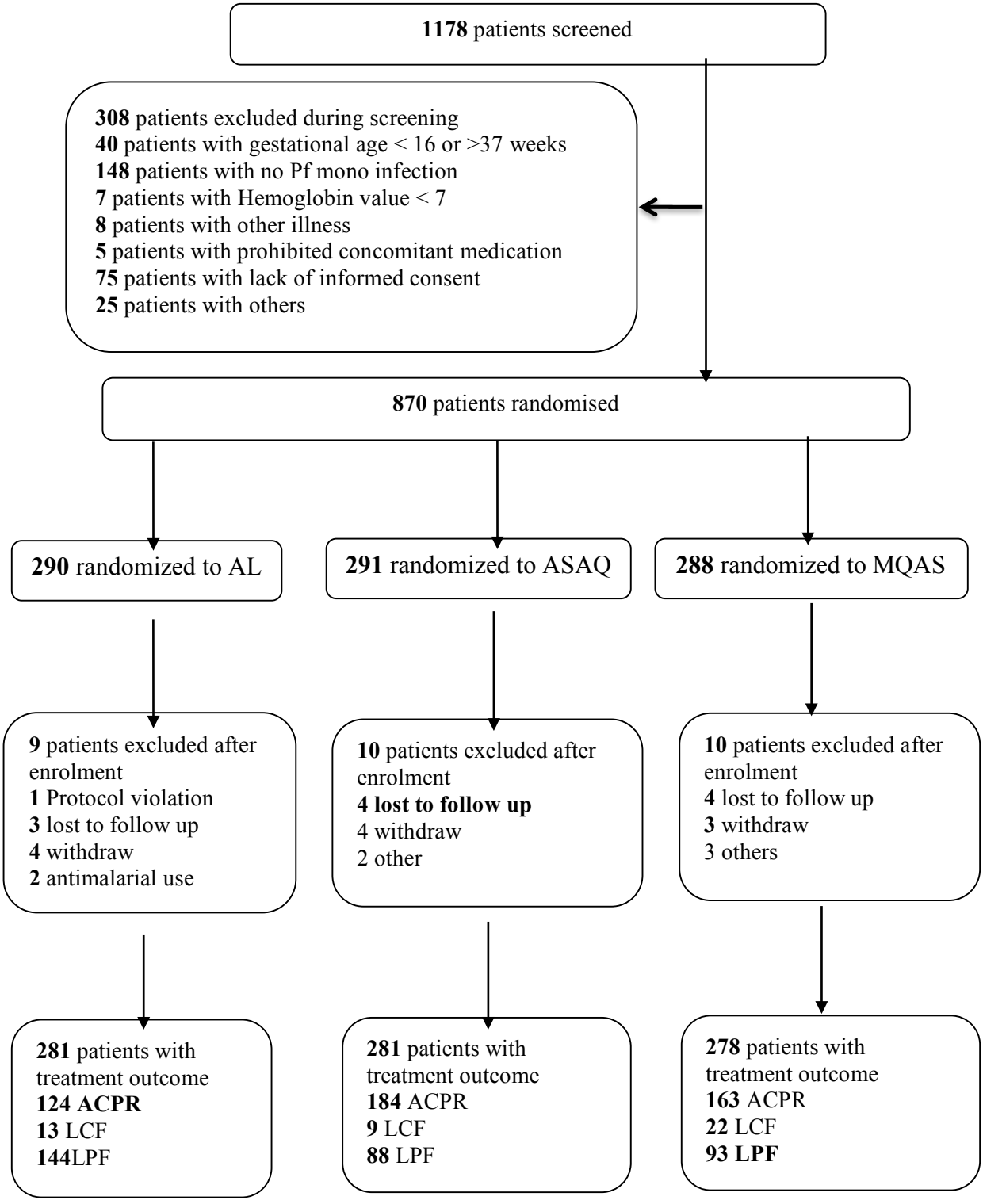


Figure 1 : Trial Profile of Pregact study (Burkina

Table 1: Baseline characteristics

	Artemether-lumefantrine (N= 290)	**Amodiaquine-Artesunate (N=290)**	**Mefloquine-Artesunate (N=288)**
Age	24.74(5.81)	24.01(5.73)	24.32(5.90)
Symptomatic malaria (%)	113(38.97)	117(40.21)	112(38.89)
Fever ≥ 37.5 °C (%)	33(11.38)	25(8.59)	36(12.50)
Parasite density ≥2000/mm^3	86(29.66)	92(31.62)	83(28.82)
≥ 3 symptoms (%)			
Gametocytes carriage (%)	11(3.79)	16(5.50)	3(1.04)
Parasite density (geometric mean)	685.14 (543.13 - 864.27)	667.86 (532.20 - 838.10)	646.50 (515.28 - 811.15)
Hemoglobin (g/dl)	10.20(1.23)	10.07(1.21)	10.06(1.22)
Gravidity (%)			
1	75(25.86)	91(31.38)	82(28.47)
2	53(18.28)	57(19.66)	61(21.18)
≥3	162(55.86)	142(48.97)	145(50.35)
Trimester of gestation			
Second	112(38.62)	122(41.92)	117(40.63)
Third	178(61.38)	169(59.08)	171(59.38)
Bed net used before trial entry (%)	146(50.34)	143(49.14)	148(51.39)
ITNs used before trial entry (%)	121(82.88)	121(84.62)	126(85.14)
Use of IPT before day 0	46(15.86)	27(9.28)	42(14.58)

Table 2: Treatment efficacy outcome

Risk category	Risk of treatment failure, %(95% CI)			Risk difference, % (95%CI)					
	AL Group	ASAQ Group	MQAS Group	AL vs ASAQ	p	AL vs MQAS	p	ASAQ vs MQAS	p
28 Day Risk									
Unadjusted by genotyping	12.01 (8.74 - 16.4)	0.7 (0.18 - 2.78)	1.06 (0.34 - 3.24)	11.31 (7.33 - 15.28)	<0.00001	10.95 (6.91 - 14.98)	<0.0001	-0.36 (-1.90 - 1.18)	0.6467
adjusted by genotyping	2.92 (1.47 - 5.75)	0.36 (0.05 - 2.51)	-	2.56 (0.4 - 4.68)	0.0173	-	-		
42 Day Risk									
Unadjusted by **genotyping**	34.28 (29.07 - 40.12)	12.30 (8.99 - 16.71)	10.66 (7.58 - 14.89)	21.98 (14.44 - 29.51)	<0.00001	23.62 (16.23 - 31)	<0.00001	1.64 (-3.80; 7.08)	0.5551
adjusted by genotyping	6.01 (3.65 - 9.8)	1.08 (0.35 - 3.32)	4.05 (2.26 - 7.19)	4.93 (1.47 - 8.38)	0.0030	1.96 (0.02 - 5.95)	0.3270	-2.97 (-5.66 ; -0.27)	0.0330
63 Day Risk									
Unadjusted by genotyping	55.48 (49.79 - 61.34)	34.19 (29 - 40)	40.99 (35.49 - 46.98)	21.29 (10.73 - 31.84)	0.0001	14.49 (3.57 - 25.40)	0.0099	-6.8 (-16.49 - 2.89)	0.1696
adjusted by genotyping	7.69 (4.87 - 12.02)	3.64 (1.91 - 6.91)	8.11 (5.29 - 12.35)	4.05 (1.11 - 9.21)	0.1037	-0.42 (-6.39 - 5.55)	0.8908	-4.47 (-9.22; 0.28)	0.0624

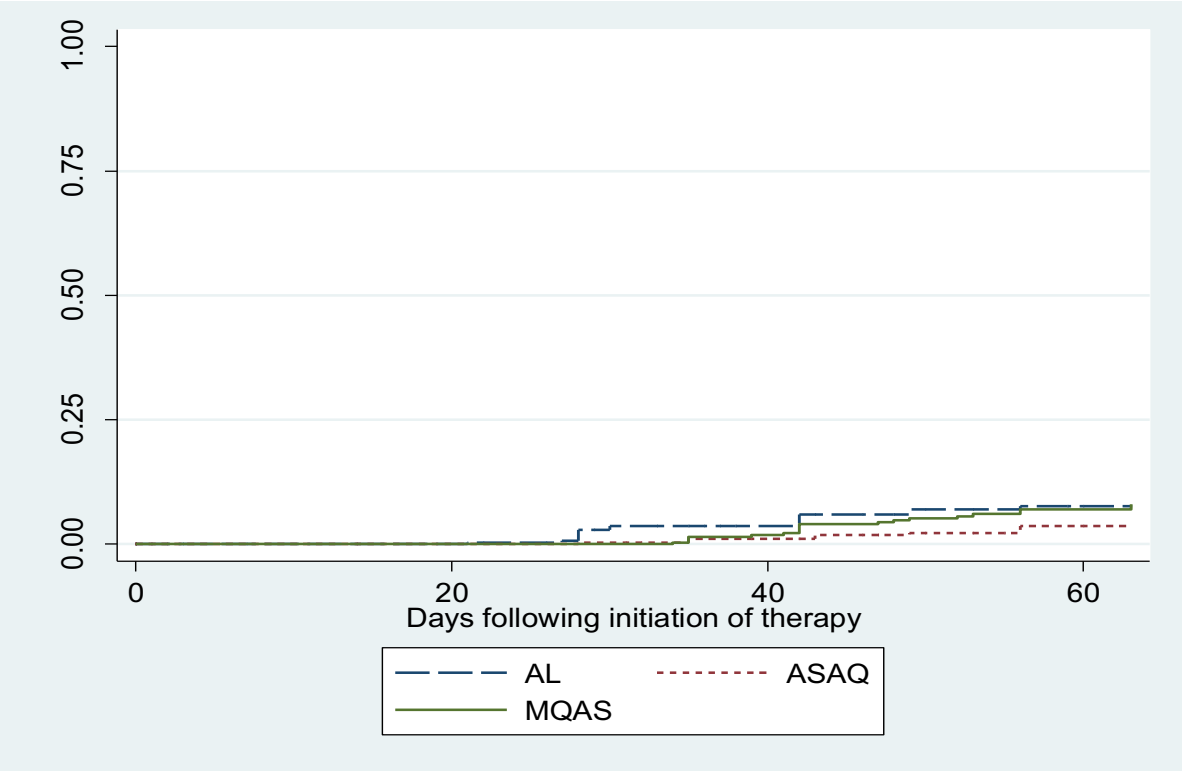


Figure 2: Risk of treatment failure

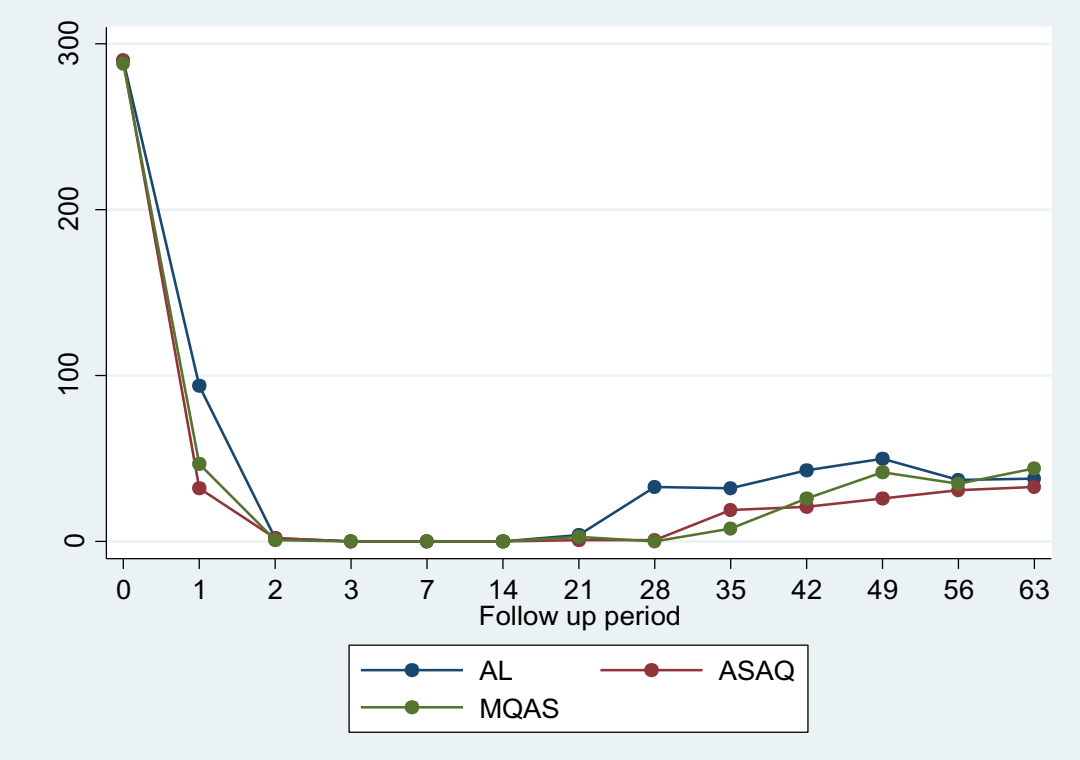


Figure 3 parasite clearance over the follow up period

Chapter 8: General discussion

Although malaria mortality has fallen, additional efforts are needed (1). Several studies conducted in the last decade have shown that MiP represents a major public health concern due to its significant adverse health effects on both mother and foetus (2). These effects include intrauterine growth retardation (3,4), preterm birth (5,6), low birthweight (LBW) (7,8) and maternal anaemia (9,10). The management of MiP relies first in the early diagnosis and treatment with safe and efficacious ACT along with preventive methods (LLINs and IPTp-SP) in the second and third trimesters (11). In this section, we will discuss our findings in relation to more recent data and current evolutions in policy.

8.1 Key findings

The presence of signs and symptoms suggestive of malaria were frequent among pregnant women but not discriminative for malaria infection. Cases and controls were not significantly different in term of parasite density and malaria infection was strongly associated with anaemia as showed in chapter 3. The absence of *dhfr* 164L and *dhps* 540E and the low prevalence of the *dhfr* triple mutation suggest IPTp-SP is still useful in pregnant women (Chapter 4). Results of chapter 5, 6 and 7 demonstrated t most ACTs recommended currently for malaria treatment are efficacious for the treatment of uncomplicated malaria, with DHAPQ as the most promising combination. In addition, our results were not different to those of others studies carried out in children and non-pregnant women in the same country.

8.2 Limitations of these findings

A limitation of the study on clinical presentation of malaria in pregnant women (Chapter 3) and the prevalence of molecular markers related to SP resistance (Chapter 4) was the

relatively short recruitment period during the time of low transmission. Indeed, we experienced a delay in the approval of the protocol by the ethics committee, which influenced the time the study started, at the end of the low transmission period. Therefore, few patients were recruited during this period compared to the majority of participants recruited during the high malaria transmission season. In addition, the one-month period delay to recruit the matched controls did not allow the recruitment of these controls during the same transmission season. Consequently, the time of recruitment of controls did not necessary matched that of the cases. Therefore, the impact of the seasonality on the differential occurrence of signs and symptoms has to be interpreted with caution. For the estimation of the ex vivo susceptibility of isolates collected from pregnant women (Chapter 5), adding SP to the panel of drug tested would have been desirable, and this is a major limitation of this study. This ex vivo study was already designed and approved before the SP study proposal draft so it was difficult thereafter to include SP. Nevertheless, SP efficacy may be estimated also by determining the prevalence of molecular markers related to drug resistance. The *ex-vivo* study (Chapter 5) included only a proportion of isolates among the total number of women recruited in the main trial. This was due to a delay in the onset of this ex vivo component that impacted the number of pregnant women included. Also, an initial parasite density of 4,000 P/µl was required but with the high sensitivity of the HRP2 method this was decrease to at least 100P/ µl at the end of the study. Consequently, a number of women were not included for a parasite density lower than 4,000P/µl. Finally, culture samples with insufficient blood volume for culture (less than 3-4 ml) or samples reaching the laboratory after 24H were rejected.

8.3 Overall interpretation

8.3.1 Is malaria clinical diagnosis relevant?

We showed in Chapter 4 that the clinical signs and symptoms suggestive of malaria although frequent, were not good predictors. When we use the microscopy as recommended by the NMCP, the prevalence of malaria infection was 40% meaning that if we consider only the symptoms most of these cases would not have been treated while infected. Malaria infections were often asymptomatic and confirm the reports from other areas of intense malaria transmission (8,12–18). However, our finding was in contrast with that reported from Ghana, a neighbouring country (19), where malaria infection in pregnant women was more symptomatic. The possible reason explaining this difference could be the study design. In Ghana, pregnant women knew already their infection status, namely if they were RDT negative, and this may have influenced their propensity to report clinical symptoms. In Burkina Faso, the NMCP recommends to confirm malaria infection (TDR or microscopy) only in women presenting signs and symptoms suggestive of malaria. Screening and treatment with SP could have been a good approach. However, its main limitation would be the sensitivity of the diagnostic test used. This strategy called Intermittent Screening and Treatment (IST) was evaluated by several studies in Africa (both East and West Africa) with different drugs (20–23). The impact of this new strategy was investigated in West Africa. Tagbor et al in Ghana (21) demonstrated that in area of moderate malaria transmission, marked seasonality with moderate SP resistance, IST-SP or IST-ASAQ could be as effective as IPT-SP. They showed that IST-SP or IST-ASAQ was not inferior to IPTp-SP in terms of preventing maternal anaemia and low birth weight. In 2015, a large multicentre study conducted in Burkina Faso, Mali, Ghana and The Gambia investigated the impact of IST-AL *vs* IPTp in preventing LBW, maternal anaemia and placental malaria (22). This study showed that IST-AL was not inferior to IPTp-SP but the incidence of visits with symptoms and

parasitaemia outside ANC schedule was higher in IST-AL group (22). This higher incidence of malaria parasitemia and clinical malaria associated with ISTp in both East and West Africa may reflect the inability of ISTp to detect low parasitemia, or occult placental infection. Such infections should be cleared by SP, but may become patent between ANC visits. From the above, IPTp-SP remains highly cost-effective in preventing the adverse consequences of MiP on mother and in the offsprings (24). This should therefore be actively scaled up in line with the recommendations of Malaria Policy Advisory Committee that did not recommend ISTp as an alternative to IPTp-SP (25).

In our study it was also noticed that few pregnant women attended ANCs during the first trimester while this is the period at greater risk of malaria (26–28). Moreover, much pregnant women first attend ANC relatively late in pregnancy (at or after20 weeks' gestation), so a substantial part of pregnancy may not be protected by IPTp (29). Community sensitization for earlier attendance of ANCs should be encouraged and would likely be efficient in preventing any adverse consequences of malaria infection. In addition, the Burkinabe government implemented recently a free health care scheme for pregnant women and children under five years old, which can improve ANCs attendance.

8.3.2 SP for the prevention of malaria in pregnancy?

In Chapter 4, the study concluded to the absence of the *dhfr* 164L and the *dhps* 540E. Consequently, the quintuple mutation was absent among Burkina samples and the prevalence of the *dhfr* triple mutation was very low. This is re-ensuring and clearly indicates that SP can still be used for IPT in Burkina Faso to prevent MiP. However, a spatial heterogeneity of the mutations was seen when comparing our results with those reported by other studies in Burkina Faso (30,31). These differences may be due to different access to treatment in different regions, which may result in higher use of SP because of ACTs unavailability

(32,33). It is then important for the NMCP to ensure the availability of recommended drugs in all health centres at the same time. Our results are in line with that of other studies carried out in West Africa (30,34,35). In contrast, many studies highlighted a decreasing IPTp-SP efficacy in Central and East Africa (36–40). Moreover, in East Africa, SP resistance was associated with an increased risk of foetal anaemia and severe malaria in the offspring (41–43). ISTp was evaluated as alternative approach in different part of Africa (11,44,45). The recent results of the multicentre study in West Africa (22), showed that although IST-AL was not inferior to IPTp-SP, it was associated with high incidence of malaria parasitaemia outside ANCs schedule. Furthermore, as the current available best options (MQ, DHAPQ) will be certainly much more expensive than SP, it is important to explore the feasibility of implementing any of these strategies where SP is no longer effective (46). In our context and more generally in Africa, we recommend the continuous use of SP as IPTp because SP remains effective even in areas where quintuple-mutant haplotypes of *P falciparum* to SP are highly prevalent (25).

Pregnant women attend ANCs but only few of them receive the minimum of three doses of SP as recommended (47). Menendez et al, described many factors such as recurrent stock outs of SP, incapacity to determine exactly the gestational age, and misinformation on the conditions of SP that can have an impact on IPT-SP coverage rate (48). These factors need to be evaluated by the NMCP to ensure a very high coverage rate. A Global Call to Action was launched in 2015 and this may help to improve IPTp-SP coverage (49). Moreover, continuous monitoring of IPTp-SP effectiveness (ex vivo *and* in vitro) is essential(44).

8.3.3 in vivo and ex vivo antimalarial drug's efficacy

Our study showed that resistance to CQ is highest among the drugs tested on *P. falciparum* isolates from Burkina Faso. We may have expected a decrease of CQ resistance, as CQ has

been withdrawn since 2005. Theoretically, in the absence of drug pressure, resistant isolates would disappear and sensitive isolates emerge, with an increased efficacy of the treatment. The persistence of this chloroquino-resistance is probably due to cross-resistance with similar drugs such as Amodiaquine and possibly the persistent use of CQ in the private sectors (50). Some isolates were found ex vivo to be resistant to MQ in Burkina and this confirms the high percentage of resistant isolates previously reported in West African regions (51,52). The latter implies that the NMCP should be cautious regarding the introduction of the combination MQAS in Burkina Faso. There is then the need to develop new compounds that have varied mechanisms of action (53).

PQ was active against both CQ-R and CQ-S isolates while DHA had the lowest IC50. This indicates that the combination DHAPQ is a very promising drug for the management of uncomplicated malaria in pregnant women. In vitro assays of a single fixed-dose combination are difficult to interpret since sensitivities are measured against individual drugs. However, analysing the ex vivo results together with the in vivo data can accurately guide the NMCP.

In the PregACT study, the combination DHAPQ was not tested in Burkina Faso arm but appears to be treatment with good safety and excellent efficacy. DHAPQ had the highest efficacy rate in addition to a long post-treatment prophylaxis period. In the context of Burkina Faso with high transmission, we would recommend the introduction of DHAPQ for the treatment of uncomplicated malaria as alternative treatment to ASAQ and AL.

However, in our context and before a possible change of the malaria treatment policy, the NMCP should:

- Conduct in vivo studies on the efficacy and safety of DHAPQ

- Ensure that all health staff member is well trained on this new policy and that sufficient stock of DHAPQ is available to avoid stock-out.

8.3.4 Conclusions

In this thesis, we demonstrated that the current recommendation of the NMCP to use ASAQ and AL as first line treatments is still a good choice. The introduction of the combination DHAPQ as alternative to ASAQ and AL needs to be evaluated. IPT-SP remains efficient but high coverage is needed. Continuous monitoring of the efficacy and safety of these drugs and the implementation of protective methods are essential.

8.3.5 Challenges

Although suitable strategies are available for the management of MiP, we are still facing several challenges:

- IPTp coverage: even if IPTp-SP is efficacious at clearing residual parasitaemia, his coverage remains far below national and global targets. Andrews et al reviewed data from 58 Demographic and Health Surveys (DHS) and concluded that IPTp coverage was not optimal despite high attendance to ANCs (54). Since 2014, the NMCP updated its malaria control strategy and now recommends IPTp at every ANC visit from the 13th week of pregnancy onward leading to the possibility of 3 or more doses per woman. Annual data from the Health Management and information System (HMIS) found that 73.2% and 18.8% received two and three doses of SP, respectively. In the meantime, 17.7% of the health centres have not started the implementation of this new recommendations of the NMCP (47). Based on this slow implementation and uptake of 3 doses of IPTp, we propose that the NMCP speed-up the translation of this recommendation into a policy by a nationwide campaign promotion. Other ways for the improvement of IPTp coverage such as the use of community health workers (CHWs) need to be explored.

- The evaluation of high IPTp coverage impact in Burkina Faso: we expect a decrease of MiP adverse events when high coverage is reached. Data on IPTp coverage in Burkina Faso are centralized by the NMCP based on health centre reports. A review of health management information system (HMIS) registers, interviews with health-care workers (HWs) in Tanzania indicated that IPTp doses administered to women were inadequate and partly inconsistent. HMIS registers were not adapted (lack of space for IPT records) forcing health staff to manipulate their record-keeping (55). A rigorous system should be set up to accurately and timely collect any information's related to pregnant women (trimester of pregnancy, date of first IPTp-SP dose, date of next dosing, malaria infections, localisation….). By using this channel, those who missed their IPTp-SP dose could be actively sought.

- Sustainability of drugs and RDT provision: early detection of malaria infection requires sufficient stocks of RDTs for pregnant women attending ANC. Stock-outs of SP are experienced in Burkina Faso (47) and this is not reassuring as SP is corner stone of the MiP prevention. This combined to the abatement of international help (World Bank, Global Funds, NGOs) condemns the government to explore others sources of funding.

- Adherence to recommended protocols: almost 20% of health centres have not started applying of the new recommendation of the NMCP for MiP prevention in Burkina Faso (47). Training and continuous monitoring of the health staff are needed to ensure that all prescribers apply current recommendations.

- Community engagement: This includes patient education to protect themselves against malaria, compliance to complete all doses correctly, encouragement of early ANC visits, etc.

- Routine monitoring of drug: the NMCP should conduct at regular intervals ex vivo and in vivo studies on the efficacy of the first line treatments drugs of malaria. The conduct of these studies will be expensive but this is the only way to timely detect the apparition of resistance.

8.3.6 References

1. WHO. World Malaria Report 2015.

2. Moya-Alvarez V, Abellana R, Cot M. Pregnancy-associated malaria and malaria in infants: an old problem with present consequences. Malar J. 2014;13(1):271.

3. Fried M, Nosten F, Brockman A, Brabin BJ, Duffy PE. Maternal antibodies block malaria. Nature. 1998;395(6705):851–52.

4. McGregor IA, Wilson ME, Billewicz WZ. Malaria infection of the placenta in The Gambia, West Africa; its incidence and relationship to stillbirth, birthweight and placental weight. Trans R Soc Trop Med Hyg. 1983;77(2):232–44.

5. Steketee RW, Wirima JJ, Slutsker L, Khoromana CO, Heymann DL, et al. Malaria treatment and prevention in pregnancy: Indications for use and adverse events associated with use of chloroquine or mefloquine. Am J Trop Med Hyg. 1996;55(1):50–56.

6. Sullivan AD, Nyirenda T, Cullinan T, Taylor T, Harlow SD, et al. Malaria infection during pregnancy: intrauterine growth retardation and preterm delivery in Malawi. J Infect Dis. 1999;179(6):1580–83.

7. Brabin B. An assessment of low birthweight risk in primiparae as an indicator of malaria control in pregnancy. Int J Epidemiol. 1991;20(1):276–83.

8. Brabin BJ. An analysis of malaria in pregnancy in Africa. Bull World Health Organ. 1983;61(6):1005–16.

9. Shulman CE, Graham WJ, Jilo H, Lowe BS, New L, et al. Malaria as an important cause of anemia in primigravidae: evidence from a district hospital in coastal Kenya. Trans R Soc Trop Med Hyg. 1996;90:535–39.

10. Cot M, le Hesran JY, Miailhes P, Roisin A, Fievet N, et al. Effect of chloroquine prophylaxis during pregnancy on maternal haematocrit. Ann Trop Med Parasitol. 1998;92:37–43.

11. Desai M, Gutman J, Llanziva A, Otieno K, Juma E, et al. Intermittent screening and

treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine versus intermittent preventive treatment with sulfadoxine-pyrimethamine for the control of malaria during pregnancy in western Kenya: an open-lab. Lancet. 2015;386(10012):2507–19.

12. Bardají A, Sigauque B, Bruni L, Romagosa C, Sanz S, et al. Clinical malaria in African pregnant women. Malar J. 2008;7:27.

13. Huynh BT, Fievet N, Gbaguidi G, Borgella S, Mévo B, et al. Malaria associated symptoms in pregnant women followed-up in Benin. Malar J. 2011;10(1):72.

14. Brabin BJ. Epidemiology of infection in pregnancy. Rev Infect Dis. 1985;7(5):579–603.

15. Verhoeff FH, Brabin BJ, Chimsuku L, Kazembe P, Broadhead RL. Malaria in pregnancy and its consequences for the infant in rural Malawi. Ann Trop Med Parasitol. 1999;93:S25–33.

16. Shulman CE, Marshall T, Dorman EK, Bulmer JN, Cutts F, et al. Malaria in pregnancy: Adverse effects on haemoglobin levels and birthweight in primigravidae and multigravidae. Trop Med Int Heal. 2001;6(10):770–78.

17. Steketee RW, Nahlen BL, Parise ME, Menendez C. The burden of malaria in pregnancy in malaria-endemic areas. In: American Journal of Tropical Medicine and Hygiene. 2001;28–35.

18. WHO. A strategic framework for malaria prevention and control during pregnancy in the African Region. 2004;1–39.

19. Tagbor H, Bruce J, Browne E, Greenwood B, Chandramohan D, et al. Malaria in pregnancy in an area of stable and intense transmission: Is it asymptomatic? Trop Med Int Heal. 2008;13(8):1016–21.

20. De Beaudrap P, Turyakira E, White LJ, Nabasumba C, Tumwebaze B, et al. Impact of malaria during pregnancy on pregnancy outcomes in a Ugandan prospective cohort with intensive malaria screening and prompt treatment. Malar J. 2013;12:139.

21. Tagbor H, Bruce J, Agbo M, Greenwood B, Chandramohan D. Intermittent screening and treatment versus intermittent preventive treatment of malaria in pregnancy: A randomised controlled non-inferiority trial. PLoS One. 2010;5(12):e14425.

22. Tagbor H, Cairns M, Bojang K, Coulibaly SO, Kayentao K, et al. A non-inferiority, individually randomized trial of intermittent screening and treatment versus intermittent preventive treatment in the control of malaria in pregnancy. PLoS One. 2015;10(8):1–17.

23. Scott S, Mens PF, Tinto H, Nahum A, Ruizendaal E, et al. Community-based scheduled screening and treatment of malaria in pregnancy for improved maternal and infant health in The Gambia, Burkina Faso and Benin. Trials. 2014;15:340.

24. Fernandes S, Sicuri E, Halimatou D, Akazili J, Bojang K, et al. Cost effectiveness of intermittent screening followed by treatment versus intermittent preventive treatment during pregnancy in West Africa: analysis and modelling of results from a non-inferiority trial. Malar J. 2016;15(1):493.

25. Malaria Policy Advisory Committee. Conclusions and recommendations. 2015.

26. Nahlen BL. Rolling back malaria in pregnancy. The New England journal of medicine. 2000;651–52.

27. Diagne N, Rogier C, Sokhna CS, Tall A, Fontenille D, et al. Increased susceptibility to malaria during the early postpartum period. N Engl J Med. 2000,343(9):598–603.

28. Huynh BT, Cottrell G, Cot M, Briand V. Burden of malaria in early pregnancy: a neglected problem? Clin Infect Dis. 2015;60(4):598–604.

29. Rogerson SJ, Unger HW. Prevention and control of malaria in pregnancy - new threats, new opportunities? Expert Rev Anti Infect Ther. 2016.

30. Coulibaly SO, Kayentao K, Taylor S, Guirou EA, Khairallah C, et al. Parasite clearance following treatment with sulphadoxine-pyrimethamine for intermittent preventive treatment in Burkina-Faso and Mali: 42-day in vivo follow-up study. Malar J. 2014;13:41.

31. Geiger C, Compaore G, Coulibaly B, Sie A, Dittmer M, et al. Substantial increase in mutations in the genes pfdhfr and pfdhps puts sulphadoxine-pyrimethamine-based intermittent preventive treatment for malaria at risk in Burkina Faso. Trop Med Int Heal. 2014;19(6):690–97.

32. Tinto H, Ouédraogo JB, Zongo I, Van Overmeir C, Van Marck E, et al. Sulfadoxine-pyrimethamine efficacy and selection of Plasmodium falciparum DHFR mutations in Burkina Faso before its introduction as intermittent preventive treatment for pregnant women. Am J Trop Med Hyg. 2007;76(4):608–13.

33. Tipke M, Louis VR, Ye M, De Allegri M, Beiersmann C, Sie A, et al. Access to malaria treatment in young children of rural Burkina Faso. Malar J. 2009;8:266.

34. Somé AF, Séré YY, Dokomajilar C, Zongo I, Rouamba N, et al. Selection of known plasmodium falciparum resistance-mediating polymorphisms by artemether-lumefantrine

and amodiaquine-sulfadoxine-pyrimethamine but not dihydroartemisinin-piperaquine in Burkina Faso. Antimicrob Agents Chemother. 2010;54(5):1949–54.

35. Bertin G, Briand V, Bonaventure D, Carrieu A, Massougbodji A, et al. Molecular markers of resistance to sulphadoxine-pyrimethamine during intermittent preventive treatment of pregnant women in Benin. Malar J. 2011;10:196.

36. Gutman J, Mwandama D, Wiegand RE, Abdallah J, Iriemenam NC, et al. In vivo efficacy of sulphadoxine-pyrimethamine for the treatment of asymptomatic parasitaemia in pregnant women in Machinga District, Malawi. Malar J. 2015;14(1):197.

37. Sridaran S, McClintock SK, Syphard LM, Herman KM, Barnwell JW, et al. Anti-folate drug resistance in Africa: meta-analysis of reported dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) mutant genotype frequencies in African Plasmodium falciparum parasite populations. Malar J. 2010;9:247.

38. Arinaitwe E, Ades V, Walakira A, Ninsiima B, Mugagga O, et al. Intermittent preventive therapy with sulfadoxine-pyrimethamine for malaria in pregnancy: a cross-sectional study from Tororo, Uganda. PLoS One. 2013;8(9):e73073.

39. Likwela JL, D'Alessandro U, Lokwa BL, Meuris S, Dramaix MW. Sulfadoxine-pyrimethamine resistance and intermittent preventive treatment during pregnancy: a retrospective analysis of birth weight data in the Democratic Republic of Congo (DRC). Trop Med Int Health. 2012;17(3):322–29.

40. Minja DTR, Schmiegelow C, Mmbando B, Bostrom S, Oesterholt M, et al. *Plasmodium falciparum* mutant haplotype infection during pregnancy associated with reduced birthweight, Tanzania. Emerg Infect Dis. 2013;19(9).

41. Harrington WE, Mutabingwa TK, Muehlenbachs A, Sorensen B, Bolla MC, et al. Competitive facilitation of drug-resistant Plasmodium falciparum malaria parasites in pregnant women who receive preventive treatment. Proc Natl Acad Sci. 2009;106(22):9027–32.

42. Harrington WE, Mutabingwa TK, Kabyemela E, Fried M, Duffy PE. Intermittent treatment to prevent pregnancy malaria does not confer benefit in an area of widespread drug resistance. Clin Infect Dis. 2011;53(3):224–30.

43. Harrington WE1, Morrison R, Fried M, Duffy PE. Intermittent Preventive Treatment in Pregnant Women Is Associated with Increased Risk of Severe Malaria in Their Offspring. PLoS One. 2013;8(2):e56183.

44. Desai M, Gutman J, Taylor SM, Wiegand RE, Khairallah C, et al. Impact of Sulfadoxine-

Pyrimethamine Resistance on Effectiveness of Intermittent Preventive Therapy for Malaria in Pregnancy at Clearing Infections and Preventing Low Birth Weight. Clin Infect Dis. 2016;62(3):323–33.

45. Gutman J, Kalilani L, Taylor S, Zhou Z, Wiegand RE, et al. The A581G Mutation in the Gene Encoding Plasmodium falciparum Dihydropteroate Synthetase Reduces the Effectiveness of Sulfadoxine-Pyrimethamine Preventive Therapy in Malawian Pregnant Women. J Infect Dis. 2015;211(12):1997–2005.

46. Gutman J, Mwandama D, Wiegand RE, Ali D, Mathanga DP, et al. Effectiveness of intermittent preventive treatment with sulfadoxine-pyrimethamine during pregnancy on maternal and birth outcomes in Machinga district, Malawi. J Infect Dis. 2013;208(6):907–16.

47. Badolo O, Nebie SP, Dodo M, Ouedraogo T, Waxman R et al. Intermittent Preventive Treatment in Pregnancy: Increasing the Doses in Burkina Faso. In: Tropical Health Matters. 2015.

48. Menendez C, Ferenchick E, Roman E, Bardaji A, Mangiaterra V. Malaria in pregnancy: challenges for control and the need for urgent action. Lancet Glob Heal. 2015;3(8):e433–44.

49. Chico RM, Dellicour S, Roman E, Mangiaterra V, Coleman J, et al. Global Call to Action: maximize the public health impact of intermittent preventive treatment of malaria in pregnancy in sub-Saharan Africa. Malar J. 2015;14:207.

50. Frosch AE, Venkatesan M, Laufer MK. Patterns of chloroquine use and resistance in sub-Saharan Africa: a systematic review of household survey and molecular data. Malar J. 2011;10(1):116.

51. Fall B, Diawara S, Sow K, Baret E, Diatta B, et al. Ex vivo susceptibility of Plasmodium falciparum isolates from Dakar, Senegal, to seven standard anti-malarial drugs. Malar J. 2011;10(1):310.

52. Nsobya SL, Kiggundu M, Nanyunja S, Joloba M, Greenhouse B, et al. In vitro sensitivities of Plasmodium falciparum to different antimalarial drugs in Uganda. Antimicrob Agents Chemother. 2010;54(3):1200–06.

53. Winstanley PA. Chemotherapy for falciparum malaria: the armoury, the problems and the prospects. Parasitol Today. 2000;16(4):146–53.

54. Andrews KG, Lynch M, Eckert E, Gutman J. Missed opportunities to deliver intermittent preventive treatment for malaria to pregnant women 2003-2013: a systematic analysis of

58 household surveys in sub-Saharan Africa. Malar J. 2015;14:521.

55. Mubyazi GM, Byskov J, Magnussen P, Bygbjerg IC, Ijumba JN, et al. Health facility-based data on women receiving sulphadoxine-pyrimethamine during pregnancy in Tanzania: lessons to learn from a cross-sectional survey in Mkuranga and Mufindi districts and other national survey reports. Reprod Health. England. 2014;11(1):6.

Chapter 9: Conclusions and perspectives

In April 2000, at the Abuja meeting, African heads of state agreed to halve malaria mortality by 2015. This would have been made possible by ensuring appropriate methods of protection and case-management for at least 60% of the continent's at-risk population. Fifteen years later, this objective has not been attained, though there has been substantial progress. Successful malaria control programs should include a combination of prevention and control strategies. Starting from the use of ITNs or LLINs, IRSs and IPTs associated with a prompt and accurate diagnostic, followed by an effective treatment.

In this thesis, we had the opportunity to investigate the possibility of early recognition of clinical malaria among pregnant women in chapter 4, and SP resistance in Burkina Faso as an important parameter of the IPTp-SP intervention strategy in chapter 5. We also examined the ex vivo sensitivity profiles of various antimalarials used in MiP in chapter 6, and finally in chapter 7 and 8, the efficacy and safety of ACTs for uncomplicated malaria in several African countries.

The main results reported in chapters 3, 4, 5, 6, and 7 will all contribute to inform policy makers involved in malaria control in Burkina Faso.

9.1 Detection of malaria infections during pregnancy: is clinical diagnosis (screening based on signs and symptoms) relevant?

Signs and symptoms usually associated with clinical malaria were common among pregnant women but non-specific. Therefore, when considering its low PPV, clinical diagnosis of malaria in pregnant women is not useful. All this indicates that malaria infection should be confirmed by a laboratory test, either microscopy or RDT. It is also important to mention that,

given the potential risks of harming the fetus, there is the need when treating pregnant women to balance the benefits against the potential risks.

9.2 Prevention of malaria in pregnancy: efficacy of SP in Burkina Faso (West Africa)

Our study was able to recruit pregnant women of any parity attending ANCs, and throughout the pregnancy. The prevalence of the triple mutation associated with pyrimethamine resistance in vitro and responsible for a 1,000-fold reduction in pyrimethamine susceptibility was low. Moreover, the mutations I164L and K540E were not found in Burkina isolates. Therefore, SP can still be used as IPTp and it is likely to have a substantial impact on maternal health and pregnancy outcomes.

The WHO recommends four ANC visits during which IPTp with SP should be implemented (updated WHO Policy 2012), with the first dose administered as early as possible during the second trimester. The relatively long half-life of SP will help suppress any new emerging infection during this period.

In our context, to ensure the efficacy of this policy some efforts should be made to break barriers that can negatively impact on SP use.

- Firstly, all pregnant women should attend as early as possible ANCs. This can be improved by using the community health workers to promote early attendance.
- Secondly, SP should be available in health centres at any time of the year during the ANCs. During our study we observed SP stock outs that may lead to a delay in its uptake.

- Thirdly, SP should be used only for IPTp and, together with amodiaquine for the seasonal malaria chemoprevention in children <5 years of age, at least in target countries. The availability of SP outside health centres should be restricted.
- Finally, a longitudinal monitoring of SP efficacy through molecular and efficacy studies in pregnant women should be done.
- Alternative drugs to SP should be tested for IPTp.

9.3 Antimalarial drugs: ex vivo efficacy of antimalarials in pregnancy

We assessed the sensitivity profile to 8 different drugs of malaria isolates collected from pregnant women included in an efficacy trial in Burkina Faso. More generally, the drugs performed well with the exception of chloroquine. This is an indication of the good efficacy of the first line treatments drugs.

Our ex vivo results also confirmed the efficacy of DHA and his partner drug, PQ. Therefore, the combination DHAPQ may be a promising drug for treating MiP. Another advantage of this combination is the long half-life of piperaquine, with can provide a relatively long period of post-treatment prophylaxis.

9.4 Efficacy and safety of ACTs use in pregnant women in Africa

Artemisinin-based combination therapies are generally efficacious for the treatment of uncomplicated malaria in pregnant women. In Burkina Faso, the recommended first line treatments are ASAQ and AL. Regarding the results of this multi-centre study, AL (one of the first line treatments in Burkina Faso) and DHAPQ had the best efficacy and safety profile. The capacity of the drug to ensure a long post-treatment prophylactic period must be taken in account when comparing treatment regimens. Although effective, AL had the shortest post-treatment prophylactic effect, which may be a disadvantage where transmission is intense as

the pregnant women could be re-infected often. In addition, the twice-per-day dosing could negatively impact on its effectiveness.

DHAPQ was not tested in Burkina Faso but had the highest PCR-adjusted cure rate at day 63 compared to the 3 others combinations and the best safety profile. The two other treatments tested in Burkina Faso, ASAQ and ASMQ, were associated with a higher occurrence of adverse events. Therefore, the combination DHAPQ is the most promising treatment when considering its efficacy and safety profile. The introduction of this combination in the Burkinabe malaria program could be considered.

9.5 Rationale and pertinence of the National malaria guidelines in Burkina Faso

ASAQ had the best efficacy profile in Burkina Faso; however, it was less well tolerated than AL. In contrast, AL had a better safety profile with a higher risk of re-infection. DHAPQ with its good efficacy profile and long post treatment prophylaxis period is a good candidate for the first line treatment. Finally, the recommendation of IPTp-SP is still a valid option as resistance to SP is still very low in our region.

9.6 Futures perspectives

- Clinical diagnosis based on signs and symptoms should be completely removed by the NMCP for malaria case management
- RDT and microscopy are useful tools for diagnosis but quality control and quality assurance systems must be in place. Also the logistics of supplies (RDTs, antimalarials) must be ensured.
- The combination DHAPQ is the suitable candidate for the malaria first line treatment
- Continuous in vivo monitoring of the first line treatments should be performed to timely detect emergence of resistance.

9.7 Conclusions

In this thesis, we have reported that:

1. Clinical signs and symptoms alone are poor predictors of malaria infections and then should be removed.
2. IPT-SP can still be used to prevent the adverse effects of MiP. The low prevalence of the triple mutation combined with the absence of the quadruple mutation confirms that IPTp-SP is still a useful intervention to prevent malaria in pregnancy. However regular monitoring of the level of SP resistance marker should be performed.
3. The four artemisinin-based combination therapies are effective in pregnant women with malaria in Africa, with relatively mild adverse events.
4. DHAPQ is a promising ACTs and should be considered for the treatment of uncomplicated malaria in pregnant women.

Photo's: © Anna Wang/MMV