Faculty of Medicine and Health Sciences

Antimalarial Treatment Efficacy and Safety in Pregnant Women

Thesis submitted for the degree of

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My son, keep my teaching in your memory, and my rules in your heart:

For they will give you increase of days, years of life, and peace.

Let not mercy and good faith go from you; let them be hanging round your neck, recorded on your heart;

So you will have grace and a good name in the eyes of God and men.

Put all your hope in God, not looking to your reason for support.

In all your ways give ear to him, and he will make straight your footsteps.

Put no high value on your wisdom: let the fear of the Lord be before you, and keep yourself from evil.

This will give strength to your flesh, and new life to your bones.

(Prov 3:1-8 (BBE))

To my Father (John Nambozi - 1931-2016) and Mother (Jenefer Bwalya – 1942-1995)

Dedication

I dedicate this book to my Lord and savior Jesus Christ who died and rose again and seated at the right hand of majesty. To Him Be glory forever and ever Amen!!

To my mentor and my father in the Lord Prophet TB Joshua who says **"Work as if it all depends on you and pray as if it all depends on God"**. Thank you for you gave your life to God to change my life and others. The work done in that way will see a malaria-free world.

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List of abbreviations

ACPR: Adequate clinical and parasitological response

ACT: artemisinin-based combination treatment

AE: Adverse event

AL: Artemether lumefantrine

ALAT: Alanine amino-transferase

AQAS: Amodiaquine artesunate

ARV: Antiretroviral

CSA: Chondroitin Sulphate A

CRF: Case report form

CS: Consortium secretariat

DRC: Democratic Republic of Congo

DHA-PQ: Dihydroartemisinin piperaquine

DNA: Deoxynucleic acid

DSMB: Data safety and monitoring board

e-CRF: electronic clinical record form

ELISA: Enzyme-linked immunosorbent assay

ETF: Early treatment failure

ETF: Early Treatment Failure

GCP: Good clinical practice

GPS: Global Positioning System

GLURP: Glutamate-rich protein

Hb: haemoglobin

HMIS: health management information system

HIV: Human immunodeficiency virus

HR: hazard ratio

HRP: Histidine-rich protein

ICH: International conference on harmonization

IRS: indoor residual spraying

ITNs: insecticide-treated mosquito nets

IPTp: intermittent preventive treatment during pregnancy

IQR: interquartile range

IRR: incidence rate ratio

ITT: intention-to-treat

LAR: Legally authorised representative

LLIN: Lasting Insecticidal Nets

LBW: low birth weight

LCF: Late clinical failure

LPF: Late clinical failure

LTF: Late treatment failure

LTFU: lost to follow-up

MiP: Malaria in pregnancy

MiPc: Malaria in Pregnancy Consortium

MQAS: Mefloquine artesunate

MSP: Malaria merozoite surface protein

OR: odds ratio

PCR: Polymerase chain reaction

PCT: Parasite clearance time

PK: Pharmacokinetics

PP: per-protocol

PRR: pooled risk ratio

RDT: rapid diagnostic test

SAE: Serious adverse event

SDV: Source data verification

SP: Sulfadoxine pyrimethamine

sSA: sub-Saharan Africa

SP: sulfadoxine-pyrimethamine

TF: Treatment failure

VCT: Voluntary counselling and testing

WBC: white blood cells

WHO: World health organization

Summary

Background and Rationale

Malaria is considered as one of the major public health problems in the world and a disease of poverty. It is among the leading cause of morbidity and mortality worldwide, severely affecting mostly under-five children and pregnant women. In 2017 there were fewer malaria cases (219 million cases) than in 2010 (239 million cases), with the decreasing trend stopping in 2016. Indeed, the estimated number of cases started to increase in 2016, about 216 million of cases in 2016 as compared to the 214 million of cases in 2015; there was a further increase in 2017, to 219 million cases. A substantial proportion of malaria cases (*Plasmodium falciparum*) occurs in a few African countries, 25% of them in Nigeria, 11% in the Democratic Republic of Congo, and 5% in Mozambique. Malaria deaths, most of them (93%) occurring in sub-Saharan Africa (sSA), have decreased globally from 607 000 in 2010 to 451 000 and 435 000 in 2016 and 2017, respectively.

In Zambia, there are three species of Anopheles mosquitoes that can transmit malaria, *An. funestus, An. gambiae s.s.* and *An. arabiensis* and predominantly transmit *P. falciparum*.

In 2011, 4.54 million malaria cases were reported in Zambia. About half of them were diagnosed clinically, i.e. without parasitological diagnosis, whilst the rest was confirmed by microscopy or rapid diagnostic test (RDT).

Malaria in pregnancy may lead to low birth weight which is the single most important risk factor of neonatal mortality and morbidity and increases the risk for infant mortality. There are few approved treatment options with good efficacy, acceptable tolerability and safety as pregnant women for long time have been systematically excluded from treatment trials and pharmacokinetic studies. Most

available information has been collected in South East Asia while in sub-Saharan Africa, until recently, such information was still limited. The effects of artemisinin-based combination therapy (ACT) on the malaria infection during pregnancy and on the offspring's first year of life as children are rarely followed up due to difficulties of adequate monitoring. Therefore, clinical research is needed to improve knowledge on the effect of ACT on pregnancy outcome and neonatal and infant morbidity and mortality.

Current World Health Organization (WHO) guidelines recommend quinine with clindamycin for 7 days (or quinine alone if clindamycin is not available) for *P. falciparum* infections during the first trimester of pregnancy. In case of treatment failure or unavailability, an ACT or oral artesunate with clindamycin for 7 days may be used. For the second and third trimester of pregnancy, ACT can be used in the same manner as in non-pregnant adults.

This thesis reports the methodology and some of the results of a trial that evaluated different options for the treatment of uncomplicated malaria in women in the 2nd and 3rd trimester of pregnancy. This also included a malariometric survey at the site where the study was implemented as study results are influenced by malaria endemicity and malaria control measures in the study area. Although artemisinin-based combination treatment of malaria during pregnancy was already recommended by WHO, information on their safety and efficacy in African pregnant women was extremely limited at the time this trial started

Methods

The main objective of this thesis was to assess the safety and efficacy of artemisinin-based combination therapies (artemether-lumefantrine, amodiaquine-artesunate, mefloquine-artesunate

and dihydroartemisinin-piperaquine) for the treatment of malaria in the second and third trimester in African pregnant women. Specifically

a. To characterize the malaria endemicity in Nchelenge district in Zambia through a cross section survey;

b. To determine the safety and efficacy of three artemisinin-based combinations, namely mefloquine–artesunate (MQAS), dihydroartemisinin–piperaquine (DHA–PQ); and artemether–lumefantrine (AL), in pregnant women in the second or third trimester with a confirmed *P. falciparum* malaria infection in Zambia in a Phase 3, non-inferiority, multicentre, randomized, open-label clinical trial

c. To assess the safety of ACT on the foetus and new-born.

Key Results

Defining the malaria burden in Nchelenge District, northern Zambia using the World Health Organization malaria indicators survey

Six hundred thirty households were selected and 782 children tested for malaria and anaemia. Prevalence of malaria infection was 30.2% (236/782), the large majority (97.9%, 231/236) being *Plasmodium falciparum* and the remaining ones (2.1%, 5/236) Plasmodium malariae. Anaemia, defined as haemoglobin concentration <11 g/dl, was detected in 51.2% (398/782) children.

In Zambia, despite the reported decline in malaria burden, pockets of high malaria endemicity, such as Nchelenge district, still remain. This is a border area and significant progress can be achieved only by concerted efforts aimed at increasing coverage of current control interventions across the border

Safe and efficacious artemisinin-based combination treatments for African pregnant women with malaria: a multicentre randomized control trial – Methods paper

The strength of this trial is the involvement of several African countries, increasing the generalisability of the results. In addition, it assesses most ACTs currently available, determining their relative '-value-' compared to others. The balanced incomplete block design was chosen because using all 4-arms in each site would have increased complexity in terms of implementation. Excluding human imunedeficiency virus (HIV)-positive pregnant women on antiretroviral drugs may be seen as a limitation because of the possible interactions between antiretroviral and antimalarial treatments. Nevertheless, the results of this trial will provide the evidence base for the formulation of malaria treatment policy for pregnant women in sub-Saharan Africa

Artemisinin-based Combination Treatments in Pregnant Women in Zambia: Efficacy, Safety and Risk of Recurrent Malaria

Nine hundred pregnant women were included, 300 per arm. PCR-adjusted treatment failure was 4.7% (12/258) (95%CI:2.7–8.0) for AL, 1.3% (3/235) (95%CI:0.4–3.7) for MQAS and 0.8% (2/236) (95%CI:0.2–3.0) for DHAPQ, with significant risk difference between AL and DHAPQ (p=0.01) and between AL and MQAS (p=0.03) treatments. Re-infections during follow up were more frequent in the AL (HR:4.71; 95%CI:3.10-7.2; p<0.01) and MQAS (HR:1.59; 95%CI:1.02–2.46; p=0.04) arms compared to the DHAPQ arm. PCR-adjusted treatment failure was significantly associated with women under 20 years (cHR:5.35; 95%CI:1.07-26.73; p=0.04) and higher malaria parasite density (cHR:3.23; 95%CI:1.03-10.10; p=0.04)], and still women under 20 years (cHR1.78; 95%CI:1.26-2.52; p<0.01) had a significantly higher risk of re-infection. The three treatments were generally well tolerated. Dizziness, nausea, vomiting, headache and asthenia as adverse events

(AEs) were more common in MQAS than in AL or DHAPQ ($p<0.001$). Birth outcomes were not significantly different between treatment arms.

As new infections can be prevented by a long acting partner drug to the artemisinins, DHAPQ should be preferred in places as Nchelenge district where transmission is intense while in areas of low transmission intensity AL or MQAS may be used.

Artemisinin–based combination treatment during pregnancy; outcome of pregnancy and infant mortality: a cohort study

3,127 live new-borns (822 in the AL, 775 in the ASAQ, 765 in the MQAS and 765 in the DHAPQ arms) were followed-up until their first birthday. Prevalence of placental malaria and low birth weight were 28.0% (738/2646) and 16.0% (480/2999), respectively, with no significant differences between treatment arms. No differences in congenital malformations ($p=0.35$), perinatal mortality ($p=0.77$), neonatal mortality ($p= 0.21$), and infant mortality ($p=0.96$) were found.

Outcome of pregnancy and infant survival were similar between treatment arms indicating that any of the four ACTs could be safely used during the second and third trimester of pregnancy without any adverse effect on the baby. Nevertheless, smaller safety differences between ACTs cannot be excluded; country-wide post-marketing surveillance would be very helpful to exclude such findings.

Conclusions and Recommendations

It is important to stress that at the time the clinical trial started, there was little information on the use of ACT in African pregnant women. The ACTs evaluated in the trial (artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine, and dihydroartemisinin-piperaquine) were highly efficacious in areas of high endemicity, with over 90% efficacy within the pre-specified equivalence

margins. These results support the WHO guidelines on the treatment of malaria during the second and third trimester of pregnancy.

Although this trial confirmed that available ACTs for the treatment of uncomplicated malaria are safe and efficacious in pregnant women, there is the need to monitor how they are used across the health system, both public and private, and the quality of case management. Until now, several studies have collected information on the safety and efficacy of ACT. The data available is not sufficient to identify rare adverse events. This can be solved by setting up a pharmacovigilance program with a pregnancy registry in which adverse events following the administration of any treatment, including antimalarial drugs, would be registered. Country-wide post-marketing surveillance would require substantial resources and collaboration between funders, policy makers and researchers.

Samenvatting

Achtergrond en rationale

Malaria wordt globaal beschouwd als een van de grote volksgezondheidsproblemen en geassocieerd met armoede. Het is een van de belangrijkste oorzaken van morbiditeit en mortaliteit wereldwijd, die voornamelijk kinderen onder de vijf jaar en zwangere vrouwen treft. In 2017 waren er minder malariagevallen (219 miljoen) dan in 2010 (239 miljoen), maar de dalende trend stopte in 2016. Inderdaad, het geschatte aantal gevallen begint opnieuw te stijgen in 2016; er zijn ongeveer 216 miljoen gevallen in 2016 in vergelijking met de 214 miljoen gevallen in 2015. Er was een verdere stijging in 2017, tot 219 miljoen gevallen. Een aanzienlijk deel van de malariagevallen (*Plasmodium falciparum*) komt voor in enkele Afrikaanse landen i.c. 25% in Nigeria, 11% in de Democratische Republiek Congo en 5% in Mozambique. Malariasterfte, voornamelijk (93%) in sub-Saharisch Afrika (sSA), is wereldwijd afgenomen van 607 000 in 2010 aan 451 000 en 435 000 in respectievelijk 2016 en 2017.

In Zambia zijn er drie soorten Anopheles muggen die malaria overdragen, *An. funestus*, *An. gambiae* s.s. en *An. arabiensis* en ze dragen vnl. *P. falciparum* over.

In 2011 werden er 4,54 miljoen malariagevallen genoteerd in Zambia. Ongeveer de helft van hen werd klinisch gediagnosticeerd, dat wil zeggen zonder parasitologische diagnose, terwijl de rest werd bevestigd door microscopie of snelle diagnostische test (RDT).

Malaria tijdens de zwangerschap kan leiden tot een laag geboortegewicht die de belangrijkste risicofactor van neonatale mortaliteit en morbiditeit en zuigelingensterfte is. Er zijn weinig bevestigde behandelingsopties met goede werking, aanvaardbare tolerantie en bijwerkingen daar zwangere vrouwen systematisch worden uitgesloten van klinische en farmacokinetische studies. De

meeste beschikbare informatie is verzameld in Zuidoost-Azië terwijl in sub-Saharisch Afrika, tot voor kort, deze informatie nog steeds beperkt was. Het effect van artemisinine gebaseerde combinatietherapie (ACT) op de malaria infectie tijdens de zwangerschap en op de borelingen tijdens het eerste levensjaar wordt zelden opgevolgd daar adequate toezicht moeilijk te organiseren valt. Daarom is klinisch onderzoek nodig om informatie te verzamelen over het effect van de ACT op de zwangerschap en bij pasgeborenen, en zuigelingen morbiditeit en mortaliteit.

De hedendaagse gidsen van de Wereldgezondheidsorganisatie (WGO) bevelen kinine met clindamycine voor 7 dagen (of kinine alleen als clindamycine niet beschikbaar is) voor *P.falciparum*-infecties aan tijdens het eerste trimester van de zwangerschap. In geval van behandelingsfalen of onbeschikbaarheid, kan een ACT of mondelinge artesunaat met Clindamycine voor 7 dagen worden gebruikt. Voor de tweede en derde trimester van de zwangerschap, kan ACT worden gebruikt op dezelfde manier als in niet-zwangere volwassenen.

Deze thesis meldt de methodologie en enkele resultaten van een studie die verschillende opties voor de behandeling van ongecompliceerde malaria in vrouwen in het 2e en 3e trimester van de zwangerschap evalueerde. Dit hield ook een malariometrische studie in op de plaats waar de studie werd uitgevoerd daar studieresultaten worden beïnvloed door malariaendemiciteit en malaria controlemaatregelen in het studiegebied. Hoewel artemisinine gebaseerde combinatiebehandeling van malaria tijdens de zwangerschap al was aanbevolen door de WGO, was informatie over hun veiligheid en de werkzaamheid in Afrikaanse zwangere vrouwen heel beperkt op het moment dat deze studie startte.

Methoden

Het hoofddoel van deze thesis was om de veiligheid en de werkzaamheid te evalueren van de artemisinine gebaseerde combinatietherapieën (artemether-lumefantrine, amodiaquine-artesunaat, mefloquine-artesunaat en dihydroartemisinin-piperaquine) voor malaria behandeling in het tweede en derde trimester in Afrikaanse zwangere vrouwen. Concreet,

a. de malaria-endemiciteit te karakteriseren in Nchelenge district in Zambia door middel van een enquete;
b. de veiligheid en de werkzaamheid te evalueren van drie artemisinine gebaseerde combinatiebehandelingen, namelijk mefloquine-artesunaat (MQAS), dihydroartemisinin-piperaquine (DHA-PQ); en artemether-lumefantrine (AL), bij zwangere vrouwen in het tweede of derde trimester met een bevestigde *P.falciparum* malaria infectie in Zambia in een fase 3, non-inferieur , multicenter, gerandomiseerde, open-label klinische proef.
c. de veiligheid van ACT te beoordelen in de foetus en de pasgeboren.

Voornaamste Resultaten

Definiëren van de malaria endemiciteit in Nchelenge District, noordelijk Zambia met behulp van de "World Health Organization malaria indicators survey"

Zes honderddertig huishoudens werden geselecteerd en 782 kinderen getest voor malaria en anemie. Prevalentie van malaria infectie was 30,2% (236/782), de grote meerderheid (97,9%, 231/236) was *Plasmodium falciparum* en de resterende *Plasmodium malariae* (2,1%, 5/236). Anemie, gedefinieerd als hemoglobinegehalte <11 g/dl, werd vastegesteld in 51,2% (398/782) van de kinderen. In Zambia blijven er nog steeds, ondanks de gemelde daling in de malaria endemiciteit, haarden van hoge malarialast zoals Nchelenge district. Dit is een grensgebied en aanzienlijke

vooruitgang kan slechts worden bereikt door gezamenlijke inspanningen gericht op een grensoverschrijdende verbetering van de dekking van de huidige controle interventies.

Veilig en doeltreffend artemisinine gebaseerde combinatiebehandelingen voor Afrikaanse zwangeren met malaria: een multicentrische gerandomiseerde klinische studie – Methode artikel

De sterkte van deze studie is de betrokkenheid van verschillende Afrikaanse landen wat het draagvlak en generaliseerbaarheid van de resultaten verhoogd. Daarnaast beoordeelt het de meeste ACTs die momenteel beschikbaar zijn, hun relatieve 'waarde' bepalend in vergelijking met andere behandelingen. De ‘balanced incomplete block design’ werd gekozen omdat het uitvoeren van alle 4-studiearmen in elke site de complexiteit van de uitvoering dermate verhoogde. Het uitsluiten van menselijke imunodeficiency virus (HIV)-positieve zwangeren op antiretrovirale behandeling kan worden gezien als een beperking omwille van de mogelijke interacties tussen antiretrovirale en antimalarial behandelingen. Niettemin, zullen de resultaten van deze studie de nodige evidentie bezorgen aan de beleidsmakers van malariabehandeling voor zwangere vrouwen in sub-Saharisch Afrika.

Artemisinine gebaseerde combinatiebehandelingen bij zwangere vrouwen in Zambia: werkzaamheid, veiligheid en risico van behandelingsfalen

Negen honderd zwangere vrouwen werden gerecruteerd, 300 per arm. PCR-gecorrigeerde behandelingsfalen was 4,7% (12/258) (95%CI:2,7-8,0) voor AL, 1,3% (3/235) (95%CI:0,4-3,7) voor MQAS en 0,8% (2/236) (95%CI:0,2-3,0) voor DHAPQ, met significante risico verschil tussen AL en DHAPQ (p=0,01) en tussen AL en MQAS (p=0,03) behandelingen. Nieuwe infecties tijdens de follow-up werden vaker gezien in de AL (Hazard Ratio (HR):4,71; 95%CI:3,10-7,2; p<0.01) en MQAS (HR:1,59; 95%CI:1,02-2,46; p=0,04) armen ten opzichte van de DHAPQ-arm. PCR-gecorrigeerd behandelingsfalen was significant geassocieerd met vrouwen onder de 20 jaar [HR:5,35;,95%CI:1,07-26,73; p=0,04) en hogere malaria parasiet dichtheid (cHR:3,23; 95%CI:1,03-10,10; p=0,04)], en opnieuw vrouwen onder de 20 jaar (cHR:1.78; 95%CI:1,26- 2,52; p<0,01)] hadden een beduidend hoger risico van herinfectie. De drie behandelingen waren over het algemeen goed getolereerd. Duizeligheid, misselijkheid, braken, hoofdpijn en asthenie als ongewenste voorvallen (AEs) werden vaker voor bij MQAS dan in AL of DHAPQ (p<0,001). Geboortepathologien waren niet significant verschillend tussen de behandelingsarmen.

Als nieuwe infecties kunnen worden voorkomen door een lang werkenden partnermedicatie naast de artemisinines, zou DHAPQ de voorkeur genieten in plaatsen zoals Nchelenge district waar de transmissie intens is terwijl in gebieden van lage overdracht, AL of MQAS is kan worden gebruikt.

Artemisinine gebaseerde combinatie behandeling tijdens de zwangerschap; uitkomst van de zwangerschap en baby mortaliteit: een cohortstudie

3,127 levend pasgeborenen (822 in de AL, 775 in de ASAQ, 765 in de MQAS en 765 in de DHAPQ arm) werden opgevolgd tot hun eerste verjaardag. Prevalentie van placenta malaria en laag geboortegewicht waren 28,0% (738/2646) en 16,0% (480/2999)respectievelijk, met geen significante verschillen tussen de behandelingsarmen. Geen verschillen in aangeboren misvormingen (p=0,35), perinatale sterfte (p=0,77), neonatale sterfte (p=0,21), en kindersterfte (p=0,96) werden gevonden.

Uitkomst van de zwangerschap en zuigelingensterfteoverleven waren vergelijkbaar tussen behandelingsarmen, wat aanwijst dat alle vier behandelingen veilig kunnen worden gebruikt tijdens de tweede en derde trimester van de zwangerschap zonder nadelige effecten op de baby. Kleinere veiligheidsverschillen tussen de behandelingen kunnen niet worden uitgesloten; hiervoor zou een nationaal postmarketing surveillance heel nuttig zijn om deze verschillen uit te sluiten.

Conclusies en aanbevelingen

Het belangrijk om te benadrukken dat op het moment dat de klinische studies werden gestart, er weinig informatie was over het gebruik van ACT bij Afrikaanse zwangere vrouwen. De ACTs die werden geëvalueerd in de studie (artemether-lumefantrine, artesunaat-amodiaquine, artesunaat-mefloquine en dihydroartemisinin-piperaquine) waren zeer doeltreffend in gebieden van hoge endemiciteit, met meer dan 90% efficiëntie binnen de vooraf opgedragen gelijkwaardigheidsmarges. Deze resultaten ondersteunen de richtlijnen van de WGO voor de behandeling van malaria tijdens het tweede en derde trimester van de zwangerschap.

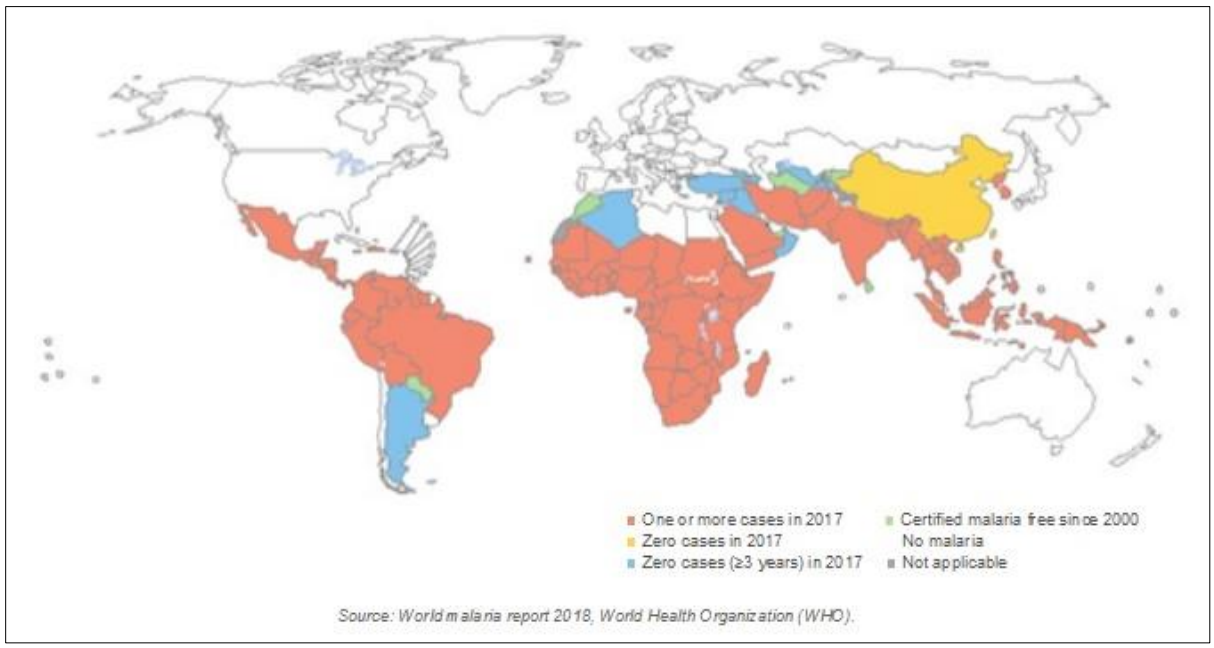
Hoewel deze studie proces bevestigt dat de beschikbare ACTs voor de behandeling van ongecompliceerde malaria veilig en doeltreffend zijn bij zwangere vrouwen, is er een noodzaak om te controleren hoe ze worden gebruikt binnen de gezondheidszorg, zowel openbare als particuliere, en de kwaliteit van de behandeling opgevolgd. Tot nu toe, hebben verschillende studies informatie verzameld over de veiligheid en de werkzaamheid van ACT.s De beschikbare gegevens zijn niet voldoende om zeldzame bijwerkingen te ontdekken. Dit kan worden opgelost door het opzetten van een geneesmiddelenbewakingsprogramma met een zwangerschapsregister waarin bijwerkingen na toediening van elke behandeling, met inbegrip van tegen anti-malaria drugs, zou worden geregistreerd. Een nationaal post marketing toezicht vereist echter aanzienlijke middelen en samenwerking tussen de financiers, beleidsmakers en onderzoekers.

Chapter 1: General Introduction

1.1 The global burden of malaria

Malaria is considered as one of the major public health problems in the world and a disease of poverty. It is among the leading cause of morbidity and mortality worldwide, severely affecting mostly under-five children and pregnant women. Although there were fewer malaria cases in 2017 (219 million cases) than in 2010 (239 million cases) [1], the decreasing trend stopped in 2016. Indeed, the estimated number of cases started to increase in 2016, about 216 million of cases in 2016 as compared to the 214 million of cases in 2015; there was a further increase in 2017, to 219 million cases [1]. The large majority (92%) of malaria cases are found in the WHO African region, accounting for about 200 million cases in 2017. A substantial proportion of malaria cases (*Plasmodium falciparum*) occurs in a few African countries, 25% of them in Nigeria, 11% in the Democratic Republic of Congo, and 5% in Mozambique [1]. In the WHO African Region, 10 countries reported >20% increase of the number of malaria cases during the period 2016-2017 [2]. Four countries have recently been certified malaria-free (achieving at least 3 consecutive years of zero indigenous cases of malaria) by WHO and these include Sri Lanka and Kyrgyzstan in 2016, and Paraguay and Uzbekistan in 2018 [3]. Figure 1 shows the malaria endemic and malaria-free countries as reported by national malaria control programs.

Figure 1. Malaria endemic and malaria-free countries [2]



Malaria deaths, most of them (93%) occurring in sub-Saharan Africa (sSA), have decreased globally from 607 000 in 2010 to 451 000 and 435 000 in 2016 and 2017, respectively. Children under 5 years of age are the most affected age group, accounting for 61% of all malaria deaths worldwide [1].

1.2 The Burden of Malaria in Pregnancy

Malaria in pregnancy can cause adverse pregnancy outcomes, including maternal and neonatal death [4], particularly in sSA where the most common malaria species is *Plasmodium falciparum* (Pf). Pregnant women and their new born babies are at higher risk and bear a high burden of malaria morbidity and mortality in endemic countries [5]. The pathophysiology of *P. falciparum* malaria in pregnancy depends partly on the capacity of infected red blood cells to sequester in the placenta intervillous space thanks to the binding of the parasite-derived protein VAR2CSA to the placental chondroitin sulphate A (CSA) receptor, especially early in gestation [6]. Such binding causes an

inflammatory response [7] that interfere with the normal exchange of nutrition between mother and fetus, resulting in intrauterine growth retardation [8] and other adverse birth outcomes [9–14], worsening with repeated infections [11, 13]. In areas of low transmission, malaria in pregnancy is usually symptomatic and the risk of severe malaria is high, resulting in abortion, stillbirths, premature deliveries and low birth weight. In areas of moderate to intense transmission, malaria-infected pregnant women are often asymptomatic, with primigravidae at higher risk of infection and of delivering low birth weight babies, who are at higher risk of dying before the first birthday compared to normal weight babies [8]. Therefore, malaria infection in pregnancy requires prompt diagnosis and treatment with effective and safe antimalarial drugs to prevent the associated adverse events in the mother and the unborn baby [15, 16].

There are few treatment options with good efficacy, acceptable tolerability and safety as pregnant women for long time have been systematically excluded from treatment trials and pharmacokinetic studies. Most available information has been collected in South East Asia while in sub-Saharan Africa, until recently, such information was still limited [15, 17–19]. Therefore, this vulnerable group lacked proven effective and safe antimalarial therapies that would mitigate the adverse effect malaria would have on their health and that of their offspring [20]. Current WHO guidelines recommend quinine with clindamycin for 7 days (or quinine alone if clindamycin is not available) for *P. falciparum* infections during the first trimester of pregnancy; in case of treatment failure or unavailability, an artemisinin-based combination therapy (ACT) or oral artesunate with clindamycin for 7 days may be used. For the second and third trimester of pregnancy, ACT can be used in the same manner as in non-pregnant adults [15, 21]. ACT include an artemisinin derivative, e.g. artemether, artesunate and dihydroartemisinin, which is rapidly eliminated, and a long-acting partner drug (lumefantrine, amodiaquine, mefloquine or piperaquine). Artemisinin derivatives

reduce rapidly the parasite density in the first few days while the long-acting drug mops out the remaining parasites [15] and provides post-treatment prophylaxis whose length depends on the drug's elimination half-life [22]. This is particularly useful in areas of high transmission as it prevents the emergence of new infections.

Some years ago, there was little information on the safety and efficacy of ACTs in sSA. This was the reason for setting up a large randomized controlled trial in 4 African countries (Burkina Faso, Ghana, Malawi and Zambia) aiming at assessing the safety and efficacy of 4 ACT, namely mefloquine-artesunate (MQAS), dihydroartemisinin-piperaquine (DHA-PQ), amodiaquine-artesunate (AQAS) and artemether-lumefantrine (AL), when administered to pregnant women in the second or third trimester with a *P. falciparum* malaria infection. These treatments are fixed dose combinations currently available on the market and have been found to have excellent cure rates among non-pregnant adults and children. To complement the efficacy and safety of these treatments when given to pregnant women, it is also important to determine any adverse effect they may have on the offspring.

1.3 Strategies to control malaria in pregnancy

1.3.1 Current control options

Pregnant women have a physiological lower immunity that makes them more vulnerable to malaria infections when compared with non-pregnant adults. This may result in a higher mortality and morbidity risk, either symptomatic malaria or placenta malaria, eventually causing adverse pregnancy outcomes [5]. In malaria endemic countries, there are about 50 million women becoming pregnant each year, and about half of them are from sSA, most of them in areas of stable transmission. WHO recommends prompt diagnosis and treatment [23], and the use of preventive interventions [5]. The latter include vector control measures such as Long-Lasting Insecticidal Nets

(LLIN), indoor residual spraying with insecticides (IRS), larval source management, and environmental modifications; and treatment-based interventions such as intermittent preventive treatment (IPTp) with sulfadoxine-pyrimethamine (SP) [24, 25]. LLIN when used in pregnancy have shown to reduce placental malaria and maternal anemia as well as the prevalence of low birth weight [26–28]. Recently, there have been concerns on increasing vector resistance to pyrethroids used on LLIN [29]. Strategies such as rational use of different classes of insecticides for IRS have therefore been proposed to manage insecticide resistance. Another approach would be the use of two different insecticides for LLIN (until recently no other class of insecticides could be put on LLIN) to reduce emergence and spread of resistance [30].

IPTp-SP is another preventive strategy that has proven to be effective in reducing the adverse effect of malaria during pregnancy, despite emerging SP resistance. Earlier studies for IPTp-SP in the second and third trimesters has shown this intervention reduces perinatal mortality, placental malaria, low birth weight and maternal illness [31, 32]. However, recent studies have shown that effectiveness of IPTp-SP may be waning, hence the need for alternative treatments such as an ACT to be used instead of SP [33]. DHAPQ has been effective in treating malaria in pregnancy and seems a promising alternative [33, 34]. Further studies are needed to assess the effectiveness of DHAPQ as IPTp.

1.4 Use of ACTs in pregnancy

Evidence on the use of ACT in pregnant women with malaria has increased substantially in the last decade [21]. Safety of ACTs during the first trimester of pregnancy for both the mother and the newborn seems acceptable although the information is still relatively limited [15, 35–37]. WHO currently recommends for the first trimester quinine plus clindamycin for seven days as first-line treatment or quinine monotherapy if clindamycin is not available; if quinine is unavailable, an ACT

or artesunate plus clindamycin can be used for *P. falciparum* uncomplicated malaria. This recommendation was based on 700 pregnant women exposed to ACT in which the risk of major congenital abnormalities when assessing the benefits and the potential risks was not increased[15]. The meta-analysis findings by Dellicour *et al* 2017 [35] however, have shown that these guidelines may need to be revised as recommended by the Malaria Policy Advisory Committee [38]. In most African national treatment guidelines, quinine rather than ACT is the treatment of choice for the first trimester of pregnancy [5].

Treatment of uncomplicated malaria during the second and third trimester of pregnancy is the same as for non-pregnant adults [15], meaning that the ACT used as for 1st line treatment can be used to treat pregnant women with *P. falciparum* malaria. These recommendations were based on the evidence from clinical trials done over the past decade which have shown that ACT are highly efficacious (>90% PCR-adjusted cure rates) [39–42], with the only exception of a study done in South-East Asia, on the Thai-Myanmar border, where artemether-lumefantrine treatment resulted in 87% efficacy (PCR-adjusted) [21]. All sub-Saharan African countries have already adopted and implemented ACT for the treatment of malaria in the second and third trimester of pregnancy. WHO recommends intravenous artesunate for severe malaria during pregnancy [15]. This is supported by a review on the treatment of severe malaria in pregnancy [43].

1.5 Malaria situation in Zambia

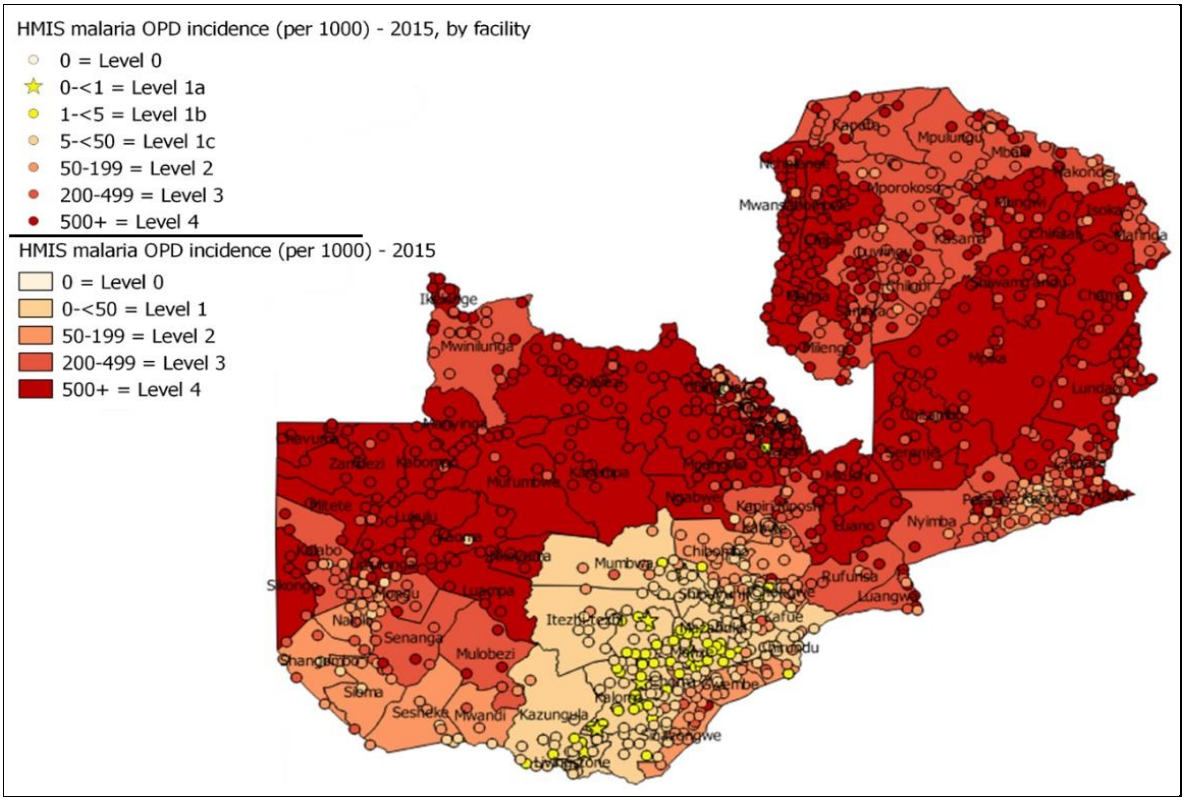
Zambia, a southern African country, is bordered by eight countries (Tanzania, Malawi, Mozambique, Zimbabwe, Botswana, Namibia, Angola and Democratic Republic of Congo). It is the 39th largest country in the world (based on total area), with a population of 15.5 million in 2015 ([44]. The climate is tropical, mostly classified as humid subtropical or tropical wet and dry. There are two main seasons, a rainy (November to April) and a dry (May to October) season. Zambia is

highly urbanized, with 44% of the population concentrated in a few urban areas while rural areas are sparsely populated. In 2014, the fertility rate was 5.3 while the average life expectancy was 55.6 years for females and 51.1 years for males. The infant mortality rate and neonatal mortality rate were 45 per 1000 live births and 24 per 1000 live births, respectively. Maternal mortality was reported at 398 deaths per 100,000 live births in 2013/2014 Zambia Demographic Health Survey (ZDHS) report [45].

1.5.1 Epidemiological stratification of malaria in Zambia

Zambia is divided in 5 levels of differing malaria epidemiology: no cases reported (no local transmission) in the health management information system (HMIS) in Lusaka and south-eastern areas (Level 0); very low malaria transmission with malaria prevalence below 1% in the south-eastern areas (Level 1); low malaria transmission with malaria prevalence between 1% and <5 % (Level 2); moderate malaria transmission with malaria prevalence of 5-15% (Level 3); high malaria transmission with malaria prevalence >15% (Level 4) (Figure 2).

Figure 2 Malaria epidemiological stratification and incidences in Zambia 2015



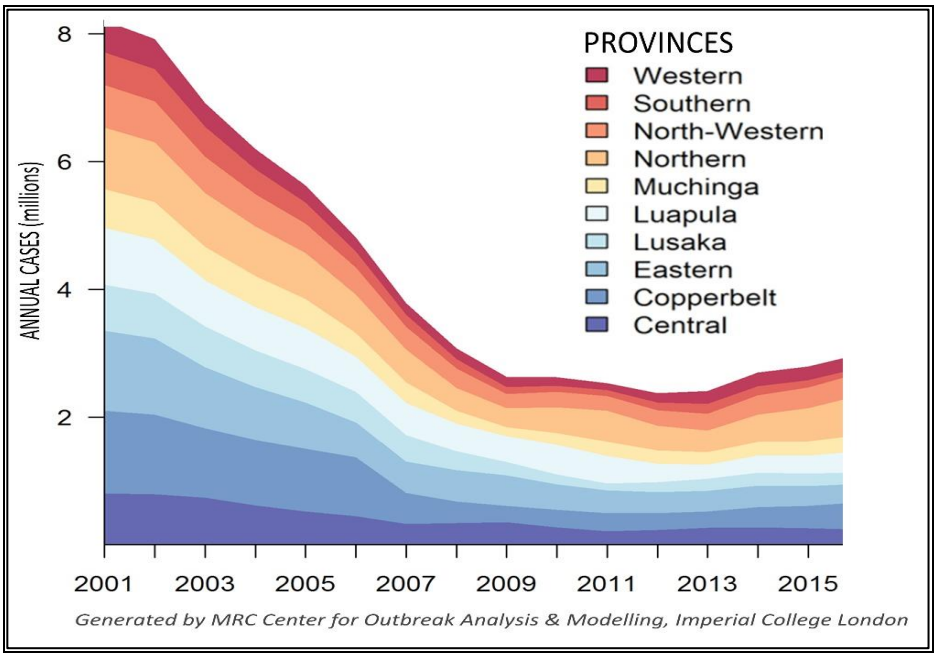
There are three species of *Anopheles* mosquitoes that can transmit malaria in Zambia, *An. funestus, An. gambiae s.s.* and *An. arabiensis.* These species are endophagic and highly anthropophilic. In areas of high transmission, in the north, the EIR is as high as 80 infective bites/person/year [46, 47]. They predominantly transmit *P. falciparum* (>95%) while other species account for some cases (*P. malariae* 3%, *P. ovale* 2%, *P.vivax* is rare) [48]. In most areas, especially northern Zambia, where prevalence of infection is above 15%, malaria transmission is complex and requires coordinated and site-tailored interventions to reduce the malaria burden [46].

1.5.2 Malaria trends in Zambia

In 2011, 4.54 million malaria cases were reported in Zambia. About half of them were diagnosed clinically, i.e. without parasitological diagnosis, whilst the rest was confirmed by microscopy or

RDT. Malaria prevalence in under-five children was 14.9% in 2012 and 19.5% in 2015, i.e. one in five children was infected with malaria, both in rural and urban areas [49] (Figure 2). By 2016, severe malaria (in-patient attendance) had decreased by 70%, from 15.8/1000 in 2010 to 6.1/1000. Similarly, malaria deaths decreased by a similar margin (by 70%), from 51.2 per 100,000 in 2010 to 31.0 per 100,000 in 2016.

Figure 3 Malaria annual cases by province and year in Zambia



1.6 Malaria treatment strategies in pregnancy in Zambia

1.6.1 Overview of malaria diagnosis in Zambia

One of the main strategies for malaria control and elimination is early diagnosis and prompt effective treatment (Figure 3). WHO recommends the parasitological diagnostic confirmation by quality microscopy and, in areas where it is not possible or available, immunochromatographic rapid diagnostic tests (RDT) in suspected malaria cases [15]. RDT detect circulating parasite–

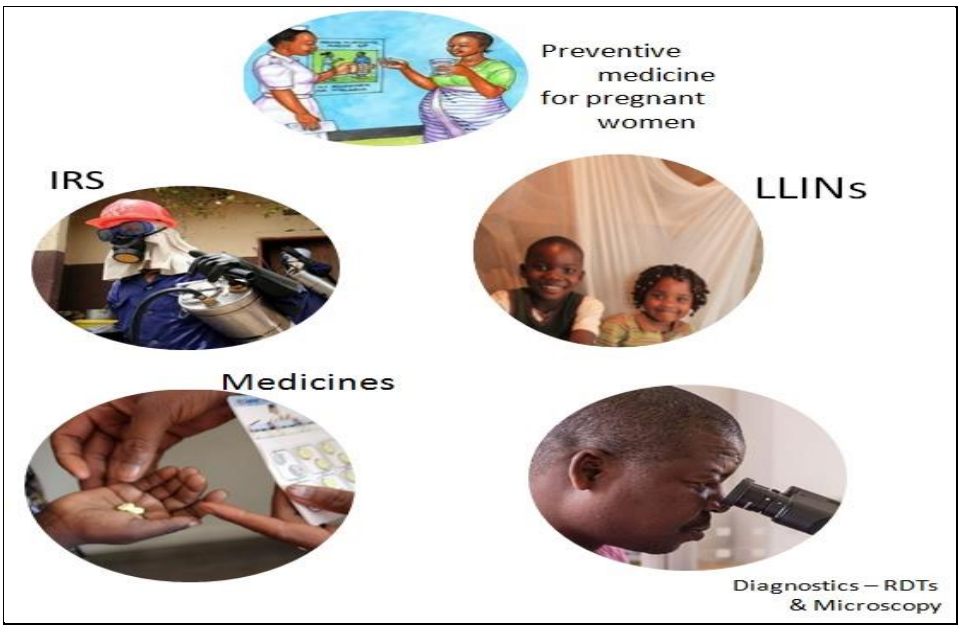
specific antigens or even enzymes (plasmodium lactate dehydrogenase and plasmodial aldolase) which are either genus or species-specific Molecular testing can also be used though usually employed in research settings. However, microscopy is the gold standard for the diagnosis of malaria in peripheral blood. Histological analysis is the reference standard for placental malaria. Some studies have been done to assess the use of RDTs to detect peripheral or placental malaria [50, 51]. A combination of RDTs based on HRP2 and the Plasmodium lactate dehydrogenase (pLDH) could have a role as tools to detect malaria in asymptomatic pregnant women in high transmission areas but its high false positivity limits its use as a single diagnostic tool [50]. In Zambia, treatment guidelines recommend confirmation of malaria by blood smear microscopy or RDT before treatment. The HMIS data has shown progressive improvement in malaria diagnosis confirmation. The HMIS in the first quarter of 2017 showed confirmed malaria cases accounting for 90% of total malaria cases, an improvement compared to 80% during the same period in 2016, and 78% in 2015 [52]. Although the number of unconfirmed cases has reduced, it shows that some cases of malaria are based on clinical judgement and may have no access to diagnostic testing. This means that a small proportion of women in high malaria transmission areas do not have access to diagnostic test during pregnancy and eventually prompt treatment. Effective malaria management requires a prompt and accurate diagnosis and this is important for high risk groups such as pregnant women [15]. This helps to reduce complications associated with malaria in pregnancy.

1.6.2 Malaria treatment strategies in Zambia

In Zambia, malaria in pregnancy in the first trimester is treated with quinine, considered to be safe and effective. It can also be used throughout pregnancy, in the second and third trimester. Some studies in Zambia have shown that AL is safe in the first trimester [49] and consequently has been included as an option in the absence of quinine where benefits outweigh risks. In the second and

third trimester, AL is recommended as first line of treatment while DHAPQ is an option if AL is not available. The second line of treatment in the second and third trimesters is quinine in all cases of treatment failure for first-line treatment. In severe malaria in pregnancy quinine is used in the first trimester while injectable artesunate is used in the second and third trimesters [48].

Figure 4 Malaria control strategies



1.7 Rationale

The risk of malaria is higher in pregnant women than in the general population, leading to higher morbidity and mortality in the mother, the fetus and the new born; maternal anemia and placental malaria may result in low birth weight, miscarriage, stillbirth and infant death [51]. Therefore, malaria in pregnancy needs to be diagnosed early and treated promptly with effective and safe antimalarial drugs to avert the adverse outcomes [15]. The safety, efficacy and pharmacokinetic data for these drugs are scarce because pregnant women have been systematically excluded from clinical trials for fear of teratogenicity and embryo-toxicity [20, 52]. This has complicated the generation of evidence-based recommendations for the prevention and treatment of malaria during pregnancy. In the past decade the Malaria in Pregnancy consortium and others have done research to increase the available data or knowledge on ACT use in pregnancy ([15, 21]. The WHO Malaria Policy Advisory Committee has made recommendations to have ACT first-line treatment options reviewed

for pregnant women in first trimester, for ACT timely inclusion in the treatment guidelines [53]. All sub-Saharan African countries have already adopted and implemented such recommendation. However, there is limited knowledge on the effect of ACT use on pregnancy outcomes and infant safety. Most of the available information originates from South-East Asia while there is not enough data from sSA [18, 19, 54]. Therefore, urgent and vigorous assessment of the safety and efficacy of ACTs in pregnancy is needed [39, 55]. To confirm this expert opinion, the study was done assess the safety and efficacy of artemisinin-based combinations (AL, ASAQ, MQAS and DHAPQ) in pregnant women in the second and third trimester with confirmed *P. falciparum* malaria infection.

The risk of malaria in pregnancy may lead to low birth weight which is the single most important risk factor (of neonatal mortality and morbidity), and eventually increases the risk for neonatal and infant mortality [56, 57]. Little information is known about the effects of ACT on the malaria infection during pregnancy on the offspring's first year of life as children are rarely followed up due to difficulties of adequate monitoring [58]. Therefore, clinical research is needed to improve knowledge on the effect of ACT on pregnancy outcome and neonatal and infant morbidity and mortality. Finally, as study results are influenced by malaria endemicity and malaria control measures in the study area, we also assessed the malaria burden in the Zambian study site by carrying out a cross-sectional survey.

1.8 Scientific objectives

The main objective of this thesis was to assess the safety and efficacy of artemisinin-based combination therapies (ACT) (artemether-lumefantrine, amodiaquine-artesunate, mefloquine-

artesunate and dihydroartemisinin-piperaquine) for the treatment of malaria in the second and third trimester in African pregnant women. Specifically

a. To characterize the malaria endemicity in Nchelenge district in Zambia;

b. To determine the safety and efficacy of three artemisinin-based combinations, namely mefloquine–artesunate (MQAS), dihydroartemisinin–piperaquine (DHA–PQ); and artemether–lumefantrine (AL), in pregnant women in the second or third trimester with a confirmed *P. falciparum* malaria infection in Zambia

c. To assess the safety of ACT on the foetus and new-born.

1.9 Study sites

The study was a multi-centre study; in Zambia the trial was done at Kashikishi and Nchelenge health centres, in Nchelenge district, Luapula province. The other sites for the PREGACT study (randomized, open label on the safety and efficacy of four artemisinin-based treatments, namely AL, ASAQ, ASMQ, and DHAPQ, when administered to African pregnant women with *P. falciparum* malaria during second and third trimester) were Nanoro and Nazoanga in Burkina Faso, Efiduase, Ejisu and Juaben in Ghana, and Chikwawa in Malawi.

1.10 Organisation of the thesis

Chapter 1: This is an introduction and rationale of this thesis. It describes the malaria burden world-wide, in sSA, and in Zambia. It also presents malaria diagnosis and treatment guidelines in Zambia, with special attention to pregnant women.

Chapter 2: This chapter defines the malaria burden in Nchelenge, Zambia, where the main clinical trial was carried out.

Chapter 3: This is the chapter on the methods for the safety and efficacy of ACT in malaria in pregnancy, a multi-center randomized controlled trial.

Chapter 4: This is a chapter on the efficacy, safety and risk of recurrent malaria in pregnant women who receive an ACT in Zambia.

Chapter 5: This chapter reports the outcomes of pregnancy and infant mortality after ACT: a cohort study

Chapter 6: In this chapter the conclusions and further perspectives in malaria in pregnancy are discussed.

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Chapter 2: Defining the malaria burden in Nchelenge District, northern Zambia using the World Health Organization malaria indicators survey.

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2.1 Abstract

Background

Malaria is considered as one of the major public health problems and among the diseases of poverty. In areas of stable and relatively high transmission, pregnant women and their newborn babies are among the higher risk groups. A multicentre trial on the safety and efficacy of several formulations of artemisinin-based combination therapy (ACT) during pregnancy is currently on-going in four African countries, including Zambia, whose study site is in Nchelenge district. As the study outcomes may be influenced by the local malaria endemicity, this needs to be characterized. A cross-sectional survey to determine the prevalence and intensity of infection among <10 years old was carried out in March-April 2012 in Nchelenge district.

Methods

The sampling unit was the household where all children < 10 years of age were included in the survey using simple random household selection on a GPS coded list. A blood sample for determining haemoglobin concentration and identifying malaria infection was collected from each recruited child.

Results

Six hundred thirty households were selected and 782 children tested for malaria and anaemia. Prevalence of malaria infection was 30.2% (236/782), the large majority (97.9%, 231/236) being *Plasmodium falciparum* and the remaining ones (2.1%, 5/236) *Plasmodium malariae*. Anaemia, defined as haemoglobin concentration <11g/dl, was detected in 51.2% (398/782) children.

Conclusion

In Zambia, despite the reported decline in malaria burden, pockets of high malaria endemicity, such as Nchelenge district, still remain. This is a border area and significant progress can be achieved only by concerted efforts aimed at increasing coverage of current control interventions across the border.

Keywords: Malaria indicator survey, Zambia, Children, Endemicity

2.2 Background

Malaria remains a major public health problem and a disease of poverty. In areas of stable and relatively high transmission, pregnant women and their newborn babies bear a high burden of malaria morbidity and mortality [1].

In Zambia, sulphadoxine-pyrimethamine (SP) is used for intermittent preventive treatment in pregnancy (SP-IPTp) during the second and third trimester. Until recently, malaria in pregnancy was treated with quinine, which has now been replaced by artemether-lumefantrine (AL) for women in the 2nd and 3rd trimester in pregnancy; quinine is used for the management of malaria during the 1st trimester and for severe cases [2,3]. To provide additional evidence on safety and efficacy of artemisinin-based combination therapy (ACT) during pregnancy, a multicentre trial (PREGACT, Clinical Trials identifier NCT00852423) is currently on-going in four sub-Saharan African countries, including Zambia.

The Zambian study site is located in Nchelenge district, Luapula province, at the border with the Democratic Republic of Congo (DRC). This is an area of intense malaria transmission as the prevalence of malaria infection in children aged <5 years was 21.8% in 2008 (national average 10.3%) and that of anaemia (Haemoglobin [Hb] <11 g/dl) 55.9% (national average 49.0%) [4]. In 2010, the prevalence in under 5 years was as high as 50.5% in some age groups and anaemia at 79.7% [5]. The same malaria indicator survey provided information on the coverage of key malaria interventions, i.e. prompt effective case management, insecticide-treated mosquito nets (ITNs), indoor residual spraying (IRS), and IPTp [6]. Considering that some of the end points of the trial on ACT in pregnancy, e.g. incidence of recurrent infections, can be influenced by the local malaria endemicity [1] and that no new information had been collected since 2008, a malariometric survey was carried out in Nchelenge district in March-April 2012 [7,8].

The rationale of this study was that it gives a more recent data on the prevalence of malaria in a high endemic region. In places like Zambia, where overall figures indicate that malaria is declining, there are still pockets of high endemicity, which are complicated by perfect breeding grounds for anopheles mosquito and cross-border human movements. This could affect national and global malaria control strategies. The primary objective was to estimate the prevalence and risk factors for malaria in Nchelenge, Luapula Province, Zambia.

2.3 Methods

Nchelenge district, Luapula province, is located on the swampy shores of Lake Mweru, and has a population of 178,000 inhabitants, mostly peasant farmers and/or fishermen. The district has one first level hospital, ten rural health centres and two health posts. The survey was done in the catchment areas (total population of 43,105) of the two health centres, Kashikishi and Nchelenge, where the PREGACT trial is currently implemented.

The household was taken as the sampling unit. The households in Nchelenge and Kashikishi health centre catchment areas were previously numbered using the Global Positioning System (GPS). Then the households were selected according to a pre-defined, computer-generated list of random numbers [9]. In selected households, all children below the age of 10 years were included in the survey after having obtained the signed informed consent from the parent/guardian. Two questionnaires were administered, the household questionnaire and the parent/guardian's questionnaire which were adapted from those recommended for the malaria indicators survey [10,11]. The household questionnaires include information on basic demographic and socioeconomic characteristics of the households.

The sample size was determined by assuming that malaria prevalence was 22% [4]; using Roasoft sample size calculator [12], 261 sampling units or households were needed to estimate such

prevalence at 5% precision with a 95% confidence interval. Nevertheless, because of possible clustering within households, was assumed a conservative design effect of 2 was assumed, and a 20% adjustment was made for non-response (from household refusals or abandoned households). Based on these assumptions, it was estimated that 630 households would provide an estimate of prevalence with the desired precision.

All children <10 years of age in the selected households were asked to be included in the survey. Each study, subject was assigned a unique identifier for identification of biological samples and questionnaire data and to guarantee confidentiality. Information on bed net use, which was by observation, including long-lasting insecticidal nets (LLIN) or previous indoor residual spraying (IRS) was collected onto the structured questionnaires. A blood sample for a malaria rapid diagnostic test (RDT), (SD BIOLINE, Malaria Ag P.f., Standard Diagnostic Inc, Korea), thick blood film and for measuring haemoglobin (Hb) (Hemocue 301 machine, Angelholm, Sweden) was collected by finger prick. Children with fever (axillary temperature ≥37.5°C) and a positive RDT or only a positive RDT were treated with AL, the first-line treatment in Zambia. Children found to be sick or anaemic were referred to the health care centres for further management.

Thick blood films were stained with 10% Giemsa for 10 minutes. Two hundred high power fields were read before declaring the slide negative. The parasite density was determined by counting the number of parasites against 200 white blood cells (WBC) and assuming 8,000 WBC per µl. All stained slides were read by two independent microscopists unaware of the RDT results. In case of discrepant results between the two independent microscopists, a third microscopist read the slide until reaching a satisfactory agreement on positivity/negativity or the parasite density.

Data were double entered, cleaned and analysed using Epi Info™ (Center for Disease Control and Prevention, version 7.1.1.14). Further multivariable logistic regression analyses were performed

using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Mean and standard deviation were computed to describe the distribution of continuous variables, while the distribution of categorical variables was described using counts and proportions. Geometric mean and median were computed for continuous variables that did not follow a normal distribution: parasite densities and Hb concentrations. Continuous variables were compared between age groups using t-tests and categorical variable proportions by $\chi 2$ test or Fisher's exact test. To account for uncertainty in the prevalence estimates, the 95% confidence intervals were computed. Anaemia was defined as $\text{Hb} < 11$ g/dl and created a dichotomous variable where $\text{Hb} \geq 11$ g/dl was defined as non-anaemic. The risk of anaemia and malaria was estimated by fitting a multivariable logistic regression model and adjusted for possible risk factors to compute odds ratios (OR) and 95% confidence intervals (95% CIs). The variables included in the multivariable logistic regression model were selected a priori via extensive search of peer-reviewed literature. A two-sided p-value of ≤ 0.05 was used as threshold for statistical significance. The 'survey logistic' procedure in SAS 9.2 (SAS Institute Inc., Cary, NC, USA) with the option 'clustering' to account for the design effect of clustering within households.

Before the survey, the study protocol was approved by the scientific and technical committee (STC) of Tropical Diseases Research Centre (TDRC), the TDRC Ethical committee and the Ministry of Health. Participation in the survey was voluntary and participants were free to withdraw any time during the capture.

2.4 Results

669 households were screened; 39 households were not responsive (21 were not available while 18 refused to participate). There was a 630/669 (94.2%) response. At this rate, the results presented are less biased. Out of the households that were responsive majority reported tubed well or borehole as

main source of drinking water (87.5, 95% CI, 84.6-89.9). 90.0% (95% CI, 87.3-92.2) households had open pits or pit latrines without slabs as toilet facilities. 96.0% (95% CI, 94.1-97.4) households reported charcoal as the main source of household fuel. Most houses were made of finished brick walls (84.8%, 95% CI, 81.7-87.4) and grass thatched roofs or sticks and mud roofs (94.6%, 95% CI). In terms of owning durable goods, 83.5% (95% CI, 79.7-87.6) households owned a landline telephone or a cell phone, while a majority (64.3%, 95% CI, 60.4-68.0) also owned a bicycle (Table 1).

Table 1 Baseline and selected socio-economic characteristics in children from Nchelenge District, Zambia (95%CI)

Variable	%
Males (n/N)	40.5 (317/782)
Median Age in years (IQR)	5 (3-8)
Bed net ownership (95%CI)	35.9 (32.6-39.4)
Slept under a bed net last night (95%CI)	78.3 (73.5-83.1)
Taken anti-malarial treatment during last month (95%CI)	63.7 (60.2-67.0)
Indoor residual spraying last year (95%CI)	11.1 (9.1-13.6)
Mother's education (95%CI)	
Primary	77.0 (73-82)
Secondary	20.0 (16-25)
Tertiary	2.0 (1-4)
Average number of individuals/household (95%CI)	3.2 (3.1-3.3)
Source of drinking water (95%CI)	
Pipe into dwelling/yard/plot/public tap	1.4 (0.4-4.5)
Tube well/borehole	87.5 (84.6-89.9)
Dug well	6.5 (4.3-10.0)
Surface water: river/dam/lake/spring/pond	4.1 (2.0-8.2)
Toilet type (95%CI)	
Flush or pour flush	2.6 (1.1-6.0)
Ventilated improved pit latrine/Pit latrine with slab	6.0 (3.8-9.5)
Pit latrine without slab/open pit	90.0 (87.3-92.2)
Compositing toilet/Hanging toilet latrine/toilet	0.8 (0.1-3.6)
House wall material (95%CI)	
Natural wall	5.7 (4.0-8.7)
Rudimentary wall	4.4 (2.6-8.3)
Finished wall bricks	84.8 (81.7-87.4)
Finished wall cement/stone with lime/cement blocks	4.6 (2.4-8.7)
Roofing material (95%CI)	
Natural roof thatch/sticks and mud	94.6 (91.7-97.61)
Rudimentary roof	0.3 (0.1-1.27)
Finished roof iron/wood/calamine/concrete/shingles	4.6 (2.8-8.4)
Source of fuel (95%CI)	
Electricity/Biogas/Kerosene/Coal/lignite	1.0 (0.1-4.6)
Charcoal	96.0 (94.1-97.4)
Firewood/straw	1.4 (0.7-2.8)
Household durable goods (95%CI)	
Radio	22.2 (19.1-25.7)
Television	3.3 (2.1-5.1)
Mobile or landline phone	83.5 (79.7-87.6)
Refrigerator	1.8 (0.9-3.2)
Bicycle	64.3 (60.4-68.0)
Motor cycle/scooter	0.48 (0.1-1.5)
Car/truck	1.1 (0.5-2.4)

Seven hundred and eighty-two children distributed in 630 households were enrolled in the study; girls (59.5%, 465/782) were more represented than boys (40.5%, 317/782) ($p < 0.005$). The median

age was five years (IQR, 3–8), with the 7–10 years age group as the largest age group (37.9%, 296/782). Only about a third of children sampled (35.9%, 281/782, 95% CI, 32.6-39.4) came from a household with a bed net. Among those coming from a household with bed net 78.3% (220/281, 95% CI, 73.5-83.1) reported to have used it the previous night. Coverage of IRS was even lower, only 11.1% (87/782, 95% CI, 9.1-13.6) households had been sprayed by the local district health team (Table 1).

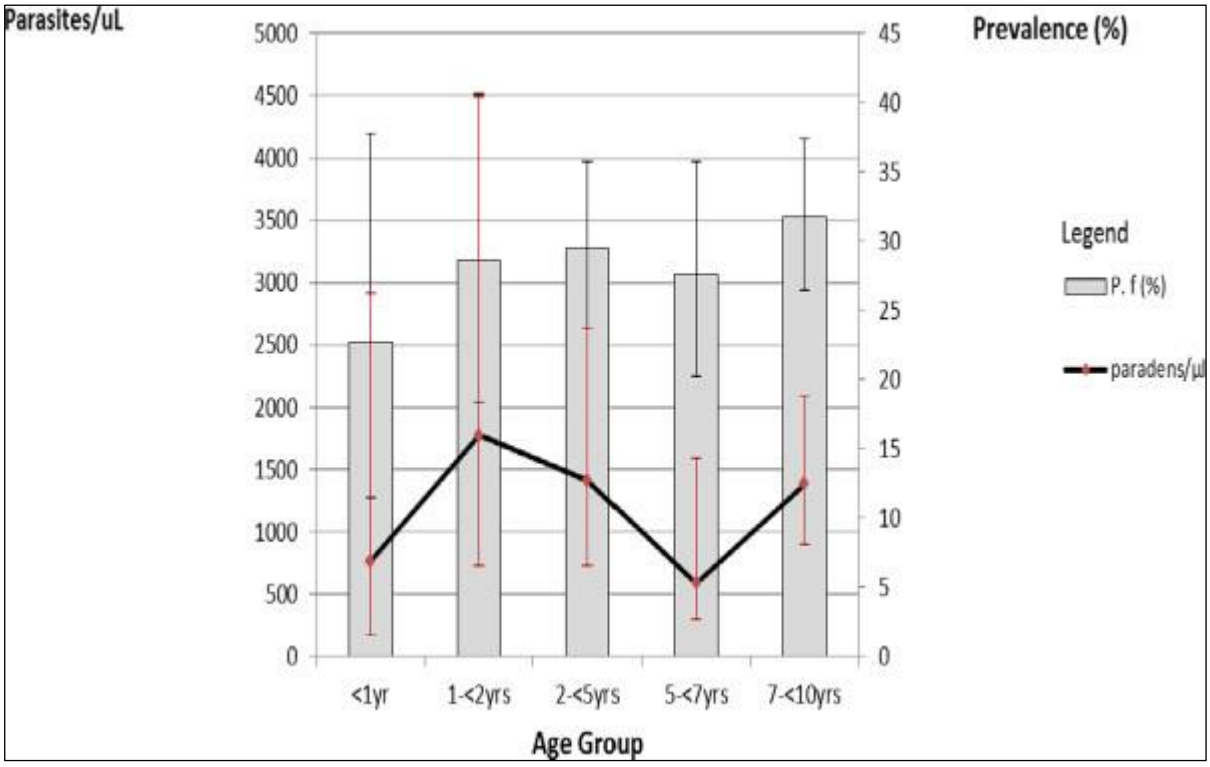
The prevalence of malaria infection was 30.2% (236/782), the large majority (98.0%, 231/236) Plasmodium falciparum and the remaining ones *Plasmodium malariae* (Table 2). No mixed infection was found. Plasmodium falciparum prevalence was the lowest in the <1-year-old children while it varied between 22.7% (1 years old) and 31.8% (7–10 years old) in the other age groups (Table 2 and Figure 1).

Table 2 Malariometric indicators by age in children under 10 years from Nchelenge District, Zambia(N)

Variable	Overall (782)	<12 m (44)	12-23 m (70)	24-59 m (234)	5- < 7 yrs (138)	7- < 10 yrs (296)	p
P. falciparum% (n)	29.5 (231)	22.7 (10)	28.6 (20)	29.5 (69)	27.6 (38)	31.8 (94)	0.5
P. malariae% (n)	0.7 (5)	0.0	2.9 (2)	0.4 (1)	0.0	0.7 (2)	0.4
Gametocytes Pf% (n)	1.5 (12)	0.0	1.4 (1)	0.9 (2)	1.5 (2)	2.4 (7)	0.4
Mean Parasite density par/µl (95% CI)	1,250 (944-1740)	765 (179-2920)	1770 (726-4490)	1410 (734-2640)	590 (299-1590)	1,380 (901-2090)	0.7
Median haemoglobin, g/dl (IQR)	10.7 (10.6-10.8)	11.0 (10.6-11.4)	10.6 (10.2-11.1)	10.7 (10.5-11.0)	10.7 (10.4-11.0)	10.6 (10.4-10.9)	0.4
Anaemia (Hb < 11 g/dl)% (n)	51.2 (398)	47.7 (21)	55.7 (39)	47.9 (112)	49.3 (67)	54.1 (159)	0.4

m=months, yrs=years. p-value used a t-test to compare proportions.

Figure 1 Mean parasite density and prevalence of infection by age group in children under 10 years of age in Nchelenge, Zambia (2013).



The geometric mean parasite density for P. falciparum was 1,250 parasites/µl (95% CI, 944–1,740) and varied with age, with the highest value in the 1–2 years old group, 1,770 parasites/µl (95% CI, 726–4,490), and the lowest, 590 parasites/µl (95% CI, 299–1,590) in the 5–7 years old group ($p = 0.7$) (Figure 1). Gametocytes were found in 12 (1.5%, 95% CI, 0.8-2.7) children, all of them P. falciparum, and were more frequent in the older age group (Table 2). No gametocytes were found in infected infants (<1-year-old).

Mean Hb was 10.7 g/dl (95% CI, 10.6-10.8), ranging between 1.2 g/dl and 15.4 g/dl. About half (51.2% (398/782), 95% CI, 47.6-54.7%) of the children were anaemic ($Hb < 11$ g/dl) while 7.4% (58/782), (95% CI, 5.6-9.5) of them were severely anaemic ($Hb < 8$ g/dl). Mean Hb and anaemia did not vary with age ($p = 0.7$). Malaria infection was significantly associated with anaemia

(AOR = 1.64, 95% CI, 1.20-2.24, p < 0.001) and such risk tended to be higher in children with the highest parasite densities (1.30, 95% CI, 0.07-22.74, p = 0. 86) (Table 3).

Table 3 Predictors for anaemia and malaria in children under 10 years from Nchelenge District, 2012, Zambia

Risk factors for anaemia	**Class**	**Unadjusted OR (95%CI)**	**Adjusted OR (95%CI)**	**P-value**
Malaria	+	2.11(1.56-2.85)	1.64 (1.20-2.24)†	<0.0001
	--	1.00	1.00	
Age-group	> 12 m	0.80 (0.42-1.53	1.22 (0.03-44.32)	0.81
	12-23 m	0.10 (0.57-1.65)	3.45 (0.50-23.94)	0.40
	24-59 m	0.80 (0.56-1.14)	1.83 (0.58-5.79)	0.91
	5- < 7 yrs	0.80 (0.54-1.19)	1.93 (0.38-0.89)	0.87
	7- < 10 yrs	1.00	1.00	-
Gender	Males	1.11 (0.83-1.49)	0.52 (0.19-1.40)	
	Females	1.00	1.00	0.46
Parasite density	>2000	1.20 (0.68-2.10)	1.30 (0.07-22.74)	0.86
	≤2000	1.00	1.00	-
Risk factors for malaria infection				
Age-group	> 12 m	1.00	1.00	-
	12-23 m	1.36 (0.57-3.26)	1.13 (0.17-7.58)	0.90
	24-59 m	1.42 (0.67-3.04)	4.23 (0.90-19.98)	0.06
	5- < 7 yrs	1.29 (0.58-2.87)	6.00 (1.22-29.51)	0.03
	7- < 10 yrs	1.58 (0.75-3.34)	2.94 (0.62-13.86)	0.17
Female	Males	1.00	1.00	
	Females	1.13 (0.82-1.54)	1.33 (0.73-2.42)‡	0.36
Mother education	Primary	1.00	1.00	
	Secondary	0.89 (0.51-1.67)	1.29 (0.53-3.13)‡	0.59
	Tertiary	0.83 (0.17-4.17)	0	0.97
Indoor residual spraying	Yes	1.00	1.00	
	No	0.80 (0.49-1.29)	0.81 (0.38-1.75)‡	0.59
Bed net use	Yes	1.00		
	No	1.48 (0.67-3.26)	1.70 (0.73-3.95)‡	0.22

† adjusted for age, gender, and parasite density. ‡ adjusted for indoor residual spraying, age, gender and mother education. Age group was adjusted for bed net use and indoor residual spraying.

The risk of malaria infection was neither different in bed net users (AOR = 0.59, 95% CI, 0.31-3.95, p = 0.22) nor in households sprayed the previous year (OR = 1.23, 95% CI, 0.77-2.01, p = 0.36). The age specific odds show that children 3- < 5 years and those from 5- > 7 years who did not use bed nets the previous night and are in homes not sprayed are more likely to have a malaria infection than those who use bed nets and have their homes sprayed (OR = 6.00, 95% CI, 1.22-29.51, p = 0.03; OR = 4.23, 95% CI, 0.90-19.98, p = 0.06) (Table 3).

2.5 Discussion

This malaria indicator survey, using the World Health Organization (WHO) survey tools, provides a recent estimation of the malaria burden in Nchelenge district. The prevalence of both malaria infection and anaemia was similar to those found in national surveys for Luapula province [13], a result that contrasts with the overall declining trend of malaria in Zambia [14]. Prior national surveys have reported a higher resurgence of malaria in Luapula province than other regions [5]. This could be attributed to seasonality of malaria.

It is important to notice that Nchelenge borders the Democratic Republic of Congo (DRC), with substantial population movements across the borders, and is difficult to reach due to poor terrain, possibly explaining the low coverage of control interventions. Indeed, only a third of the children included in the survey stated to have a bed net and even less used it the previous night. As IRS, only a small proportion of the sampled houses had received this intervention so that any impact on the local vector population is unlikely. It is unknown what the coverage of control interventions may be on the other side of the border but probably not higher than that in Nchelenge, more likely lower. In these conditions, the prevalence of malaria and anaemia found by this survey are not surprising as they reflect the extremely low coverage of ITN and IRS. However, even in case of a better coverage, prevalence may still have been high as the important population movements between

Zambia and DRC would maintain the local human reservoir and hence malaria transmission. A significant reduction in the malaria burden in this region may be achieved only through a close collaboration between the national control programmes of both countries. Other countries sharing borders like South Africa, Mozambique and Swaziland in a programme called the Lubombo Spatial Development Initiative (LSDI) have achieved spectacular successes in reducing cross-border malaria burden [15].

The mean parasite density in the children included in this survey, most of them asymptomatic, was relatively high, particularly in the 1–2 years old age group, confirming Nchelenge as an area of intense malaria transmission. Prevalence of malaria would probably have been higher if molecular methods to detect infection had been used. Following continuous exposure to malaria infection, children develop partial immunity and are able to tolerate relatively high parasite densities. Access to treatment seems high as more than 60% of children's parents stated they had taken an antimalarial treatment the month prior to the survey. However, information on the quality and the source of treatment was not collected during the survey so that it is impossible to know if this was of adequate quality and dosage. The apparently frequent administration of antimalarial treatment is just an indicator of the importance of malaria in this setting.

The prevalence of gametocytes was relatively low though probably a higher number of children with gametocytaemia could have been identified by using molecular methods. Detecting gametocytes in thick blood smears, particularly when present at low densities, is a challenge even for experienced microscopists [16]. Indeed, detecting of P falciparum gametocytes by microscopy can miss most of the gametocyte carriers [17-19] as they are present at an extremely low density though they can still be infectious to mosquitoes [20-22]. This was the weakness of this study in which gametocytes were examined on thick smears.

Coverage by bed nets and IRS was low and this may explain the higher prevalence of malaria infection. However, recent data seem to indicate a problem of emerging resistance to pyrethroids and DDT among the vector population in Nchelenge [23]. Neighbouring countries, such as Mozambique, have found similar patterns of resistance to pyrethroids [24]. If this is confirmed, increasing coverage of both insecticide-treated bed nets and IRS would have a lower than expected impact as ITN would just act as mechanical barriers.

The logistic multivariable analyses found that children did not use bed nets and are in households not sprayed have a higher risk of malaria infection in the age 2 to 7 years. This is the age group that is just weaned from the mother and would not be under a bed net nor cover themselves from mosquito bites. This study also confirms that malaria affects haemoglobin levels in communities where children bear the brunt of the malaria burden [25,26] and high levels of anaemia are seen as in other parts of Africa as Kenya and Tanzania [27,28]. Further, children with malaria infection have a higher risk of anaemia, having higher anaemia levels than those without malaria affecting children in many ways including poor performance [29].

The limitation of this work is that it was carried out in relatively smaller or narrow geographical focus. This could over- or under-estimate the bed net or IRS coverage and indeed malaria prevalence. Since malaria presents with some seasonality, the study was done at the end of the rainy season but there could be differences in the dry season. Sociodemographic data were collected via self-reporting and, therefore, self-report, recall bias, and social desirability bias cannot be ruled out. Cognisant of this potential bias, the study interviewers made efforts to enhance truthful reporting. The nature of the cross-sectional survey does not permit causal inference of the observed association.

2.6 Conclusion

In conclusion, even though overall figures indicate that malaria is declining in Zambia, there are still pockets of high endemicity such as the Nchelenge district. This district is on the shores of Lake Mweru and neighbouring DR Congo. Malaria will be controlled only by concerted efforts across borders aiming at increasing coverage of preventive intervention and prompt access to diagnosis and antimalarial treatment.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors contributed to the design of the study and assisted with data interpretation. MN and PM coordinated the study and supervised the enrolment, data collection and entry. PM did the laboratory analysis and study participants enrolment. JPVG, MM and UDA participated in analysis of data. All authors participated in the preparation of the manuscript and approved the final version. All read and approved the final manuscript.

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Chapter 3: Safe and efficacious artemisinin-based combination treatments for African pregnant women with malaria: a multicentre randomized control trial – Methods paper

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3.1 Abstract

Background

Asymptomatic and symptomatic malaria during pregnancy has consequences for both mother and her offspring. Unfortunately, there is insufficient information on the safety and efficacy of most antimalarials in pregnancy. Indeed, clinical trials assessing antimalarial treatments systematically exclude pregnancy for fear of teratogenicity and embryotoxicity. The little available information originates from South East Asia while in sub-Saharan Africa such information is still limited and needs to be provided.

Design

A Phase 3, non-inferiority, multicentre, randomized, open-label clinical trial on safety and efficacy of 4 ACT when administered during pregnancy was carried out in 4 African countries: Burkina Faso, Ghana, Malawi and Zambia. This is a four arm trial using a balanced incomplete block design. Pregnant women diagnosed with malaria are randomised to receive either amodiaquine-artesunate (AQ-AS), dihydroartemisinin-piperaquine (DHA-PQ), artemether-lumefantrine (AL), or mefloquine-artesunate (MQAS). They are actively followed up until day 63 post-treatment and then monthly until 4–6 weeks post-delivery. The offspring is visited at the time of the first birthday. The primary endpoint is treatment failure (PCR adjusted) at day 63 and safety profiles. Secondary endpoints included PCR unadjusted treatment failure up to day 63, gametocyte carriage, Hb changes, placenta malaria, mean birth weight and low birth weight. The primary statistical analysis will use the combined data from all 4 centres, with adjustment for any centre effects, using an additive model for the response rates. This will allow the assessment of all 6 possible pair-wise treatment comparisons using all available data.

Discussion

The strength of this trial is the involvement of several African countries, increasing the generalisability of the results. In addition, it assesses most ACTs currently available, determining their relative '-value-' compared to others. The balanced incomplete block design was chosen because using all 4-arms in each site would have increased complexity in terms of implementation. Excluding HIV-positive pregnant women on antiretroviral drugs may be seen as a limitation because of the possible interactions between antiretroviral and antimalarial treatments. Nevertheless, the results of this trial will provide the evidence base for the formulation of malaria treatment policy for pregnant women in sub-Saharan Africa.

Trial registration: NCT00852423

Keywords: Artemisinin-based therapy, Malaria in pregnancy, Pregnant women, Malaria, Sub-Saharan

3.2 Background and Rationale

The risk of malaria is higher in pregnant women than in the general population. There is insufficient information on the safety and efficacy of most antimalarial drugs in pregnancy [1] as they are systematically excluded from clinical trials for fear of teratogenicity and embryotoxicity [2]. This has complicated the generation of evidence-based recommendations for the prevention and treatment of malaria during pregnancy. Though the experience on the use of ACTs and their safety and efficacy in pregnancy is increasing (over 1,000 documented pregnancies, mainly in South East Asia), such information is still limited in sub-Saharan Africa [3]. Preclinical data indicate that the artemisinin derivatives were embryotoxic and potentially teratogenic in several animal species, without maternal toxic effects or impaired fertility [2]. More recent studies confirm these findings. One important aspect is that the critical window for drug exposure is approximately 10–14 days in the rat and their extrapolation to humans would indicate a sensitive period of weeks 2–6 of pregnancy [2]. There is increasing experience with the use of artemisinin derivatives in the second and third trimesters and there have been no reported adverse effects on the mother or foetus [4]. Despite limited data the World Health Organization (WHO) recommends effective artemisinin-based combination treatments (ACT) in the second and the third trimester and several African countries are already implementing it [3].

We propose to assess the efficacy and safety of the most important ACTs currently available, namely artemether-lumefantrine (AL), amodiaquine-artesunate (AQ-AS), mefloquine-artesunate (MQ-AS) and dihydroartemisinin-piperaquine (DHA-PQ). The choice of ACTs is based on several criteria, including known treatment efficacy in children, safety in pivotal phase-3 trials in children and adults, practicality of the dosing regimen (duration, e.g. 3 vs. 7 days), fixed dose combinations, drug tolerance, current availability in the population and affordable cost. The rationale for

contemporaneously testing several drug regimens is to shorten the time of data collection and determine the relative 'value' of each treatment, providing the basis for an informed choice by malaria control programmes and policy makers.

Reliable data on the safety and efficacy of ACTs in pregnant women can be rapidly collected only within the context of a randomized-controlled trial. The production of such a large dataset will advance considerably the knowledge on the treatment of malaria in pregnancy in a relatively short period of time compared to pregnancy registers. One of the major issues for this trial is the altered pharmacokinetic of antimalarial drugs during pregnancy and the influence on the outcome of treatment. There has been a recent increase in trials that measure the pharmacokinetics of antimalarial drugs in pregnant women [5]. Recent information on the pharmacokinetics of both artemisinins [6] and partner drugs in the ACT is reassuring as amodiaquine [7], mefloquine [8] and piperaquine [9] do not seem to need dose adjustment. Nevertheless, the pharmacokinetic of lumefantrine (when administered as co-formulated artemether-lumefantrine) seems altered in pregnancy [10]. The clinical implications of the PK findings may not be clear from small rich PK studies alone and, unless the effects are important, may not be apparent in a pilot study in one site. Therefore, the proposed study aims not only at collecting safety and efficacy data on ACTs in a systematic and standardized manner but also explanatory variables (pharmacokinetic and in vitro drug sensitivity) that may help in interpreting the observed results.

ACTs have been shown to be extremely efficacious in children [11]. Efficacy is determined by the drug partnering the artemisinin derivative and, for artesunate–mefloquine, artemether–lumefantrine, and dihydroartemisinin–piperaquine, this usually exceeds 95% [12]. Amodiaquine-artesunate has proved to be an efficacious combination (range 90-95%) in areas where 28-day cure rates with amodiaquine monotherapy exceed 80% [13]. Few data on pregnant women are available but

efficacy should be as good, if not better, than in children. In all the three areas where AQAS is assessed, efficacy of AQ monotherapy exceeds 80%. Therefore, it is expected that all the four treatments will have an efficacy of about 95%.

Trial objectives and purpose

The main objective of the trial is to determine the safety and efficacy of 4 ACTs when administered to pregnant women with P. falciparum infection during the second and the third trimester, and collect explanatory variables for therapeutic response. The primary hypothesis is the clinical equivalence (pair-wise non-inferiority) of the 4 treatment regimens, with clinical equivalence defined as difference in treatment failure rates (PCR corrected) of 5% or less.

Specific objectives are:

a) to compare the efficacy of AL, AQ-AS, MQ-AS and DHA-PQ in terms of treatment failure by 63 days after start of treatment with or without genotyping; Parasite clearance time; Haematological recovery by 14, 28, 42 and 63 days post-treatment and at delivery; Birth weight measured within 72 hours of delivery; and prevalence of placenta P. falciparum malaria;

b) to describe the safety profile of AL, AQ-AS, MQ-AS and DHA-PQ in terms of tolerability; incidence of adverse events until one year post-partum;

c) to determine the relation between drug pharmacokinetics (Day 7 levels of the partner drug) and response to treatment;

d) to assess the in vitro susceptibility of P. falciparum isolates collected before treatment to several drugs, including the partner drug tested, and to correlate their IC50 to treatment response.

3.3 Methods

Study design

This is a non-inferiority, multicentre, randomized, open label study on 4 antimalarial treatments, namely DHA-PQ, MQAS, AQAS and AL, assessed in each site using a "balanced incomplete block design" – with 3 out of 4 arms used in each site. There are 7 sites in the study distributed in the four countries, i.e. Burkina Faso (Nanoro and Nazoanga), Ghana (Effiduase, Ejisu and Juaben), Malawi (Chikwawa) and Zambia (Nchelenge).

The treatments tested are distributed in a way to allow a head-to-head comparison and the establishment of the treatment's relative value according to a series of outcomes (Table 1). This approach has the advantage of testing several treatment options at the same time, maximizing the use of resources, and is the most likely to achieve our aim of identifying at least 2 antimalarial treatments suitable for use in pregnancy and one rescue/alternative treatment.

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

West Africa: comparator AQAS			
Burkina (870)	AL (290)	AQAS (290)	MQAS (290)
Ghana (870)	AQAS (290)	MQAS (290)	DHA-PQ (290)
Eastern-Southern Africa: comparator AL			
Malawi (870)	DHA-PQ (290)	AL (290)	AQAS (290)
Zambia (870)	MQAS (290)	DHA-PQ (290)	AL (290)

AQAS: amodiaquine-artesunate; DHA-PQ: dihydroartemisinin-piperaquine; AL: artemether-lumefantrine; MQAS: Mefloquine-artesunate.

The primary analysis is the assessment of therapeutic equivalence of the 4 treatments (clinical non-inferiority) with respect to therapeutic success at day 63 and their safety throughout the follow up, i.e. up to one year after delivery.

The following procedures are used to ensure an unbiased assignment of treatment safety and efficacy:

1. The randomization list is generated prior to the beginning of the study.

2. The interpretation of the PCR reading is blinded or masked with regard to the treatment allocation of the patients.

3. An independent Data Safety and Monitoring Board (DSMB) reviews all safety data.

Primary endpoint

There are two primary end points:

1. Treatment Failure (TF) (PCR adjusted) at day 63 defined according to the WHO criteria [14] as the sum of early and late treatment failures. Early Treatment Failure (ETF) could be one of the following: Development of danger signs or severe malaria on Day 0, Day 1, Day 2 or Day 3, in the presence of parasitaemia; Parasite density on Day $2 >$ Day 0 count, irrespective of axillary temperature; Presence of parasitaemia on Day 3 with fever (axillary temperature $\geq 37.5°C$);

Parasitaemia on Day $3 \geq 25\%$ of count on Day 0. Late treatment failure (LTF) is divided in late clinical and late parasitological failure. Late Clinical Failure (LCF): Development of danger signs or severe malaria on any day after Day 3 in the presence of parasitaemia, without previously meeting any of the criteria of Early Treatment Failure; Presence of parasitaemia and fever on any day after Day 3, without having previously meet the criteria of ETF. Late Parasitological Failure (LPF): Presence of parasitaemia on any day from day 7 onwards and axillary temperature <37.5°C, without previously meeting any of the criteria of ETF or LCF. The Adequate Clinical and Parasitological Response (ACPR) is 1-TF. It is defined as absence of parasitaemia at the end of the follow up period, irrespective of axillary temperature without previously meeting any of the criteria of early and late treatment failure. In the adjusted estimates, patients with late asexual parasite reappearance (with or without fever) are considered ACPR if the PCR analysis shows a new infection rather than a recrudescence.

2. Safety profiles including significant changes in relevant laboratory values. Subjects are monitored for 63 days for possible development of adverse events. All adverse events are recorded on the specific form in the electronic CRF. Vital signs, blood chemistry and haematology are monitored and changes in relevant laboratory parameters are assessed.

Secondary endpoints

PCR unadjusted treatment failure up to day 63 (TF63U); Time to treatment failure (PCR adjusted and unadjusted) during 63 days of active follow-up after treatment; Asexual parasite clearance time (PCT): Asexual parasite clearance time is defined as the time (in days) from time of randomization to 2 consecutive negative blood slides (collected at different days) - the time to the event is taken as the time to the first negative slide; Gametocytaemia (prevalence and density) at day 7, 14, 21, 28 and 63 after treatment; Hb changes day 14, 28, 42 and 63; Acute, chronic or past infection of the placenta (prevalence); Mean birth weight and prevalence of low birth weight.

Laboratory procedures

Haematology

Maternal haemoglobin is measured using Hb301 Hemocue®, Angelholm, Sweden, according to manufacturer's instructions.

Peripheral malaria infection

Blood samples are collected by finger prick at specified time points during the trial for blood slides (thick blood film) and blood spots on filter paper. Thick blood smears are stained with 3% Giemsa for 30 minutes and read by trained microscopists at each site. Parasite densities are calculated by counting the number of asexual parasites per 200 leukocytes (or per 500 leukocytes if the count is <10 asexual parasites/200 leukocytes), assuming a leukocyte count of 8,000/µl. A blood smear is considered negative when the examination of 100 high power fields does not reveal asexual parasites. Each slide is read separately by two experienced microscopists and discrepancies resolved by a third reader.

Blood spots on filter paper are used for genotyping recurrent malaria infections during follow up and compared them with pre-treatment samples. This is done by characterizing MSP1, MSP2 and GLURP, single-copy genes in the *Plasmodium falciparum* genome. For the three genes, each PCR-amplification product of a different size is considered to originate from a different clone of *Plasmodium falciparum*, reflecting a different genotype. For the samples collected from the same patient at day 0 and day of recurrent parasitaemia, the length polymorphism of MSP1, MSP2 and GLURP are determined, i.e. the number of bands in each PCR reaction and their respective size. Results are interpreted as follows:

- Recrudescence: For each marker (MSP1, MSP2 and GLURP), at least one identical length polymorphism is found in the sample collected at day 0 and day of recurrent parasitaemia.

- New infection: For at least one marker, length polymorphism is different between the sample collected at day 0 and that at day of recurrent parasitaemia.
- Indeterminate: Samples that fail to produce a result due to an inability to amplify DNA at day 0 and/or day of recurrent parasitaemia.

Placental malaria

A 1 cm^3 biopsy specimen is obtained from the maternal-facing side of the placenta as soon as possible after delivery. Biopsy specimens are preserved in 10% neutral buffered formalin which are processed and embedded in paraffin wax by standard techniques. Pending histological evaluation, all biopsies are kept at 4°C. Paraffin sections 4 mm thick are stained with haematoxylin-eosin stain. Placental biopsies are classified according to the following definitions [15]:

1.Acute infection (parasite present, malaria pigment absent)

2.Chronic infection (parasites and malaria pigment present)

3.Past infection (no parasite but pigment present)

4.No infection (both parasites and malaria pigment absent)

PK assessment

Individual pharmacokinetic studies are often underpowered for identifying the factors that influence antimalarial pharmacokinetic parameters, which may have a major influence on the observed therapeutic response. Complementing efficacy data with some information on the pharmacokinetic properties of the treatment used allows a better interpretation of the observed recurrent infections or true recrudescences/new infections, as these may be the results of inadequate drug levels because of altered distribution, poor absorption or metabolism. The day 7 drug concentration has been shown to be the most important single measure, in terms of correlation with the area under the concentration time curve and association with treatment response, for lumefantrine, piperaquine and mefloquine [16]. Therefore, a blood sample of 2 ml is collected from all women at day 7 with the aim of

measuring with an appropriate assay the concentration of the partner drug to the artemisinin derivative. Not all samples will be analysed; instead a smaller number of samples will be chosen for the analysis according to the observed therapeutic response, i.e. in each arm women having experienced a true recrudescence will be compared with those having had a new infection and with those with an adequate clinical and parasitological response. Analysis of the blood samples will be carried out within the Malaria in Pregnancy Consortium.

In-vitro tests

This component is carried out at the sites in Burkina Faso only. The sensitivity of the parasites to the drugs used is determined by carrying out *in vitro* tests. Venous blood samples (5 ml) are collected at day 0 before treatment from women with a parasite density of at least 4,000/μL. The HRP2 ELISA [17, 18] is used to measure the proliferation of *P. falciparum* in the presence of lumefantrine, monodesethylamodiaquine (active metabolite of amodiaquine), mefloquine, piperaquine and dihydroartemisinin (active metabolite of artemisinin derivatives).

Sample size

The sample size for this study was determined by simulation with the following assumptions and requirements: (1) study conclusions are determined by two-sided 95% confidence intervals for difference in response rates (% of therapeutic success), with decision rule as described below, (2) all 4 treatments have identical true response rates of 95%, (3) 95% power for each of the 6 pair-wise comparisons and 80% power for the combined hypothesis that all treatments are therapeutically equivalent is required. With these assumptions, approximately 700 patients/treatment arm are needed. If the true response rate for one of the treatments is lower than 95%, then power is reduced to 80% (for a true response rate of 94%) or 50% (for a true response rate of 93%). Allowing for a 20% loss to follow-up, a total of 870 patients are recruited to each treatment; this is equivalent to 290 patients in each treatment group in each centre (i.e. a total centre sample size of 870 patients) –

and hence a total study sample size of 3480 patients. Inclusion of HIV-infected women is not expected to have a major influence on the sample size calculation. The percentage of therapeutic success may be slightly but not dramatically lower in this subgroup of patients.

For safety, when combined, the trial is able to detect with 90% power major adverse events occurring at the frequency of at least 2-3%.

Statistical analysis

A detailed analysis plan is drawn up prior to the analysis.

1.Baseline comparability: Patients in each treatment group in each site are described separately with respect to baseline characteristics. The clinical importance of any imbalance will be noted, though statistical tests of significance are not undertaken.

2.Primary analyses: The primary analysis of the study is the assessment of therapeutic equivalence of the 4 treatments (clinical non-inferiority) with respect to therapeutic success at day 63 and their safety throughout the follow-up, i.e. up to one year after delivery.

Efficacy

Therapeutic equivalence is assessed using the pair-wise difference in response rates (percentage of women with therapeutic success). Assessment of the difference in true response rates is performed by calculating the two-sided 95% confidence interval for the difference in response rates from the observed data, using the following decision rule:

- if the two-sided 95% confidence interval for the difference in response rates lies entirely between −5% and +5%, then therapeutic equivalence of the two treatments is concluded;
- if the 95% confidence interval for the difference in response rates includes −5% or +5%, then therapeutic equivalence cannot be established;
- if the 95% confidence interval for the difference in response rates lies entirely below −5% or entirely above 5%, then one treatment is clinically inferior to the other.

The primary analysis uses the combined data from all 4 centres together, with adjustment for any centre effects, using an additive model for the response rates (i.e. a generalized linear model with Bernoulli error distribution and an identity link function). This allows the assessment of all 6 possible pair-wise treatment comparisons using all available data. Equivalence will be established using two-sided confidence intervals. Though 6 treatment comparisons will be performed, no adjustment for multiplicity is needed as the focus of the study is on the individual pair-wise treatment comparisons. In addition, combined hypotheses of interest (e.g. all 4 treatments are therapeutically equivalent) require each of the individual hypotheses to be accepted. Consequently, there is no inflation of the type I error rate due to multiple testing. However, the power for combined hypotheses is lower than for the individual pair-wise comparisons. Thus, the power calculation of this study required a high (95%) power for individual pair-wise treatment comparisons, resulting in an acceptable (80%) power for the combined hypothesis. For the efficacy analysis, both an intention-to-treat and a per-protocol approach are adopted, with the per-protocol analysis being the primary approach, as recommended for equivalence studies.

Safety

For safety analysis, all non-serious and serious adverse events (SAE) are grouped according to a pre-specified side-effect coding system and tabulated. The number (and percentage) of patients experiencing any adverse event, any SAE, and any drug-related SAE are compared between treatment groups using Fisher's exact test. Safety is analyzed using the all-patients-treated approach.

Selection of the patients

All pregnant women in the second and third trimester (<37 weeks) and attending the antenatal clinic of the study health facilities are systematically screened for malaria infection with a rapid diagnostic test; if positive they are further assessed for eligibility. They are included if they are at least 15 years old, with a pregnancy of at least 16 weeks, a *P. falciparum* monoinfection of any density, regardless

of symptoms, and a Hb concentration of at least 7 g/dL. Pregnant women with a negative blood slide are not included in the study and go through the routine antenatal clinic procedures according to national policy and receive a dose of sulfadoxine-pyrimethamine (SP) as intermittent preventive treatment (IPT). Exclusion criteria include history of allergic reactions to the study drugs, of known pregnancy complications or bad obstetric history such as repeated stillbirths or eclampsia, of presence or history of major illnesses likely to influence pregnancy outcome, e.g. diabetes mellitus, severe renal or heart disease, or active tuberculosis, current cotrimoxazole prophylaxis or ARV treatment. Table 2 provides the full list of inclusion/exclusion criteria. The reason of including women with any parasite density is justified by the important adverse outcomes any malaria infection has on the mother's and her offspring's heath. Limiting the inclusion to women at 16 weeks or more of gestation is justified by the uncertainty on the safety of ACT when administered during the first trimester of pregnancy. The gestational age was confirmed by measuring symphysio-fundal height and the foetal viability by using an ultra-sonography. Exclusion criteria are formulated because of possible safety issues, e.g. history of allergic reactions to the study drugs, or the need for a clear interpretation of the therapeutic response, e.g. recent exposure to antimalarial treatments.

Table 2 Inclusion and exclusion criteria

Inclusion criteria	Patients eligible for inclusion in the trial must fulfil all of the following criteria
	Gestation ≥16 weeks and <37 weeks;
	P. falciparum monoinfection of any density, with or without symptoms
	Hb ≥ 7 g/dL;
	At least 15 years old;
	Residence within the health facility catchment's area;
	Willing to deliver at the health facility;
	Willing to adhere to the study requirements (including, in Zambia and Malawi, HIV VCT)
	Ability to provide written informed consent; if the woman is minor of age/not emancipated, the consent must be given by a parent or legal guardian according to national law (however, in this case, the investigator is responsible to check that the woman herself is also freely willing to take part in the study).
Exclusion criteria	Patients who meet any of the following criteria are not eligible for the study
	History of allergic reactions to the study drugs;
	History of known pregnancy complications or bad obstetric history such as repeated stillbirths or eclampsia;
	History or presence of major illnesses likely to influence pregnancy outcome including diabetes mellitus, severe renal or heart disease, or active tuberculosis;
	Current cotrimoxazole prophylaxis or ARV treatment;
	Any significant illness at the time of screening that requires hospitalization, including severe malaria;
	Intent to move out of the study catchment area before delivery or deliver at relative's home out of the catchment area.
	Prior enrollment in the study or concurrent enrollment in another study.
	Unable to take oral medication
	Clear evidence of recent (1 week) treatment with antimalarials or antimicrobials with antimalarial activity (clindamycin; azythromycin; etc.)

Informed consent

For the informed consent, all interviews are conducted in the native language of the patients by a qualified person identified by the Investigator. Written information and consent forms in the local language are provided to the women or Legally Authorized Representatives (LAR) for their review. After the interview, the patients and, in case they are of minor age/not emancipated, the parents or

guardians are asked to confirm their willingness to participate in the study by signing (or thumb-printing if illiterate) the consent form.

Each eligible pregnant woman who agrees to give informed consent is assigned a unique study number and enrolled. Besides malaria infection and parasite density, Hb, total white blood cell count, differential count, total bilirubin, ALAT and creatinine are measured. In addition, a blood sample is collected on filter paper for later genotyping.

Randomization and treatment

Randomisation is carried out according to a pre-established list comprising blocks of varying size and stratified according to the number of recruitment points in each site. Allocation of treatment according to the randomization list is in sealed envelopes labelled with the patient's unique code, guaranteeing concealment until recruitment.

The study treatment is administered during the first 3 study days (days 0–2) by the study doctor or nurse and the patient kept for one hour in the clinic. If vomiting occurs within 30 minutes, a full treatment is re-administered, half a dose if after 30 minutes. In case of persisting vomiting, an alternative treatment, e.g. quinine, is provided.

Patients follow up

Scheduled visits are at day 3, 7 and then every week until day 63 post-treatment. However, women are encouraged to attend the antenatal clinic if they felt ill between scheduled visits. At the end of the active follow-up period, women are asked to attend the antenatal clinic monthly and at any time they feel unhealthy until delivery. At each visit, scheduled and unscheduled, the medical history since the last visit (including any treatment taken), current signs and symptoms (if any) are

collected. A blood sample for thick and thin blood film and later genotyping to determine the rate of re-infection is collected and the body temperature checked. Haematology (Hb, total white blood cell count, differential count) is measured at day 7, 14, 28 and 63; biochemistry (total bilirubin, ALAT and creatinine) at day 7 and 14.

If pregnant women recruited during the third trimester deliver before the end of the 63-day active follow, scheduled visits continue after delivery until the day 63 is completed. The outcome of pregnancy, including any congenital abnormality, the birth weight and maternal Hb are collected as soon as possible after delivery. In addition, a placenta impression smear and a placenta biopsy for later histopathological analysis are collected. Both the mother and the new-born are reassessed twice after delivery for any adverse event: between 4 and 6 weeks and then after one year (Table 3). Antimalarials or antibiotics with antimalarial activity (erythromycin or other macrolides, co-trimoxazole or other sulphonamides, any tetracycline including doxycycline, and quinolones, clindamycin) cannot be administered during the active follow up as it would lead to withdrawal of the patient from the study.

Table 3 Study procedures/study visit schedule

Day	0	1	2	3	7	14	21	28	35	42	49	56	63	Any other day[1]	Delivery	4-6 weeks post-partum	EPI clinics	1 year post-partum
History (symptoms)	X																	
Informed consent	X																	
Examination (clinical)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Foetal viability	X			X	X	X	X	X	X	X	X	X	X	X				
Blood Pressure	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Temperature	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Blood film	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Filter paper PCR	X				X	X	X	X	X	X	X	X	X	X	*X* [2]			
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Hb	X				X	X		X		X			X		X			
Haematology	X				X	X		X					X		X			
Treatment	X	X	X															
Blood sample for PK					X													
Pop PK	3 samples/woman according to a predefined schedule (120 women in 3 sites each)																	
In vitro test	X	and time of recurrent infection																
Biochemistry	X				X	X		X[3]					X[4]					
Placenta sample															X			
New born assessment															X	X		
Infant assessment																	X	X

[1]Spontaneous attendance to the health facility.
[2]Includes placental blood sample.
[3]Only ALAT and total bilirubin.
[4]Only ALAT and total bilirubin, and only if abnormal at Day 28.

Patients with treatment failure, including parasitological failure, are treated with rescue treatment (quinine 10 mg/kg orally three times a day for 7 days or an anti-malarial treatment according to the country's national guidelines) and their active follow up stopped. Nevertheless, they are still followed up (safety data) until one year post-delivery.

Safety variables

Safety is closely monitored during the course of the study in compliance with ICH/GCP guidelines. At each visit, the investigator ascertains the occurrence of any adverse events since the previous visit; including those involving laboratory values which are out of normal range and are of clinical importance to be considered as AEs, and the proper AE reporting procedure is followed. The severity of a clinical adverse event is scored according to the following scale: mild, moderate, severe and life-threatening.

Serious Adverse Events (SAEs) are defined as any untoward medical occurrence that, at any dose of the medication given, fulfilled the following criteria; death, life-threatening, requiring hospitalization or prolongation of hospitalization, resulting in persistent or significant disability or incapacity, congenital anomaly/ birth defect, or other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. The reporting investigator assesses the relationship between investigational product and the occurrence of each AE/SAE. The relationship of an adverse event to study drug is assessed according to the following definitions: 'Definitely unrelated', 'Unlikely', 'Possible', 'Probable' and 'Definitely related'. The outcome of each AE is assessed according to the classification as follows – 'Completely recovered', 'Not yet completely recovered', 'Deterioration', 'Permanent damage', 'Death', 'Ongoing' and 'Unknown'.

Each SAE has to be reported to the sponsor and to the concerned ethical bodies in the study countries within 24 working hours since the time the study staff becomes aware of it, and any reporting delay has to be explained. All the SAE forms are further sent by the sponsor to the concerned ethical bodies in Belgium and to the independent DSMB. Each SAE is followed up until resolution.

Monitoring and quality assurance

Each site is visited at least 3 times during the conduct of the trial plus a study initiation visit at the start of clinical activities and a close-out visit after the last patient has completed the follow up. The monitor will perform the tasks as described in International Conference on Harmonisation (ICH)-Good Clinical Practice (GCP) E6, section 5.18 and will carry out at least 10% source data verification (SDV). For all sites, the SDV percentage will be increased by the monitor if the quality of data entry is found not to be satisfactory.

Case report form and data management

Each patient has her own source document file, according to a common source document template provided by the Sponsor, with all the original documents, e.g. laboratory results. This data is captured into an electronic case report form (e-CRF) developed in the GCP-compliant software MACRO (InferMed, UK) for clinical trials. The e-CRF has in-built consistency checks; data can be entered either online or offline and then uploaded to be sent to the central server. The final database is obtained after the resolution of all queries and then locked for later statistical analysis done according to a pre-established statistical data analysis plan.

Study committee

Consortium secretariat

The Consortium Secretariat (CS) acts as a steering committee. It comprises one investigator from each site and will assess the progress of the trial. The members of the CS will address policy and operational issues related to the protocol. The CS has responsibility for protecting the scientific conduct and integrity of the trial. Its functions include review of the protocol before ethical approval; and formulation of recommendation for any change in the design and operations of the trial during the course of the trial, when needed.

Data safety and monitoring board

The independent Data Safety and Monitoring Board (DSMB) is composed by four independent scientists, i.e. a paediatrician, a gynaecologist, a statistician and a malariologist. They meet every three months during recruitment or can be called together if the necessity arises.

History of amendments to the study protocol

Several amendments (Table 4) have been made to this study protocol based on emerging information and were approved by the relevant ethical committees. Before study start the ASAQ manufacturer provided information regarding potential transitory increase of ALAT at day 28 post-treatment. Therefore, ALAT measurement at day 28 was introduced. In addition, an overview of the reproductive toxicity of DHAPQ and mefloquine was made available by the manufacturer and showed in the animal model prolonged length of gestation and dystocic pup expulsion in animals treated close to delivery. It was therefore, decided to modified the original inclusion criterion of gestation ≥16 weeks to gestation ≥16 weeks and <37 weeks.

Table 4 Major amendments

Amendment version	**Basis/rationale of amendment**	**Amendment**
Amendment 1	The information provided by the ASAQ manufacturer of some transitory increase of ALAT at day 28 post-treatment.	ALAT measurement at day 28 was introduced
Amendment 2	The reproductive toxicity of DHAPQ and mefloquine was made available by the manufacturer and showed in the animal model prolonged length of gestation and dystocic pup expulsion in animals treated close to delivery.	Modification of the original inclusion criterion of gestation ≥16 weeks to gestation ≥16 weeks and <37 weeks
Amendment 3		paragraph on placenta biopsy is added to the ICF (was omitted by mistake in the previous versions)
Amendment 4	Amendment was done on the basis of the modification of blood piperaquine concentrations by food intake which could result in a QTc prolongation, a risk factor for serious cardiac arrhythmia.	• DHA/PQP tablets should be administered with water only and at least 3 hours apart from meal, mainly for the second and third administration.
		• correction of dosage of DHA/PQP, should be 3 tablets for 3 consecutive days instead of 2 tablets for 3 consecutive days as erroneously stated in the previous versions of the protocol.
		• notification of change in sites in Malawi.
		• % of SDV reduced to 10%
Amendment 5	Need for baseline drug plasma concentrations	PK sample at day 0 before study drug administration for the patients participating in the population PK study
Amendment 6		one additional site

Another amendment was done on the basis of the modification of blood piperaquine concentrations by food intake which could result in a QTc prolongation, a risk factor for serious cardiac arrhythmia. Therefore, the manufacturer advised to administer DHAPQ tablets with water only and

at least 3 hours apart from meal, mainly for the second and third administration, and advice the woman not to eat for the next 3 hours.

3.4 Discussion

Pregnant women are one of the high risk groups affected by the malaria burden and few antimalarials are available to treat them. This study assesses the efficacy and safety of four ACTs for the treatment of uncomplicated malaria in pregnant women in Africa. This is the largest clinical trial of its kind and will provide the evidence base for the formulation of treatment guidelines for malaria in pregnancy.

Evidence of the interaction between malaria and HIV-1 has already been reported. HIV-1 infected pregnant women have a higher prevalence of peripheral parasitaemia and placental malaria [19, 20] and their infants experience higher postnatal mortality when both diseases are present [4, 21]. HIV-1 infected adults have a higher risk of malaria infection and clinical malaria, the latter increasing with falling CD4-cell count [22, 23]. Therefore, offering adequate and efficacious antimalarial treatment and prevention is extremely important for this high risk group. However, little is known about the safety and efficacy of antimalarial drugs in HIV-infected individuals and much less on the interaction between antimalarials and antiretrovirals [24] and reliable data are urgently needed [3]. HIV-infected individuals have a higher risk of experiencing treatment failure and this depends on the degree of immune-suppression [25]. However, no major safety problems related to ACT treatment in pregnant women have been identified so far.

Considering that most pregnant women recruited in the study will have an infection with a relatively low parasite density and that they will be treated with an ACT, it is expected that the number of treatment failures in this specific sub-group would be extremely low. They will have to be treated in

any case as the consequences of the malaria infection on the woman's health and that of her offspring are well known.

In the second and third trimester of pregnancy, WHO recommends the use of ACT known to be effective in the country/region. Despite this recommendation, it should be recognized that the available information on the treatments to be used in this trial is limited. ACT should not be used in the first trimester of pregnancy, the time of greatest concern for potential teratogenicity, and particular care will be taken in excluding women of this gestational age. DHA-PQ is the least used of the 4 ACTs studied in this trial and it is not among the WHO recommended ACTs during pregnancy because of insufficient information [3]. However, DHA-PQ is the first line treatment in Papua Indonesia, where it is also used to treat pregnant women with malaria; an observational study reported significant benefits of DHA-PQ over quinine-based regimens in reducing recurrent malaria and poor foetal outcome in pregnant women in the second or third trimester [26]. This trial will increase significantly the knowledge on the use of DHA-PQ in African pregnant women.

Trial status

Data collection completed.

Abbreviations

ACPR: Adequate clinical and parasitological response; ACT: Artemisinin combination therapy; AE: Adverse event; AL: Artemether lumefantrine; ALAT: Alanine amino-transferase; AQAS: Amodiaquine artesunate; ARV: Antiretroviral; CRF: Case report form; CS: Consortium secretariat; DHA-PQ: Dihydroartemisinin piperaquine; DNA: Deoxynucleic acid; DSMB: Data safety and monitoring board; ELISA: Enzyme-linked immunosorbent assay; ETF: Early treatment failure; GCP: Good clinical practice; GLURP: Glutamate-rich protein; HIV: Human immunodeficiency virus; HRP: Histidine-rich protein; ICH: International conference on harmonisation; IPT: Intermittent preventive therapy; ITN: Insecticide treated net; LAR: Legally authorised representative; LCF: Late clinical failure; LPF: Late clinical failure; LTF: Late treatment failure; MIP: Malaria in pregnancy; MQAS: Mefloquine artesunate; MSP: Malaria merozoite surface protein; PCR: Polymerase chain reaction; PCT: Parasite clearance time; PK: Pharmacokinetics; SAE: Serious adverse event; SDV: Source data verification; SP: Sulfadoxine pyrimethamine; TF: Treatment failure; VCT: Voluntary counselling and testing; WHO: World health organisation.

Declarations

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Competing interests

The research was funded by EDCTP and Co-funding from Bill and Melinda Gates foundation (BMGF) - USA, Directorate-General for Development Cooperation (DGDC) – Belgium, London School of Hygiene and Tropical Medicine (LSHTM) – United Kingdom, Netherlands-African Partnership for Capacity Development and Clinical interventions Against Poverty-related diseases (NACCAP) – Netherlands, and Medical Research Council (MRC) – UK. The authors declare that they have no competing interests.

Authors' contributions

UDA and JPV proposed the ideas of the paper. MN contributed to the writing of the paper. UDA, JPV, JM, RR, YC, contributed to the ideas of the original protocol. RR organized the first investigators meeting in Dakar, Senegal. JM and UDA contributed to the analytical plan. MN, MM, TH, HT, VM, LKP, GK, IV, MT, DM, YC, CS, MDC, JM, RR, KT, JPV, TM, UDA reviewed the manuscript. UDA is the corresponding author/main contact for the PREGACT study. All authors read and approved the final manuscript.

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Chapter 4: Artemisinin-based Combination Treatments in Pregnant Women in Zambia: Efficacy, Safety and Risk of Recurrent Malaria.

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4.1 Abstract

Background

In Zambia, malaria is one of the leading causes of morbidity and mortality, especially among under five children and pregnant women. For the latter, the World Health Organization recommends the use of artemisinin-based combination therapy (ACT) in the second and third trimester of pregnancy. In a context of limited information on ACT, the safety and efficacy of three combinations, namely artemether–lumefantrine (AL), mefloquine–artesunate (MQAS) and dihydroartemisinin–piperaquine (DHAPQ) were assessed in pregnant women with malaria.

Methods

The trial was carried out between July 2010 and August 2013 in Nchelenge district, Luapula Province, an area of high transmission, as part of a multi-centre trial. Women in the second or third trimester of pregnancy and with malaria were recruited and randomized to one of the three study arms. Women were actively followed up for 63 days, and then at delivery and 1 year post-delivery.

Results

Nine hundred pregnant women were included, 300 per arm. PCR-adjusted treatment failure was 4.7% (12/258) (95% CI 2.7–8.0) for AL, 1.3% (3/235) (95% CI 0.4–3.7) for MQAS and 0.8% (2/236) (95% CI 0.2–3.0) for DHAPQ, with significant risk difference between AL and DHAPQ ($p = 0.01$) and between AL and MQAS ($p = 0.03$) treatments. Re-infections during follow up were more frequent in the AL (HR: 4.71; 95% CI 3.10–7.2; $p < 0.01$) and MQAS (HR: 1.59; 95% CI 1.02–2.46; $p = 0.04$) arms compared to the DHAPQ arm. PCR-adjusted treatment failure was significantly associated with women under 20 years [Hazard Ratio (HR) 5.35 (95% CI 1.07–26.73; $p = 0.04$)] and higher malaria parasite density [3.23 (95% CI 1.03–10.10; $p = 0.04$)], and still

women under 20 years [1.78, (95% CI 1.26–2.52; p < 0.01)] had a significantly higher risk of re-infection. The three treatments were generally well tolerated. Dizziness, nausea, vomiting, headache and asthenia as adverse events (AEs) were more common in MQAS than in AL or DHAPQ (p < 0.001). Birth outcomes were not significantly different between treatment arms.

Conclusion

As new infections can be prevented by a long acting partner drug to the artemisinins, DHAPQ should be preferred in places as Nchelenge district where transmission is intense while in areas of low transmission intensity AL or MQAS may be used.

Keywords: Zambia Sub-Saharan Africa Artemisinin-combination therapy Treatment failure

4.2 Background

Malaria is a poverty-related disease and a major public health problem in many sub-Saharan African countries where over 90% of the cases worldwide are found. Pregnant women and children are at higher risk of malaria infection and of developing serious complications related to the disease. Malaria in pregnancy is associated with higher risk of maternal anaemia, low birth weight, spontaneous abortion, stillbirths and maternal mortality [1, 2, 3].

There are few treatments with known safety and efficacy for the treatment of malaria in pregnancy. Some anti-malarials known to be efficacious e.g. quinine, are not well tolerated, resulting in poor compliance and higher risk of treatment failures [4]. For other treatments, there are insufficient data as pregnant women are systematically excluded from treatment efficacy studies. Therefore, pregnant women lack proven effective and safe anti-malarial therapies [5]. In such context of limited information, and weighing risks and benefits, the World Health Organization (WHO) allows the use of artemisinin-based combination therapy (ACT) during the second and third trimester of pregnancy [1].

To confirm this expert opinion, we assessed the safety and efficacy of three artemisinin-based combinations, namely mefloquine–artesunate (MQAS), dihydroartemisinin–piperaquine (DHA–PQ) and artemether–lumefantrine (AL), in pregnant women in the second or third trimester with a confirmed Plasmodium falciparum malaria infection. This study was part of a multi-centre trial carried out also in Burkina Faso, Ghana and Malawi. Each site tested 3 ACT medicines so that each country dataset could be analysed separately [6] and give detailed site specific data. This paper reports results collected in the Zambian site, Nchelenge, Luapula Province. Knowing that anti-malarial drug's efficacy depends not only on the parasite susceptibility to the drug and on its blood concentration but also on the host's immunity which may be affected by factors such as pregnancy

itself, age, parasite density and malaria transmission intensity, the impact of these factors on the treatment outcome were assessed [7, 8]. The results of this study provide the national policy makers the information for a wider and alternative choice of treatments to be used during pregnancy.

4.3 Methods

The trial was conducted between June 2010 and August 2013 in Nchelenge district in Luapula Province, Zambia; one of the provinces where malaria prevalence is higher than the national average (32.1% vs 14.9% in 2012) [9]. Nchelenge district is located in the northern part of the province on the swampy shores of Lake Mweru, borders with the Democratic Republic of Congo (DRC) and has an estimated population of 178,000 inhabitants, mostly peasant farmers and/or fishermen. The district has three seasons: cool dry winter, hot dry and rainy season. Malaria transmission is perennial because of the presence of Anopheles funestus during the dry season and Anopheles gambiae in the wet season [10]. In 2012-2013, the entomological inoculation rate (EIR) was estimated at 70 infective bites/person/year [11], and annual malaria incidence at more than 700/1000 person years in the general population and more than 1900/1000 person years among under-five children [12]. The study protocol of this trial is been described in detail elsewhere [13]. Briefly, pregnant women aged at least 15 years, in the second and third trimester, with Hb ≥7 g/dL, HIV negative, a P. falciparum mono-infection of any density and irrespective of having symptoms (excluding illness at time of screening that required hospitalization such as severe malaria) were recruited into the trial and randomized to one of the following treatments: artemether–lumefantrine (AL), mefloquine–artesunate (MQAS) and dihydroartemisinin–piperaquine (DHA–PQ) using a randomization list provided of 300 participants in each arm. Sealed envelopes labeled with patient's unique code and containing treatment allocation were provided according to randomization list. A woman was defined as symptomatic if any of the following were present: fever (temperature >37.5

°C) at baseline with parasitaemia (any density); parasite count >2000/µL, regardless of symptoms; at least 3 or more of the following symptoms: fever in the past 24 h, weakness/fatigue; muscle and/or joint aches, headache, convulsion, with parasitaemia of any density). Gestational age was estimated by symphysio-fundal height and then confirmed by obstetric ultrasound, including the fetal viability assessment [14, 15]. A blood sample of about 5 mL was collected before treatment for the assessment of haematological and biochemistry parameters. All study drugs were given on days 0, 1 and 2 under direct observation and according to the manufacturer's recommendations (Eurartesim® from Sigma-Tau Industrie Farmaceutiche Riunite S.p.A., 40 mg of dihydroartemisinin and 320 mg of piperaquine phosphate per tablet, 3 tablets once per day over 3 days; mefloquine–artesunate from Far-Manguinhos Ministério da Saúde-Fundação Oswaldo Cruzm, 100 mg artesunate and 220 mg mefloquine per tablet at 3 tablets once per day over 3 days; Coartem® from Novartis Pharma AG, 20 mg artemether and 120 mg lumefantrine per tablet at 4 tablets twice per day over 3 days). After completing the 3-day treatment, patients were asked to return to the clinic for follow up visits on day 3, 7 and then once every week until day 63. At each visit, a medical history, and current clinical signs and symptoms were collected, including information on any adverse events (AE), a blood sample for malaria smears and dried blood spots (DBS) for later genotyping, for full blood counts (days 7, 14, 28 and 63 only) and for total bilirubin, alanine aminotransferase (ALAT) and creatinine (days 7 and 14 only). Rescue treatment (Quinine) for recurrent infections was according to local national guidelines [16]. (In Zambia, AL is used for treatment of uncomplicated malaria in second and third trimester of pregnancy). At the end of the active follow-up period, women were asked to continue with the antenatal clinic monthly or when they felt unwell until delivery. Recurrent malaria episodes after day 63 were treated with quinine.

Giemsa-stained thick and thin blood films were read independently by two readers, followed by a third reader in case of significant discrepancy. Parasite density was estimated by counting the number of asexual parasites per 200 white blood cells (WBCs) assuming a WBC count 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 (Hb) was measured using Hemocue (Angelholm, Sweden). For polymerase chain reaction (PCR) analysis, DBS were prepared on filter paper (Whatman 3MM), and were subsequently transported to the Institute of Tropical Medicine (ITM), Antwerp, Belgium, where centralized genotyping (GluRP, MSP2 and MSP1) was conducted [17]. Samples that failed to produce a result were classified as indeterminate.

Consent was obtained in all cases from study participants and/or legal representative for those between 15 and 17 years old. The study was approved by the Institutional Review Board of the ITM and the Ethics Committee of the Antwerp University Hospital. In addition, the study was also approved by Tropical Diseases Research Centre (TDRC) Ethics Review Committee, the Zambia Medicines Regulatory Authority and Zambia Ministry of Health. The trial was registered at clinicaltrials.gov (NCT00852423).

The primary endpoints were the PCR-adjusted cure rates at day 63 and the safety outcomes as described elsewhere [13]. AEs and serious AEs (SAEs) were recorded and monitored regularly throughout the study by an independent Data and Safety Monitoring Board (DSMB). Secondary endpoints were PCR-unadjusted cure rates at day 63, PCR adjusted and unadjusted time to treatment failure, asexual parasite clearance [18], gametocytaemia (prevalence and density) and Hb changes during follow up.

The study was designed to show that all 3 treatments had similar (PCR-adjusted) cure rates (within 5% difference), with 95% power for each of the 3 pair-wise comparisons and 80% power for the combined hypothesis that all treatments were therapeutically equivalent [13].

Data were captured into an electronic clinical record form (e-CRF) developed in MACRO (InferMed©). A statistical analysis plan was pre-specified before the database lock. For the primary outcome, three analysis populations were used: (1) per-protocol (PP), (2) intention-to-treat (ITT) that excluded lost to follow-up (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 the primary analysis approach. Major protocol violators, defined prior to analysis, were excluded from the PP analysis.

PCR-adjusted treatment failure rate between pair-wise treatment groups was compared using a Chi square test. The 95% exact confidence intervals for the difference in failure rates were determined. If the difference in true (PCR adjusted) failure rates was less than 5%, treatments were considered therapeutically equivalent. Briefly, risk difference was computed for the following groups: AL and DHAPQ; AL and MQAS; and MQAS and DHAPQ. The 95% confidence interval for a proportion was calculated using the Wilson score method. Baseline variables to be included in the Cox-regression model to compute the adjusted hazards of re-infection (new infection) and recrudescence were selected using the log-rank test for equality across strata. The covariates were included if the p value was 0.25 or less except study treatment dosage. The starting covariates were treatment, symptomatic malaria, parasite density, maternal age, gravidity, anaemia, study treatment dosage, gestational age, haematological and biochemical parameters. Covariates in the multivariable model that were not statistically significant (>0.05) were dropped off except where literature shows them as important variables [gravidity and gestational age (dropped for new infection)] to have in the

final model. The proportion hazard assumptions for the Cox-regression model were evaluated using graphical approach [19].

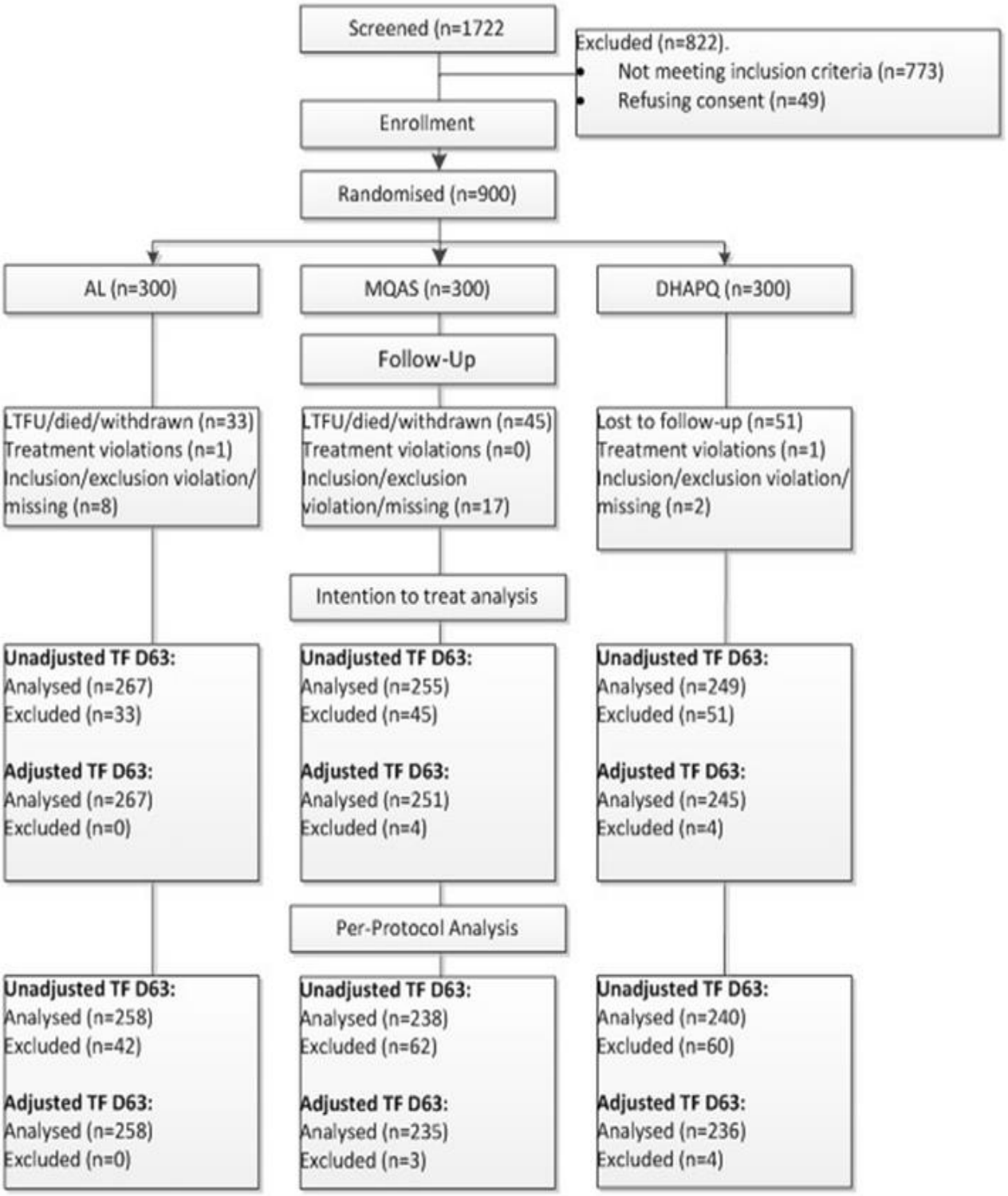
The hematological and biochemistry profiles by day of follow-up were assessed using box-plots plotted at each time point. Differences in these parameters between treatment arms at each day of follow-up were assessed using Kruskal–Wallis test.

Firth logistic regression was used to assess impact of placental malaria (categorized as placental malaria or no infection) on birth outcomes (still birth, miscarriage, premature live delivery, intrauterine fetal death and term live birth) for separation and 'empty cells' in the model. A "stillbirth" was defined as a baby born dead after 24 weeks gestation; a baby born dead before 24 weeks gestation or during the 24th week was considered a "miscarriage". "Preterm live born" was defined as a delivery before 37 weeks of gestation following echography. This was calculated as date of delivery minus date of echography (in weeks) plus gestational age determined through echography. Or based on the Ballard score which determines gestational age based on the sum of neuromuscular and physical scores [20]. A neonate with a score of 30 or lower was labeled "preterm" using this method. Logistic regression was used to assess impact of placental malaria (categorized as placental malaria or no infection) on birth weight. It was also used to assess risk factors for malaria. Placental malaria was classified as acute infection; chronic infection; past infection or no infection and analysed as binary outcome, placental malaria or no infection. For safety, all individuals having received at least one treatment dose were included and analysed in terms of proportions with Chi square test for the difference. Delivery related AEs, caesarean sections or reasons for caesarean sections and pregnancy outcomes were not included in the AE report. Also SAEs which were pregnancy related were excluded.

4.4 Results

A total of 1722 pregnant women were screened for malaria infection, regardless of symptoms. Out of these, 900 met the inclusion criteria and were randomized to one of the three study arms: 300 to AL, 300 to MQAS and 300 to DHAPQ. The ITT analysis included 900 pregnant women. The PP analysis included 729 women, i.e. 258 in the AL, 235 in the MQAS and 236 in DHAPQ arms (Fig. 1). The main reasons for exclusion from the PP analysis were lost to follow-up and withdrawals. The baseline characteristics (age, gravidity, parasite density, Hb, symptoms) of the excluded patients were similar to those included in the PP analysis.

Figure 1 Trial flow chart of the PREGACT trial at Nchelenge, Zambia (2010–2014)



Baseline characteristics were similar between treatment arms (Table 1); all gravidities were equally represented and the median gestational age by obstetric ultrasound (echography) was 25.0 weeks [IQR: 20.5–29.0] and similar between the treatment arms. The median parasite density was 1540/µL (IQR: 480–4540) and similar between the treatment arms [AL 1360/µL (IQR: 480–4280), MQAS 1610/µL (IQR: 540–4340) and DHAPQ 1640/µL (IQR: 520–4880)]; over 40% of women had a parasite density ≥2000/µL and 49% were symptomatic.

Table 1 Baseline characteristics of pregnant women with malaria episode Nchelenge, Zambia (2010–2014)

	AL (N=300)	MQAS (N=300)	DHAPQ (N=300)
Age (years): median (IQR)	20 (18–24)	19 (18–24)	20 (18–24)
Symptomatic malaria (%)	46.0	48.7	52.7
Fever (temperature ≥ 37.5°C) (%)	2.0	3.3	3.7
Parasite density >2000/µL (%)	41.0	42.7	45.7
At least 3 symptoms[a] (%)	11.0	12.3	12.0
Gametocytes present (%)	2.3	0	1.3
Parasite density (/µL): median (IQR)	1360 (480–4280)	1610 (540–4340)	1640 (520–4880)
Haemoglobin (g/dL): median (IQR)	10.0 (9.1–11.0)	10.0 (9.0–10.9)	10.0 (9.2–10.9)
Gravidity			
1st Pregnancy (%)	33.3	35.7	31.3
2nd Pregnancy (%)	30.3	30.3	32.0
3rd Pregnancy or more (%)	36.3	34.0	36.7
Gestational age[b]			
2nd Trimester (%)	50.0	50.0	43.7
3rd Trimester (%)	50.0	50.0	56.3
Bed net used before study entry (%)	30.7	28.7	27.3
ITN used before study entry[c] (%)	25.3	21.0	22.7
IPT use (before day 0) (%)	9.7	7.0	11.7

[a]At least 3 or more of the following symptoms: fever in the past 24 h, weakness/fatigue; muscle and/or joint aches, headache, convulsion, with parasitaemia of any density
[b]2nd Trimester were patients ≤24 weeks gestation and 3rd trimester >24 weeks gestation
[c]Women were provided with ITN at study start

The gametocyte prevalence at baseline was low, 2.3% (95% CI 1.1–4.7) in AL, 1.3% (95% CI 0.5–3.4) in DHAPQ and 0% (95% CI 0.0–1.3) in MQAS. The range of the gametocyte density, if present, was 40–440 gametocytes/µL. Gametocyte carriage remained low during the follow-up

period and only appeared in AL (4/297; 1.3%) and DHAPQ (6/292; 2.1%) arms and none in MQAS. Use of preventive measures, i.e. ITN and IPTp, at recruitment was low (Table 1).

In the PP analysis, the graphs for the global proportional hazards (PH) assumptions testing for treatment failure adjusted for several variables (treatment, anaemia, gestational age, gravidity, parasite density, maternal age and malaria symptoms at baseline) were roughly parallel and met the PH assumptions. The day 63 PCR-adjusted treatment failure rate was 4.7% (12/258) (95% CI 2.7–8.0) for AL, 1.3% (3/235) (95% CI 0.4–3.7) for MQAS and 0.8% (2/236) (95% CI 0.2–3.0) for DHAPQ (Table 2), with significant risk difference between AL and DHAPQ ($p = 0.01$) and between AL and MQAS ($p = 0.03$) treatments. Figure 2 which shows the time to PCR adjusted and unadjusted treatment failure confirms this difference. Figure 3 presents the risk difference computed for the pairwise comparisons conducted for PCR-adjusted and unadjusted treatment success rates at day 63. AL showed somewhat higher (about 3%) PCR-adjusted treatment failures. Therapeutic equivalence could be shown for MQAS and DHAPQ but not for AL as compared to the other 2 treatments. The ITT analysis gave similar results (Table 2). When considering recrudescence, i.e. treatment failure due to the reappearance of the same strain as identified by PCR analysis, its hazard was higher in patients treated with AL than in those treated with DHAPQ (HR: 10.47; 95% CI 2.18–50.19; $p < 0.01$) although the estimates were unstable probably due to the small or low number of observations. The hazard was not significantly different in the MQAS than in the DHAPQ arm (HR: 1.56; 95% CI 0.26–9.38; $p = 0.63$) (Table 3). The hazard of treatment failure was higher in younger women than in those over 20 years (HR: 5.07; 95% CI 1.01–25.43; $p = 0.05$). Higher parasite density at baseline was associated with a higher hazard of PCR-adjusted treatment failure (HR: 3.35; 95% CI 1.07–10.45; $p = 0.04$) (Table 3).

Table 2 Malaria treatment outcome of pregnant women with malaria episode in Nchelenge, Zambia (2010–2014)

	AL (N=300)	MQAS (N=300)	DHAPQ (N=300)
Efficacy outcomes, n (%)			
Early treatment failure[a]	0	0	1
Late clinical and parasitological treatment failure[a]	126	60	36
Recrudescence	12	5	2
New infection	114	55	34
Adequate clinical and parasitological response[a]	132	175	199
Cannot be determined	42	65	64
Did not meet inclusion/exclusion criteria/missing	8	17	8
Treatment violations	1	0	1
No PCR sample	0	3	4
LTFU/died/withdrawn	33	45	51
Treatment failure rate % (95% CI)			
PP-analysis: PCR-unadjusted	48.4 (42.4–54.5)	23.9 (19.0–29.8)	16.3 (12.1–21.4)
PP-analysis: PCR-adjusted	4.7 (2.7–8.0)	1.3 (0.4–3.7)	0.8 (0.2–3.0)
ITT-analysis: PCR-unadjusted	47.6 (41.7–53.5)	25.1 (20.2–30.8)	16.5 (12.4–21.6)
ITT-analysis: PCR-adjusted	4.9 (2.9–8.2)	2.0 (0.9–4.6)	1.2 (0.4–3.5)
Placental malaria	N = 235	N = 228	N = 227
Acute infection, n (%)	3 (1.3)	2 (0.9)	0 (0.0)
Chronic infection, n (%)	75 (31.9)	70 (30.7)	67 (29.5)
Past infection, n (%)	148 (63.0)	139 (61.0)	146 (64.3)
No infection, n (%)	9 (3.8)	17 (7.5)	14 (6.2)

LTFU Lost to follow-up; *PP* per-protocol; *ITT* intention-to-treat.

[a]Early Treatment Failure (ETF) defined as one of the following: (i) development of danger signs or severe malaria or worsening of clinical conditions on day 0, day 1, day 2 or day 3, in the presence of parasitaemia, (ii) parasitaemia on day 3 ≥count on day 0, (iii) parasitaemia on day 3 and fever (axillary temperature ≥37.5°C). Late clinical failure (LCF) defined as (i) development of danger signs or severe malaria or worsening of clinical conditions on any day after day 3 in the presence of parasitaemia, without previously meeting any of the criteria of Early Treatment Failure or (i) presence of parasitaemia and fever on any day after day 3, without having previously meet the criteria of ETF. Late parasitological failure (LCF) defined as presence of parasitaemia on any day from day 4 onwards and axillary temperature <37.5°C, without previously meeting any of the criteria of ETF or LCF. Adequate clinical and parasitological response (ACPR) defined as absence of parasitaemia at the end of the follow up period (day 63), irrespective of axillary temperature without previously meeting any of the criteria of early and late treatment failure. In the PCR-adjusted estimates, patients with late asexual parasite reappearance (with or without fever) are considered ACPR if the PCR analysis shows a new infection rather than a recrudescence. Placental malaria classified as: acute infection (parasite present, malaria pigment absent); chronic infection (parasites and malaria pigment present); past infection (no parasite but pigment present); no infection (both parasites and malaria pigment absent)

Fig.2 Time to PCR adjusted and unadjusted treatment failure in Zambian leg of PREGACT study in Nchelenge, Zambia (2010–2014)

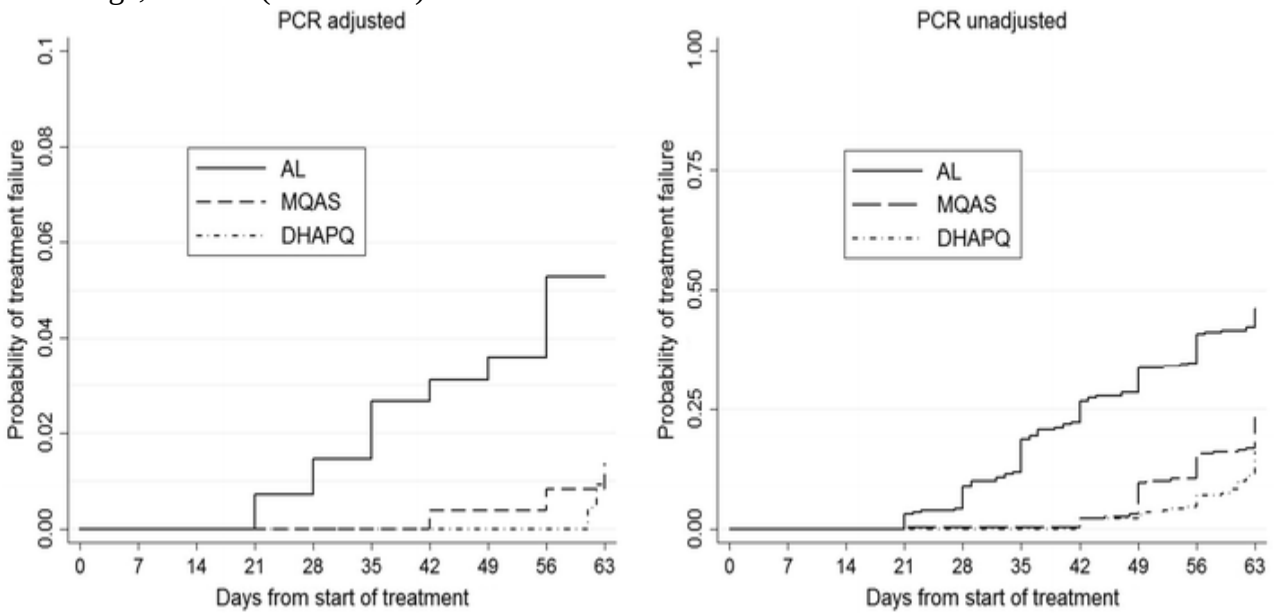
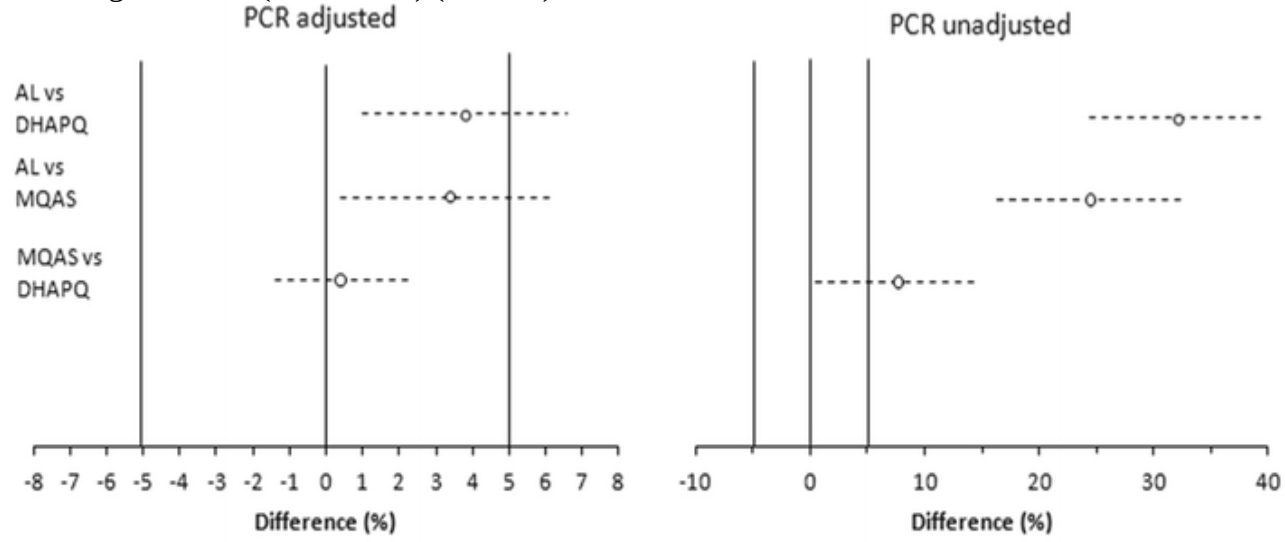


Fig. 3 Pair-wise comparisons for PCR adjusted and unadjusted ACPR at days 63 (PP population). Nchelenge, Zambia (2010–2014) (95% CI)



In the PCR-adjusted cure rates, recurrent infections confirmed by genotyping to be same infections as those before treatment (recrudescence) were considered as treatment failures. The estimation of PCR-unadjusted cure rates, all recurrent infections were considered to be treatment failures. A positive value in the difference reflects a higher cure in the treatment listed last. If the difference in the cure rates was less than 5 percentage point, the treatments were considered therapeutically equivalent.

Table 3 Risk factors associated with recrudescence and new infection after anti-malarial treatment in pregnant women in Nchelenge, Zambia (2010–2014)

	Hazard ratio	95% CI	*p* value
Risk factors associated with recrudescence			
Treatment			
AL	10.47	2.18–50.19	
MQAS	1.56	0.26–9.38	
DHAPQ	1	1 (reference)	<0.01[a]
Maternal age (15–19 years)	5.07	1.01–25.43	0.05
Gestational age			
2nd Trimester	2.35	0.76–7.40	
3rd Trimester	1	1 (reference)	0.14
Gravidity			
Primigravidae	1.44	0.51–4.38	
Multigravidae	1	1 (reference)	0.47
Parasite density (>2000/µL)	3.35	1.03–10.10	0.04
Study treatment dosage (mg/kg for 3 days)	2.10	0.75–5.89	0.16
Risk factors associated with malaria new infection			
Treatment			
AL	4.71	3.10–7.15	
MQAS	1.59	1.02–2.46	
DHAPQ	1	1 (reference)	<0.01[a]
Maternal age (15–19 years)	1.78	1.26–2.52	<0.01[a]
Parasite density (>2000/µL)	1.46	1.09–1.94	0.01
Anaemia (Hb <11.0 g/dL)	1.56	1.05–2.32	0.03
Gravidity			
Primigravidae	1.11	0.80–1.54	
Multigravidae	1	1 (reference)	0.52
Study treatment dosage (mg/kg for 3 days)	1.00	0.74–1.37	0.98

[a]p value of the joint effect of treatment

New infections were more frequent in the AL (HR: 4.71; 95% CI 3.10–7.15; p < 0.01) and MQAS (HR: 1.59; 95% CI 1.02–2.46; p = 0.04) arms compared to the DHAPQ arm. The risk of re-infections was higher in women between 15 and 20 years (HR: 1.78; 95% CI 1.26–2.52; p < 0.01) than in women older than 20 years. Anaemic mothers had a higher hazard of new infection during follow up (HR: 1.56; 95% CI 1.05–2.32; p = 0.03). Similarly, mothers with higher parasite density a higher hazard of new infection (HR: 1.46; 95% CI 1.09–1.94; p = 0.01). All the other risk factors analysed were not significantly associated with new infection (Table 3).

Placental malaria infection (acute and chronic) was similar between the treatment arms (p = 0.47) (Table 2). Treatment allocation, AL and MQAS (DHAPQ as reference group), was not significantly related to placental malaria (OR 1.22; 95% CI 0.45–3.31 and OR 0.76; 95% CI 0.34–1.72, respectively) (p = 0.58). Women with recrudescence and new infections were at higher risk of placental malaria (OR 4.46; 95% CI 1.01–19.70; p = 0.05). Placental malaria was significantly higher in women between 15 and 20 years (OR 4.56; 95% CI 1.48–14.04; p = 0.01) than in women older than 25 years. Placental malaria was significantly associated with low birth weight (<2500 g) (OR 4.37; 95% CI 1.04–18.39; p = 0.04) but not with adverse birth outcomes (stillbirth, preterm, miscarriage, intrauterine fetal death). (OR 5.47; 95% CI 0.33–90.62; p = 0.24).

The study drugs were generally safe with a total of 7 SAEs for mother. A woman treated with MQAS died 41 days after treatment, probably because of meningitis. There were three SAEs in DHAPQ [low haemoglobin, measles and sickle cell mother in haemolytic crisis (vaso-occlusive)]. An additional SAE in the MQAS arm, severe vomiting, was considered related to study treatment and recovered completely. The other two in MQAS were asthmatic attack and pneumonia. They all recovered.

The proportion of women with AEs in each treatment arm (82.7% in AL, 84.9% in MQAS and 79.3% in DHAPQ) were not significantly different (p = 0.19) (Table 4). The drug-related AEs (dizziness, nausea, headache, vomiting and asthenia) were more common in the MQAS arm (67.9%; 95% CI 62.4–72.9) than the AL (12.7%; 95% CI 9.3–16.9) and DHAPQ arms (23.3%; 95% CI 18.9–23.4) (p < 0.01). There were significant differences in median Hb at day 7 between AL (10.1 g/dL) versus MQAS (9.9 g/dL)), p = 0.01; and AL vs DHAPQ (9.9 g/dL), p = 0.04; and at day 63 between AL (10.7 g/dL) vs DHAPQ (11.0 g/dL), p = 0.01 (Fig. 4). There were no significant differences in systolic and diastolic blood pressures between the treatment arms (p = 0.07 and p =

0.20 respectively). The median biochemical (creatinine, ALAT and bilirubin) safety values between treatment groups did not differ significantly during the follow-up period (p=0.69, p=0.92 and p=0.88 respectively) (Fig.4).

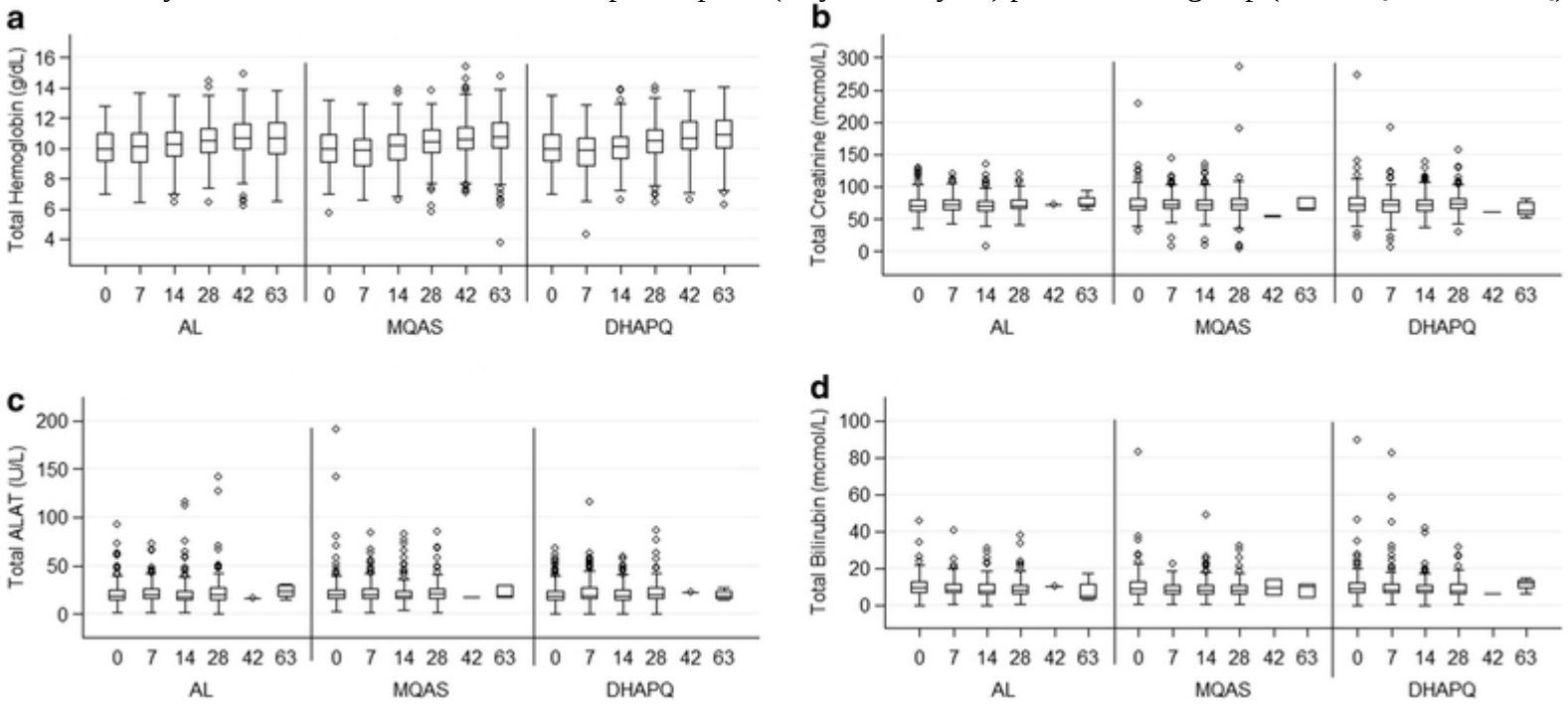
Table 4 Pregnant women with an adverse event till 63 days having received at least one malaria treatment dose in Nchelenge, Zambia (2010–2014)

Safety population	AL (N=300)	MQAS (N=299)	DHAPQ (N=300)
At least one AE, n (%)	248 (82.7)	254(84.9)	238 (79.3)
Most common AEs[a], n (%)			
Headache	136 (45.3)	142 (47.5)	134 (44.7)
Nausea	8 (2.7)	39 (13.0)	23 (7.7)
Cough	99 (33.0)	116 (38.8)	120 (40.0)
Asthenia	36 (12.0)	69 (23.1)	49 (16.3)
Dizziness	11 (3.7)	88 (29.4)	17 (5.7)
Vomiting	14 (4.7)	47 (15.7)	26 (8.7)
Abdominal pain	73 (24.3)	71 (23.7)	70 (23.3)
Musculoskeletal pain	43 (14.3)	47 (15.7)	30 (10.0)
Backache	51 (17.0)	40 (13.4)	28 (9.3)
Influenza	24 (8.0)	32 (10.7)	40 (13.3)
At least one related AE, n (%)	54 (18.0)	127 (42.5)	72 (24.0)
Most common related AEs[a], n (%)			
Dizziness	5 (1.7)	72 (24.1)	8 (2.7)
Nausea	5 (1.7)	34 (11.4)	16 (5.3)
Vomiting	5 (1.7)	43 (14.4)	17 (5.7)
Asthenia	9 (3.0)	38 (12.7)	14 (4.7)
Headache	14 (4.7)	16 (5.4)	15 (5.0)
SAE n (%)	0	4 (1.3)	3 (1.0)
Related SAE, n (%)	0	1 (0.3)	0
At least one SAE which caused death, n (%)	0	1 (0.3)	0
Birth outcomes, n (%)			
Still birth	8 (2.8)	3 (1.1)	10 (3.7)
Miscarriage	0 (0)	2 (0.7)	1 (0.4)
Prematurity	13 (4.6)	6 (2.2)	10 (3.7)
Congenital abnormality	7 (2.6)	4 (1.5)	4 (1.6)

AE adverse event; *SAE* serious adverse event; *Related SAE* serious adverse event which the investigator classified as possibly, probably or definitely related to study drug

[a]AEs and related AEs recorded in, respectively, at least 10 and 5% of patients in any treatment group

Fig.4 Box plots showing median and interquartile range as well as outlying values for total hematological and biochemical parameter levels in the *y*-axis and *x*-axis for each follow-up time point (Day 0 to Day 63) per treatment group (AL, MQAS, DHAPQ).



a Comparison of hemoglobin level between treatment groups by day of follow-up. b Comparison of creatinine level between treatment groups by day of follow-up. c Comparison of ALAT level between treatment groups by day of follow-up. d Comparison of bilirubin level between treatment groups by day of follow-up

There were 21 stillbirths: 8 (2.7%) in AL, 3 (1.0%) in MQAS and 10 (3.3%) in the DHAPQ arms and three miscarriages (two in MQAS, one in DHAPQ arms, and none in the AL arm). The preterm delivery was 4.3% in AL, 2.0% in MQAS and 3.3% in the DHAPQ arm. There were 15 congenital malformations [3 cleft lip and palate, one club foot, one ear tag, 6 polydactyl, one syndactyl, one umbilical hernia, one depression on parietal bone, one tongue tie) observed (4 (1.3%) in each of DHAPQ and MQAS arms and 7 (2.3%) in AL arm] with no significant difference between the arms (p=0.54).

4.5 Discussion

With the range of 0.8–4.7% recrudescences, the three artemisinin-based combinations used for the treatment of uncomplicated malaria in the second and third trimester of pregnancy were efficacious, in an area of high endemicity in Nchelenge district, Zambia. Therapeutic equivalence could be shown for MQAS and DHAPQ but not for AL as compared to the other two treatments. In Nchelenge Zambia, there were significantly more treatment failures in the AL arm compared to the other two arms, though AL efficacy was still above the 90% cure threshold recommended by WHO for adopting new anti-malarial treatments as policy [21]. In Uganda, in an area with malaria transmission as high as that in Nchelenge, AL administered to pregnant women was also extremely efficacious, with even less treatment failures (0.7%) than in this trial [22].

In Zambia, ACT has been shown to have excellent cure rates among children and adults [23, 24]. Their efficacy, determined by the drug partnering an artemisinin derivative, namely mefloquine, lumefantrine, and piperaquine for the treatment tested in this study, usually exceeds 95% [25]. However, there have been reports pointing to the effect of the physiological changes during pregnancy, e.g. as increased volume of distribution, reduced gut motility, possibly altering drug

disposition and metabolism, and thus leading to incorrect dosing [26, 27, 28]. This does not seem to apply to the results observed in Nchelenge as treatment efficacy was very high, possibly due to the underlying anti-malarial immunity in the Nchelenge district population, including pregnant women, due to the intense malaria transmission and high exposure to infection. The importance of pre-existing immunity on the therapeutic response is also supported by the association between treatment failure (both new infections and recrudescences) and young age [29]. Also transmission intensity may not influence the risk difference between treatments but may influence individual failure rates.

Pregnant women have an increased susceptibility to malaria, and this susceptibility is greatest in the first pregnancy (primigravidae) [30]. The decreasing prevalence and intensity of infection in successive pregnancies mirrors the acquisition of antibody immunity to the variant surface antigens, expressed on the parasitized red blood cells infecting the placenta. Antibodies titres against VSA-PAM are associated to clinical outcomes [31, 32] and opsonizing antibodies that allow phagocytic clearance of infected erythrocytes are associated with a better treatment outcome in pregnant women [33]. Results from Nchelenge and other studies suggest that antibodies to VSA-PAM might have important roles in determining both pregnancy outcomes and the effectiveness of anti-malarial drugs in pregnancy. Other factors such as cellular immunity, cytokines, and hormonal changes might also influence outcomes in pregnancy [29] and also affect treatment outcome.

In Nchelenge, pregnant women treated with AL had a higher risk of new infection than the other two treatments. This is probably due to the shorter post-treatment prophylaxis offered by lumefantrine which is eliminated more rapidly [34] than piperaquine [35]. When the artemisinin component is rapidly eliminated, a new infection would encounter only the partner drug and this may explain the association between the risk of new infection and treatment given. It indirectly confirms that the distinction between recrudescence and new infection and genotyping is reasonably

reliable. Considering that Nchelenge women who experienced a new infection during follow up had a higher risk of acute or chronic placenta malaria, both conditions associated to the delivery of low birth weight babies, a longer post-treatment prophylaxis would be extremely important in this area of intense malaria transmission. Therefore, DHAPQ could be preferentially chosen for such conditions, while AL could be used where transmission is low.

Recrudescence may easily occur in the context of emergence or spread of parasite resistance to a given anti-malarial when the partially efficacious anti-malarial may fail to clear the resistant strain or simply select for mutant parasites. In Zambia, artemisinin resistance has not been reported yet. Recrudescence can be caused by the parasites surviving the effect of a shorter-acting ACT [6] in this case AL. Low study drug dosage may play an important role in recrudescence in the AL group as the point estimate indicates low study drug dosage suggests a double independent risk for recrudescence. However, the power of the study was to assume a clear association. Besides parasite sensitivity to drug and the level of the concentration of the drug in the blood, host immunity and parasite density at presentation contributes to positive treatment outcome. Immunity can be affected by different factors, including age, body temperature, pregnancy and parity [29, 36]. The Nchelenge study has shown that younger women and high malaria parasite density at baseline are associated with recrudescence and could not demonstrate a significant association between treatment failure and parity.

Other studies have shown that high parasite density at presentation is associated with treatment failure [30, 31, 32, 33, 36, 37, 38] and that age, temperature and parasite density are predictors of anti-malarial treatment failure [36].

The three artemisinin-based combinations tested are generally safe in second and third trimester of pregnancy in Zambia. Patients on MQAS had higher rates of treatment-related AE. Dizziness was the most common, followed by vomiting and weakness. Dizziness has been reported even in other

studies as related to MQ treatment [39]. On the pregnancy outcomes, there was no significant difference between treatments for stillbirths, miscarriages, congenital malformations and prematurity, a finding similar to those of other studies on AL [22, 40, 41], mefloquine [5] and DHAPQ [42].

This trial was done in an area where the majority of the population practice farming and fishing as a source of livelihood and they migrate to farming areas for a considerable period [43], possibly explaining the relatively high number of lost-to-follow-ups and withdrawals. Nevertheless, considering that the post-treatment follow-up was up to day 63 and that pregnant women are a group particularly difficult to follow, the sample size had been estimated assuming a dropout rate of 20%, while the actual figure was 16%. Such a relatively high dropout rate is unlikely to have had a major influence on the trial's results as the patients excluded and those included did not differ significantly on their baseline characteristics.

4.6 Conclusion

The study has shown that both AL and DHAPQ were well tolerated in second and third trimester pregnant women, with low treatment failures. MQAS was less well tolerated than the other two treatments though it had similar low treatment failure. DHAPQ seems to be well tolerated and has low treatment failure with a longer post-treatment prophylaxis. As new infections can be prevented by a long acting partner drug to the artemisinins, DHAPQ should be preferred where transmission is intense as in Nchelenge while and in areas of low transmission intensity AL or MQAS may be used.

Abbreviations

ACPR: adequate clinical and parasitological response; AE: adverse events; AL: artemether–lumefantrine; ACT: artemisinin-based combination treatment; DHAPQ: dihydroartemisinin–piperaquine; ETF: Early Treatment Failure; e-CRF: electronic clinical record form; Hb:

haemoglobin; HR: hazard ratio; ITT: intention-to-treat; IQR: interquartile range; ITN: insecticide treated net; LTFU: lost to follow-up; MQAS: mefloquine–artesunate; OR: odds ratio; PP: per-protocol; PCR: polymerase chain reaction; SAE: serious adverse event

Declarations

Authors' contributions

MN, UDA and JPV proposed the ideas of the paper. MN, UDA and JPV were major contributors to the writing of the paper. MN and JB contributed to data analysis. MN, MM, JBBK, SH, JB, WK, JMM, DM, JPV, UDA reviewed the manuscript. MN is the corresponding author/main contact for this paper. All authors read and approved the final manuscript.

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Competing interests

No competing interests stated except UDA who reports receiving grant support from Sigma-Tau Industrie Farmaceutiche Riunite.

Availability of data and materials

The data that support the findings of this study are available from Institute of Tropical Medicine, Antwerpen, Belgium Clinical Trials Unit but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Institute of Tropical Medicine, Antwerpen, Belgium (http://www.itg.be).

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Antwerp University Hospital [Universitair Ziekenhuis Antwerpen (UZA)] and Tropical Diseases Research Center Ethics Committee in Zambia. All study participants consented to participate in the study.

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Chapter 5: Artemisinin–based combination treatment during pregnancy; outcome of pregnancy and infant mortality: a cohort study

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5.1 Abstract

Background: The World Health Organization (WHO) recommendation of treating uncomplicated malaria during the second and third trimester of pregnancy with an artemisinin-combination therapy (ACT) has already been implemented by all sub-Saharan African countries. However, there is limited knowledge on the effect of ACT on pregnancy outcomes, and on new-born and infant's health.

Methods and Results: Pregnant women with malaria in four countries (Burkina Faso, Ghana, Malawi and Zambia) were treated with either artemether-lumefantrine (AL), amodiaquine-artesunate (ASAQ), mefloquine-artesunate (MQAS), or dihydroartemisinin-piperaquine (DHA-PQ); 3,127 live new-borns (822 in the AL, 775 in the ASAQ, 765 in the MQAS and 765 in the DHAPQ arms) were followed-up until their first birthday. Prevalence of placental malaria and low birth weight were 28.0% (738/2646) and 16.0% (480/2999), respectively, with no significant differences between treatment arms. No differences in congenital malformations (p=0.35), perinatal mortality (p=0.77), neonatal mortality (p= 0.21), and infant mortality (p=0.96) were found.

Conclusion: Outcome of pregnancy and infant survival were similar between treatment arms indicating that any of the four ACTs could be safely used during the second and third trimester of pregnancy without any adverse effect on the baby. Nevertheless, smaller safety differences between ACTs cannot be excluded; country-wide post-marketing surveillance would be very helpful to confirm such findings.

Trial registration: ClinicalTrials.gov, NCT00852423, Registered on 27 February 2009, https://clinicaltrials.gov/ct2/show/NCT00852423

5.2 Background

Pregnant women are more vulnerable to malaria than non-pregnant women because of altered immunity. Each year, about 85 million pregnant women in sub-Saharan Africa (SSA) are at risk of Plasmodium falciparum (Pf) infection [1,2]. Pf malaria in pregnancy (either asymptomatic or symptomatic) can result in serious adverse outcomes for both the mother and the infant; maternal anaemia and placental malaria may result in low birth weight (LBW), miscarriage, stillbirth and infant death [3-6]. Effective and safe anti-malarial treatments decrease the occurrence of such adverse outcomes. The World Health Organization (WHO) recommends the use of artemisinin-combination therapy (ACT) for the treatment of Pf uncomplicated malaria during the second and third trimester of pregnancy [7]. All sub-Saharan African countries have already adopted and implemented such recommendation. However, there is limited knowledge on the effect of ACT use on pregnancy outcomes and infant's safety.

We recently reported the results of the safety and efficacy of four ACT, namely artemether-lumefantrine (AL), amodiaquine-artesunate (ASAQ), mefloquine-artesunate (MQAS), dihydroartemisinin-piperaquine (DHA-PQ), in African pregnant women with malaria [8,9]. AL had the best tolerability profile, acceptable cure rates but the shortest post-treatment prophylaxis, while DHA-PQ seemed the most suitable treatment in terms of safety and efficacy, including its longer post-treatment prophylaxis [8,9]. Here, we report the effect of the four ACT on pregnancy outcome, and on neonatal and infant morbidity and mortality.

5.3 Methods

The study was an open label, randomized controlled clinical trial comparing the efficacy and safety of four ACT in pregnant women with Pf uncomplicated malaria in the second and third trimester of pregnancy. The trial protocol has been reported in detail elsewhere (NCT00852423) [10]. Briefly,

the trial was carried out between June 2010 and April 2015 at seven sites across four countries, namely Burkina Faso, Ghana, Malawi and Zambia. Pregnant women in their second or third trimester were enrolled and randomized to one of four treatment arms (AL, ASAQ, MQAS and DHAPQ). Participants were followed up until day 63 and then were seen at delivery.

Infant follow-up: Mothers were asked to attend study health facilities with their babies at 4-6 weeks post-delivery and/or at the time of due vaccinations, 6 months later and at around the baby's first birthday. Infants were examined by a study nurse or doctor and their medical conditions, if any, managed as appropriate, including hospital admissions. Congenital anomalies or birth defects and deaths were reported as serious adverse events (SAEs), and relevant information was collected by interviewing the mother and/or from hospital records. SAEs were codified using Medical Dictionary for Regulatory Activities (MeDRA) preferred term.

Perinatal mortality rate was defined as the number of perinatal deaths (stillbirths and early neonatal deaths) per 1000 total births [11]. Neonatal mortality rate was defined as the number of neonatal deaths (death during the first 28 days of life) per 1000 live births (first week of life: early neonatal death; 8-28 days of life: late neonatal death) [12]. Infant mortality rate was defined as the number of infant deaths (<1 year of age) per 1000 live births. Placental malaria was classified as acute and chronic infection (presence of parasites with or without malaria pigment), past infection (presence of malaria pigment) and no infection (no parasites or malaria pigment).

The trial was approved by the ethics committee of the Antwerp University Hospital, Belgium, the relevant national or local ethics committees, and the national drug regulatory authorities of the four African participating countries.

Statistical analysis

The data analysis was based on an intention-to-treat (ITT) analysis, i.e. all infants born to mothers included in the study and randomized to one of the four treatments were included, regardless of the number of doses taken. Twins were excluded from the analysis as mortality is higher in this group [13]. Descriptive statistics were used to summarise socio-demographic and clinical characteristics of the mothers and infants. The difference between the treatment arms was calculated using logistic regression with fixed effects for treatment and country. The same approach was applied for the incidence of fever and other symptoms such as cough, diarrhoea, and difficulty in feeding at 4-6 weeks of life per 1000 live births. Continuous variables were compared between treatment groups using ordinary least square regression adjusted for country.

Incidence of SAEs in infants between treatment groups was compared by using a logistic regression model with fixed effects for treatment and country, to correct for possible imbalances in the reporting between countries.

Population-attributable fraction was used to assess the effect of placental malaria on LBW. The prevalence ratio (Pr) was set as the proportion of infected placentas (acute and chronic infections) with LBW divided by the proportion of uninfected placenta (past infections and uninfected) with LBW. The infected attributable fraction was calculated as the percentage of infected placenta with LBW that was due to malaria [(Pr-1)/Pr] while the population-attributable fraction was the percentage of LBW cases that were due to malaria infection [14].

Infant mortality and hospital admissions rates were calculated as number of subjects with the event over the time at risk. The rate was compared between treatment arms using Poisson regression or negative binomial regression model [15] adjusted for covariates, including country, treatment, maternal age, etc. In the univariate analysis, covariates were selected for inclusion in multivariable model if the p-value was ≤ 0.25. In the final model covariates with a p-value of >0.2 were dropped

step by step. Stata v14 (Stata Corp, USA) was used for all statistical analyses. The significant level was set at $p \leq 0.05$.

5.4 Results

A total 3,258 out of the 3,428 mothers enrolled had a recorded delivery outcome. Maternal characteristics such as gestational age (estimated by the total Ballard score), and maternal malaria infection status at delivery were not significantly different between treatment groups (Table 1). Out of the total recorded delivery outcomes, 3,127 mothers (excluding mothers with twins) delivered live babies: 822 in the AL, 775 in the ASAQ, 765 in the MQAS and 765 in the DHAPQ arms. Fifty two mothers delivered twins and were excluded from analysis. There was no difference in the proportion of live births, adjusted for country, between treatment groups (p=0.85) (Table 2). There were 13 (0.39%) miscarriages and 78 (2.36%) stillbirths [8]. Among all live births, 118/3174 (3.7%) were lost during the 1-year follow-up while 6 mothers (0.2%) withdrew their consent.

Prevalence of LBW was 16.0% (480/2999), and was significantly more frequent among women with acute and chronic placenta malaria (21.0%, 152/723) than in those with past or no infection (13.7%, 259/1892) (p<0.01). Nevertheless, LBW (p=0.54) and mean birth weight were similar between treatment arms (Table 2). Prevalence of both cord blood anaemia and congenital malaria infection (malaria parasites in cord blood) was low and not significantly different between treatment arms. Similarly, there were few congenital abnormalities identified at birth (1.4%, 44/3065), with no statistically significant difference between study arms (Table 2) [8].

The overall prevalence of acute and chronic placental malaria was 28.0% (738/2646). Attributable fraction analysis suggests placenta malaria (acute and chronic) was responsible for 35% of the LBW among women with placental malaria infection (infected attributable fraction). At the prevalence

found in this trial, the percentage of all LBW babies due to malaria (population-attributable fraction) was estimated at 13% (Table 3).

Besides congenital abnormalities (almost half of them (46%, 23/50) polydactyly), the most commonly reported SAEs among infants were infections and infestations (Table 4), the most frequent being pneumonia (26.1%, 12/46), neonatal sepsis (21.7%, 10/46), sepsis (17.4%, 8/46), and malaria (8.7%, 4/46), with no statistically significant difference between treatment arms.

Reported hospital admissions (episodes per 1000 live birth/year) were similar between treatment arms: AL: 90, ASAQ: 104, MQAS: 56, DHAPQ: 85 (p= 0.91) (Table 5).

During the 1-year follow up, when adjusting for country and other potential risk factors, babies born at home (IRR 1.44, 95% CI: 1.02-2.03) (p=0.04) or with a congenital abnormality (IRR 5.12, 95% CI: 2.09-12.51) were more likely to be admitted in hospital. Infants sleeping under bed nets (0.36, 95% CI: 0.21-0.62) (p<0.01) were less likely to be admitted in hospital than those who were not (Table 6).

There were 70 deaths within the first month of life, representing 59.3% (70/118) of all infant deaths, with no difference between study arms (55.2% in AL, 74.1% in ASAQ, 41.7% in MQAS and 63.2% in DHAPQ) (p=0.47). The large majority of neonatal deaths (71.4%, 50/70) occurred in the first week of life, at the mean age of 1.3 (SD 1.9) days, with no statistically significant difference between treatment arms (AL: 2.1; ASAQ: 1.5; MQAS: 0.3; DHAPQ: 1.0) (p=0.19). Neonatal mortality (early and late) rate was 22.4 deaths per 1000 live births (AL: 19.5; ASAQ: 25.8, MQAS: 13.1; and DHAPQ: 31.4) (p= 0.21); perinatal mortality rate was 21.9 deaths per 1000 total births (AL: 17.9; ASAQ: 17.7, MQAS: 28.0; and DHAPQ: 24.2) (p= 0.77), with no significant difference between the treatment groups (Table 7). The overall infant mortality rate was 41.0 per 1000 live

births per year, and similar between treatment arms (AL: 38.7, ASAQ: 38.0, MQAS: 33.4 and DHAPQ: 54.1) (p= 0.96) (Table 5). Infant mortality was significantly higher in Zambia than in Burkina Faso but not in Ghana or Malawi (Table 6).

When adjusting by country and other potential risk factors, LBW babies had an almost 2-fold higher risk of dying during the first year of life than other babies (IRR 1.78, 95%CI: 1.03-3.06, p=0.04). Babies born with birth asphyxia (IRR 10.89, 95% CI: 4.48-26.46) (p<0.01) and congenital abnormality (25.47, 95% CI: 10.46-62.02) (p< 0.01) had significantly higher risk of dying than other infants (Table 6).

5.5 Discussion

The PREGACT study aimed at evaluating the safety and efficacy of four ACT when administered to pregnant women with malaria [8]. The follow-up included also the offspring's first year of life to identify any potential problem the treatment may have. Results are reassuring as no significant differences between study arms in terms of reported illness, hospital admissions or infant mortality were found. It is extremely difficult to estimate cause-specific mortality as most infant deaths occurred outside a health facility [16-19]. Some of the most frequent SAEs reported in this study, namely neonatal sepsis, respiratory infections, malaria and prematurity, are also among the most common causes of infant deaths in malaria-endemic African countries, where infant mortality rates are the highest in the world [19,20], and were probably among the most frequent cause of infant death.

More than two third of all neonatal deaths occurred in the first week of life. In this age group, malaria is usually an indirect cause of death as it causes LBW which in turn increases the risk of dying in the first year of life [16,20]. This is confirmed by the significant association between LBW and placenta malaria (acute and chronic) and the substantially higher mortality in LBW babies. Few

trials have evaluated the effect of chemoprevention during pregnancy on miscarriages, stillbirths, perinatal deaths, or neonatal deaths, and usually these studies are underpowered to detect clinically important differences [21]. Such a difference may be found when comparing an intervention to a placebo arm, as in Mozambique where Intermittent preventive treatment during pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) was compared to a placebo arm [22]. Indeed, in this trial significantly fewer neonatal deaths, particularly early infant deaths, occurred in the intervention than in placebo group despite no difference in prevalence of anaemia, LBW or placenta malaria (active and past infection) [22]. However, infant mortality did not differ between study arms although among the 58 infant deaths reported, more than half occurred in the placebo arm [16]. When all women included in a trial receive a malaria-preventive intervention, significant differences in neonatal and/or infant mortality are unlikely to be found unless one of the interventions has either a negative or positive effect on infant survival. For example, a trial comparing IPTp with either SP (IPTp-SP) or mefloquine (MQ) did not report any difference in neonatal and infant mortality between the two arms as both treatments are efficacious against malaria [23]. Therefore, the similar outcomes in the four arms of our trial are not surprising as all treatments were extremely efficacious against malaria [8]. A difference may have occurred if any of the treatments tested would have had an adverse effect on the foetus or infant survival or if the four tested ACT were compared to a non-ACT. Indeed, in Uganda spontaneous abortions and neonatal deaths were less frequent among women treated with AL than in those treated with quinine although the difference was not statistically significant [24].

Placenta sequestration of malaria parasites can lead to inflammatory response, particularly in first-time mothers who often have high density infections, and also affect development of the foetal circulation. This decreases the supply of nutrients and oxygen to the foetus, resulting in intrauterine

growth retardation and LBW, which is a risk factor for higher infant mortality [25]. More than a quarter of women had an active placenta infection, mainly chronic ones, i.e. with both parasites and malaria pigment, while evidence of past (non-active) infection was found in more than 60% of women. As the trial recruited pregnant women with malaria, such a high prevalence is understandable. In addition, women were actively followed up during the 63 days post-treatment, and passively until delivery. Most women were treated during the second trimester and the early third trimester of pregnancy, and the treatment by itself could not prevent women to be re-infected near delivery. Indeed, longitudinal genotyping of Pf isolates during gestation in Cameroonian pregnant women showed that 77% of placental parasites were acquired from 30 weeks' gestation onwards [25].

Foetal anaemia plays a role in neonatal survival [16,26]. Cord blood anaemia was particularly low, just around 1% and similar in all treatment arms, although it increased the risk of infant death almost 3-fold, an association that was of borderline significance. Such low prevalence is surprising when considering that a recently published study on a trial assessing IPTp with either SP or MQ reported a prevalence of about 10%, with a definition of anaemia that was more conservative than in this study (Hb<12.5 g/dl versus Hb<14.0g/dl) [23]. The reason for such difference is unclear.

The longer recall period between the post-partum visits, at 4-6 weeks, and then at 6 and 12 months could have affected the accuracy of information on morbidity. Nevertheless, it is unlikely the study team missed any infant death. Moreover, such recall bias would have equally affected the 4 study arms.

5.6 Conclusion

In summary, there were no major differences between treatment arms in terms of perinatal, neonatal and infant mortality, nor on the overall occurrence of SAEs in babies, indicating that any of the

tested ACT can be used for the treatment of malaria during the second and third trimester of pregnancy without any adverse effect on the baby. As women were treated only once, repeated treatments may have resulted in a different outcome. It is reassuring that repeated administration of DHAPQ as IPTp did not increase the occurrence of adverse birth outcomes, indicating that at least this ACT could be safely administered 2-3 times during the second and third trimester of pregnancy [27]. Although the number of women treated and infants followed up is substantial, smaller safety differences between ACTs cannot be excluded. Country-wide post-marketing surveillance would be very helpful in confirming that any of the 4 ACTs tested can be safely used to treat malaria during pregnancy.

List of abbreviations

AE, adverse events; AL, artemether-lumefantrine; ACT, artemisinin-based combination treatment; DHAPQ, dihydroartemisinin-piperaquine, Hb, Haemoglobin; IRR, incidence rate ratio; IQR, interquartile range; ITN, insecticide treated net; LBW, low birth weight; LTFU, Lost To Follow-Up; MQAS, mefloquine-artesunate; PRR, pooled risk ratio; PCR, Polymerase Chain Reaction; SAE, serious adverse event.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Antwerp University Hospital (Universitair Ziekenhuis Antwerpen (UZA)) and National Ethics Committee in Burkina Faso, Ghana, Malawi and Zambia. All study participants consented to participate in the study.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from Institute of Tropical Medicine, Antwerp, Belgium, Clinical Trials Unit. Restrictions apply to the availability of these data due to ethical and privacy concerns. Data can however be made available after approval of a motivated and written request to the Institute of Tropical Medicine at ITMresearchdataaccess@itg.be/.

Competing interests

No conflict of interest stated except UDA who reports receiving grant support from Sigma-Tau Industrie Farmaceutiche Riunite.

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The funding body had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript should be declared.

Authors' contributions

PREGACT Consortium proposed the manuscript. MN, UDA and JPV wrote the manuscript. JB and MN did the statistical analysis. Manuscript was reviewed by MN, HT, VM, HT, JBBK, SH, MT, IV, MCT GA JB, RR, DA, KT, MM, JPV, UDA

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Tables and figures

Table 1. Gestational age and maternal malaria infection status at delivery

	AL		ASAQ		MQAS		DHAPQ		p-value[1]
	N		*N*		*N*		*N*		
Maternal age median (IQR)	881	21 (18-26)	842	22 (19-27)	850	22 (19-27)	855	20 (18-25)	0.67
Gestational Age (week) median (IQR)	804	38 (36-38)	729	38 (38-40)	733	38 (36-40)	709	38 (36-38)	0.30
Gravidity n (%)	880		842		850		855		0.55
1st Pregnancy		319 (36.3)		315 (37.4)		278 (32.7)		343 (40.1)	
2nd Pregnancy		204 (23.2)		187 (22.2)		201 (23.7)		216 (25.3)	
3rd or more		357 (40.6)		340 (40.4)		371 (43.7)		296 (34.6)	
Malaria prevalence (peripheral blood) n (%)	829	120 (14.5)	756	95 (12.6)	752	123 (16.4)	748	75 (10.0)	0.21
Parasite density; median (IQR)	120	1560 (440-6804)	95	1800 (320-8220)	123	1729 (561-9000)	75	2320 (480-8960)	0.09
% ≤2000/ µL (n)		55.0 (66)		53.7 (51)		52.0 (64)		46.7 (35)	
% >2000/µL (n)		45.0 (54)		46.3 (44)		48.0 (59)		53.3 (40)	
Gametocyte carriage n (%)	829	3 (0.4)	756	4 (0.5)	752	4 (0.5)	748	2 (0.3)	0.89
Maternal Hb median (IQR)	828	11.2 (10.1-12.2)	757	11.5 (10.4-12.4)	752	11.3 (10.3-12.3)	749	11.2 (10.3-12.2)	0.30
Placenta Malaria n(%)[2]	711		655		674		664		
Acute Infection		7 (1.0)		14 (2.1)		13 (1.9)		11 (1.7)	
Chronic infection		191 (26.9)		168 (25.6)		177 (26.3)		171 (25.8)	
Past infection		444 (62.4)		382 (58.3)		398 (59.1)		409 (61.6)	
No infection		69 (9.7)		91 (13.9)		86 (12.8)		73 (11.0)	

[1]adjusted by country

[2]the proportion of current (acute and chronic) and past infection vs. no infection, adjusted for country, p=0.47. Proportion of acute and chronic infection vs. past or no infection, adjusted for country p=0.52

Table 2. Baseline characteristics of study infants at delivery (%)

	AL		ASAQ		MQAS		DHAPQ		p-value[1]
	N	Value	*N*	Value	*N*	Value	*N*	Value	
Live births by country									0.85
Burkina Faso	280	278 (99.3)	279	278 (99.6)	275	268 (97.5)			
Ghana			245	235 (95.9)	241	229 (95.0)	252	243 (96.4)	
Malawi	276	269 (97.5)	269	262 (97.4)			270	268 (99.3)	
Zambia	282	275 (97.5)			273	268 (98.2)	264	254 (96.2)	
Birth asphyxia	706	19 (2.7)	635	42 (6.6)	658	44 (6.7)	648	39 (6.0)	0.91
Congenital abnormality[2]	815	17 (2.1)	757	8 (1.1)	751	13 (1.7)	742	6 (0.8)	0.35
Prematurity[3]	796	272 (34.2)	720	74 (10.3)	720	255 (35.4)	698	226 (32.4)	0.64
Congenital malaria[4]	808	7 (0.9)	727	2 (0.3)	719	1 (0.1)	711	1 (0.1)	0.15
Anaemia at birth[4]	793	12 (1.5)	720	7 (1.0)	713	10 (1.4)	696	10 (1.4)	0.36
Birth weight mean (SD)	804	2856 (452)	742	2873 (463)	733	2860 (460)	720	2889 (463)	0.56
LBW <2500g	804	138 (17.2)	742	118 (15.9)	733	119 (16.2)	720	105 (14.6)	0.54

[1]adjusted by country
[2]Reported as SAE at delivery
[3]Calculated by Ballard score
[4]Cord blood at 14g/dl cut off for congenital anaemia and cord blood for malaria

Table 3. Risk of LBW associated with placental malaria

Placenta malaria prevalence	Prevalence of LBW		Prevalence ratio	Infected attributable fraction	Population-attributable fraction
	Placenta malaria	No Placenta malaria			
27.89 (738/2646)	21.0 (152/723)	13.7 (259/1892)	1.535	34.87	12.99

The prevalence ratio (Pr) is the proportion of infected women with LBW divided by the proportion of uninfected women with LBW. The infected attributable fraction is the percentage of infected women with LBW that is due to malaria [(Pr-1)/pr]. The population-attributable fraction is the percentage of LBW cases that are due to malaria infection: [PM(Pr-1)]/{1+[PM(Pr-1)]} where PM is the proportion with placental malaria infection

Table 4: SAEs in infants by treatment arm

SAE by system organ class	AL (N= 822)		ASAQ (N= 775)		MQAS (N= 765)		DHAPQ (N= 765)	
	%	n	%	n	%	n	%	n
Congenital disorders	2.1	17	1.5	12	1.6	12	0.8	6
Infection and infestations	1.5	12	1.5	12	0.9	7	2.0	15
Respiratory disorders	1.0	8	0.8	6	0.9	7	1.0	8
General disorders	0.6	5	0.4	3	0.8	6	1.0	8
Gastrointestinal disorders	0.4	3	0.0	0	0.3	2	0.0	0
Perinatal complications	0.4	3	0.5	4	0.1	1	0.9	7
Blood disorders	0.2	2	0.0	0	0.1	1	0.1	1
Hepatobiliary disorders	0.1	1	0.0	0	0.0	0	0.0	0
Metabolism and nutrition disorders	0.1	1	0.1	1	0.0	0	0.3	2
Reproductive and breast disorders (e.g. labia enlarged)	0.1	1	0.0	0	0.0	0	0.0	0
Nervous system disorders	0.0	0	0.1	1	0.1	1	0.1	1

Table 5: Incidence of hospital admissions, infant mortality

Variable		*N/PYAR*	IR	*IRR (95%CI)*[1]	*p-value*
Hospital admissions	AL	67/748.6	0.09	Reference	
	ASAQ	74/710.6	0.10	1.01 (0.71-1.43)	
	MQAS	40/718.4	0.06	0.88 (0.57-1.35)	
	DHAPQ	60/702.9	0.09	1.01 (0.70-1.47)	0.91
Infant mortality	AL	29/748.6	0.04	Reference	
	ASAQ	27/710.6	0.04	0.96 (0.44-2.09)	
	MQAS	24/718.4	0.03	0.94 (0.47-1.86)	
	DHAPQ	38/702.9	0.05	1.12 (0.57-2.20)	0.96

[1]IRR (incidence rate ratio)

Table 6: Risks (incidence rate ratio) of infant mortality and hospital admission in PREGACT study

Infant mortality	IRR*	95% CI	P-value
AL	1	-	0.96
ASAQ	0.96	0.44-2.09	
MQAS	0.94	0.47-1.86	
DHAPQ	1.12	0.57-2.20	
Congenital anaemia	2.93	0.96-8.96	0.06
Low birth weight	1.78	1.03-3.06	0.04
Birth asphyxia	10.89	4.48-26.46	<0.01
Congenital abnormality	25.47	10.46-62.02	<0.01
Burkina Faso	1	-	<0.01
Ghana	1.02	0.31-3.36	
Malawi	2.14	0.89-5.15	
Zambia	4.02	1.85-8.73	
Hospital Admission	IRR*	95% CI	P-value
AL	1	-	0.91
ASAQ	1.01	0.71-1.43	
MQAS	0.88	0.57-1.35	
DHAPQ	1.01	0.70-1.47	
Baby ITN use	0.36	0.21-0.62	<0.01
Born at home	1.44	1.02-2.03	0.04
Congenital malaria	3.80	0.93-15.56	0.06
Congenital abnormality	5.12	2.09-12.51	<0.01
Burkina Faso	1	-	<0.01
Ghana	1.02	0.68-1.54	
Malawi	1.89	1.33-2.69	
Zambia	0.49	0.31-0.79	

*IRR= incidence rate ratio

Table 7: Perinatal and neonatal mortality, and morbidity during the first month of life by treatment (%)

	AL		ASAQ		MQAS		DHAPQ		p-value[1]
	N	Value[2]	*N*	Value[2]	*N*	Value[2]	*N*	Value[2]	
Perinatal mortality	837	15 (17.9)	789	14 (17.7)	787	22 (28.0)	784	19 (24.2)	0.77
Neonatal mortality	822	16 (19.5)	775	20 (25.8)	765	10 (13.1)	765	24 (31.4)	0.21
Morbidity									
Fever (4-6 [3]wk)	764	37 (48.4)	718	34 (47.4)	715	42 (58.7)	680	23 (33.8)	0.82
Diarrhea (4-6 wk)	764	14 (18.3)	717	22 (30.7)	715	19 (26.6)	679	4 (5.9)	0.62
Cough (4-6 wk)	764	34 (44.5)	717	33 (46.0)	715	56 (78.3)	679	52 (76.6)	0.02
Difficulty in feeding (4-6 wk)	764	2 (2.6)	717	5 (7.0)	715	6 (8.4)	679	1 (1.5)	0.76
Jaundice (4-6 weeks)	764	0 (0.0)	717	1 (1.4)	714	2 (2.8)	679	1 (1.5)	0.90
Other symptoms (4-6 wk)	764	33 (43.2)	717	44 (61.4)	715	51 (71.3)	679	16 (23.6)	0.33

[1]p-values are from logistic regression adjusted for country.
[2]values are n (/1000 live births) except perinatal mortality which has n (/1000 total births)
[3]wk (weeks)

Chapter 6: Conclusions and future perspectives of malaria in pregnancy

6.1 Conclusions

This thesis reports the methodology and some of the results of a trial that evaluated different options for the treatment of uncomplicated malaria in women in the 2nd and 3rd trimester of pregnancy, including a malariometric survey at the site where the study was implemented. Although artemisinin-based combination treatment of malaria during pregnancy were already recommended by WHO [1], information on their safety and efficacy in African pregnant women was extremely limited at the time this trial started. The trial was nested within the research activities of the Malaria in Pregnancy Consortium (https://www.mip-consortium.org/), coordinated by Liverpool School of Tropical Medicine, that included four research themes and cross-cutting activities, namely:

1. Treatment (Africa, Asia, and Latin America) aiming at identifying at least 2 antimalarial drug combinations that are safe, practical to use (3 day regimen or shorter) and highly effective for the treatment of uncomplicated *falciparum* and *vivax* malaria in pregnancy in Africa, Asia and Latin America;
2. Prevention (Africa) aiming at 1. Identifying at least one safe and effective alternative to SP for IPTp in Africa, and 2. Determining whether IPTp can be restricted to the main malaria transmission season in areas with highly seasonal transmission and to determine the optimal dosing frequency for IPTp in the context of integrated use with insecticide treated nets.
3. Prevention (Asia and Latin America) aiming at defining the malaria burden and at determining the optimal strategy for the control of MiP in areas with low or moderate transmission of falciparum and vivax malaria in Asia and Latin America.
4. Public health impact aiming at determining optimal ways of scaling up the use of existing and new tools to control MiP.

5. Cross-cutting activities divided into 4 areas:
    a. Pharmacokinetics: establishing the pharmacokinetic profiles and tolerability of the antimalarial candidate drugs and optimizing the dose regimens for use in subsequent treatment and prevention trials;
    b. Pharmacovigilance: establishing a centralized pharmacovigilance database for safety reporting of pregnant women who are exposed to antimalarial drugs when given for treatment, prevention or inadvertently during pregnancy.
    c. Pathogenesis & immunity: consisting of laboratory-based studies aiming at improving the interpretation of the results of trials by relating host immunity, placental histology and drug resistance to the outcome of interventions against MiP, and determining the impact of these interventions on the development of pregnancy specific malaria immunity in the mother and infant.

6.2 Malaria situation in Zambia

According to the results of the last malaria indicator survey, the prevalence of malaria among children under five in Zambia has decreased from 19.4% found in 2015 to 9.1% in 2018 [2], a change attributed to better coverage of control interventions such as IRS, ITNs, case management, and improved knowledge of malaria and attitudes towards control intervention among the local communities [2]. In addition, malaria-related mortality has also decreased [3]. Nevertheless, malaria prevalence varies from north to south, with northern Zambia bearing the highest burden, particularly Luapula province, the site where our clinical trial was implemented. Indeed, in 2018 malaria prevalence was 30.4%, despite relatively good coverage of malaria control interventions. Such high residual transmission may be explained by environmental factors favoring transmission, Nchelenge is located along the marshy swamps of Lake Mweru in the Congo River Basin, and by socio-economic factors such as agriculture and fishing which

encourage population movements across the lake and the interior hampering indoor-residual spraying effectiveness. Indeed, the ecology of Nchelenge district, with its swamps and water bodies, provide a perfect habitat for the malaria vectors, both in the dry and the wet seasons, supporting perennial transmission. This is also illustrated by the higher malaria risk among people living close to streams [4]. In addition, implementing indoor residual spraying once a year, at the beginning of the rainy season and targeting two major malaria vectors, *An. funestus* and *An. gambiae s*.s., may not be effective. These species have different seasonal patterns, with *An. funestus* abundancy peaking in the dry season, seven to nine months after IRS, at the time when the insecticidal effect would have disappeared, while *An. gambiae s.s.* peaks in the rainy season [5]. In this area, insecticide resistance, particularly to pyrethroids and carbamates, remains a challenge [6, 7]. Fortunately, there has been no recorded IRS resistance to the currently used insecticide organophosphate pirimophos-methyl (Actellic® 300cs) in both *An. funestus and An. gambiae s.s* [6, 7]. Moreover, substantial population movements, from the lakeside fishing areas to more fertile agricultural areas inland during the fishing ban, at the time of the rains, may reduce the protective efficacy of IRS as residents, when farming, would stay in unsprayed houses [5]. This seems to indicate the need for implementing IRS twice a year, once at the beginning and once at the end of the rainy season. Other strategies against resistance would need highly effective and long lasting insecticides such as Fludora® by Bayer Crop Science or SumiShield® by Sumitomo Chemical Company Limited which are currently undergoing assessment.

6.3 Methodology of the clinical trial

Our clinical trial was done in four sub-Saharan African countries which increases the generalizability of its results. We chose a balanced-incomplete block design that simplified the implementation of the trial, allowing at the same time the evaluation of four different treatments.

Indeed, in each site only 3 treatments were tested, with amodiaquine-artesunate taken as the control arm in West Africa and artemether-lumefantrine as control arm in Eastern and Southern Africa. Such choice was driven, at the time of trial implementation, by the antimalarial treatment policies of the participating countries.

HIV-positive pregnant women on antiretroviral drugs were excluded from the trial because of concerns about the possible interactions between antiretroviral and antimalarial treatments. Nevertheless, artemisinin-based combination treatments, or at least AL, seem to be safe when administered to HIV-infected adults on efavirenz-based antiretroviral therapy [8, 9]. It is unclear whether these findings can be extrapolated to pregnant women.

The pregnant women included in this trial were closely and extensively followed up, with a last visit performed one year after delivery, to detect and possibly exclude any adverse effect the antimalarial treatment may have had on the offspring [10]. Nevertheless, the active follow up until Day 63 post-treatment should have been able to capture all or most recrudescences due to lower treatment efficacy. Such a long follow up is challenging as it increases the risk of loss to follow up and also the probability of detecting a new infection, particularly in areas of intense transmission such as Nchelenge. Although recurrent infections can be genotyped to establish whether they are the same infection before treatment or a new one, such methods have limitations and may misclassify them [11, 12]. Some studies done in Africa and Asia, recrudescence has been observed even after 100 days [13–17].

Although it is unlikely that a single antimalarial treatment during pregnancy would have deleterious effect on the growth and development of the offspring, it is important such information is collected in a few studies, to provide a complete safety profile of the ACT [18].

Our trial did a head-to-head comparison of different ACTs which were expected to have, all of them, high efficacy against malaria and similar safety profile. This makes it difficult to detect small differences in terms of adverse effects on the foetus or on infant survival. A difference may have occurred if any of the treatments tested would have had an adverse effect on the foetus or infant survival or if the four tested ACT were compared to a non-ACT [19].

6.4 Safety, efficacy and pharmacokinetics of ACTs during pregnancy

ACT were already recommended by WHO for treatment of uncomplicated malaria in second and third trimester pregnant women [1]. However, it is important to stress that at the time the clinical trial started, there was little information on the use of ACT in African pregnant women. Most of the available information was from South-East Asia [1, 15, 20, 21]. The ACTs evaluated in the trial (artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine, and dihydroartemisinin-piperaquine) were highly efficacious in areas of high endemicity, with over 90% efficacy within the pre-specified equivalence margins [22, 23]. These results support the WHO guidelines on the treatment of malaria during the second and third trimester of pregnancy.

The pharmacokinetic data for ACT use during pregnancy are limited. Physiological changes during pregnancy can alter the absorption, disposition, metabolism and excretion of drugs [24]. These changes could result in under-exposure or overexposure to antimalarial treatments. The available data show that the pharmacokinetic properties of these drugs are often altered in pregnancy. These alterations are insignificant to warrant dosage modifications during pregnancy [1]. The limited available data for dihydroartemisinin-piperaquine have shown lower exposure of dihydroartemisinin in pregnant women with *P. falciparum* malaria while pharmacokinetic modelling showed similar results with no major differences in piperaquine overall exposure. Nevertheless, a shortened elimination half-life for piperaquine was noted [25]. Valea and

colleagues in Burkina Faso found no relevant differences in plasma levels of mefloquine exposure between pregnant women in the second and third trimester and non-pregnant women [26]. Artesunate-mefloquine pharmacokinetic data are insufficient to recommend dosage adjustments [1]. Pharmacokinetic data for artesunate-amodiaquine have only been collected in pregnant and non-pregnant women with *vivax* malaria and drug exposure in these groups were similar. No data are available for *P. falciparum* malaria [27]. Therefore, WHO has not recommended any dosage adjustments for amodiaquine because there have been no significant changes in the pharmacokinetics of amodiaquine or its metabolite derivatives seen during second and third trimesters of pregnancy [28]. As for artemether-lumefantrine pharmacokinetics most data have shown a decreased overall exposure during the second and third trimesters but standard dosages above 3 days improves the exposure [29, 30]. More data are, therefore, needed for a change of this regimen to be recommended. A systematic review concluded that more data are needed to identify the exact influence of pregnancy on the pharmacokinetic properties of artemisinin-based combinations [31].

6.5 Remaining Gaps in terms of treatment in pregnancy

In the past decade, evidence on treatment of malaria during pregnancy has substantially increased and the available ACT on the market can be used to treat malaria in the second and third trimester of pregnancy. WHO recommends the use of quinine and clindamycin in the first trimester of pregnancy for *P. falciparum* malaria infection, or quinine alone if clindamycin is not available, or an ACT or artesunate with clindamycin in case of treatment failure or if quinine is unavailable. Therefore, use of ACTs vs quinine and clindamycin in the first trimester would need to be assessed. These studies are of concern in terms of risk to the pregnant women. The available WHO data from 700 pregnant women exposed to artemisinin derivatives in the first trimester would exclude at least a 4.2-fold increase in risk of major congenital defects [1, 32].

And as Dellicour et al 2017 [33] have reported, these guidelines may need to be revised as recommended by the Malaria Policy Advisory Committee [34]. Fears of conclusively including ACT in the first trimester are based on the artemisinins adverse effects observed in animal models. Artemisinin derivatives have shown embryotoxic effects such as death and organ malformations in pregnant rats due to embryonic erythroblasts deaths [35]. This effect is mostly seen at a gestational age of 10-14 days, corresponding to 3-9 weeks gestation in humans [36]. Nevertheless, malaria infection may protect against artesunate-induced toxicity. Indeed, in rats artemisinins accumulate in infected red blood cells [37] while malaria causes hypoferraemia, and both these effects (ferrous iron activates the drug to toxic free radicals) may protect against artemisinin-induced decrease in reticulocytes [36]. This means that artemisinins are a risk for pregnant women without malaria as they may cause embryotoxicity [32]. Nevertheless, the adverse effects described in animal models have not been observed in pregnant women. Gordi and colleagues have suggested the prolonged presence of artemisinins intramuscular formulations (which is a slow release oil-based) as the main cause of the observed toxicity in laboratory animals. But in humans, the commonly used oral formulations result in rapid clearance of these artemisinins and their derivatives which would unlikely cause any toxicity [38]. Therefore, the different toxicity compared to humans of artemisinin derivatives observed in animals is most likely due to different routes of administrations resulting in different pharmacokinetic profiles [38].

The drugs partner of the artemisinin derivatives in ACT (amodiaquine, piperaquine, mefloquine, lumefantrine and now pyronaridine) do not cause embryo deaths or malformations in animal models. Pyronaridine causes embryo deaths at excessively high doses [39]. Therefore, amodiaquine, piperaquine and now pyronaridine in animal models did not show findings that would be of concern when given to animals throughout organogenesis [39]. In 2018, a study was initiated in Zambia to evaluate the use of Pyramax® (pyronaridine-artesunate) in asymptomatic

carriers to provide information on the global efforts to reduce the burden and eradicate malaria. Another Phase IV real-life study in five African countries is underway to evaluate the safety of Pyramax® [40].

Malaria prevention is also key in control strategies for malaria in pregnancy. This is mainly based on IPTp-SP and the use of Long-Lasting Insecticidal Nets. IPTp-SP is threatened, at least in some sub-Saharan countries, by emerging resistance to SP [41], which call for alternatives to SP. Several options have been considered, e.g. mefloquine, chloroquine-azithromycin, but the most promising seems dihydroartemisinin-piperaquine ([42]. Two trials in areas of high SP resistance have shown that dihydroartemisinin-piperaquine was well tolerated when given as IPTp, with greater reductions in the mothers for clinical malaria, malaria infection during pregnancy and at delivery, and placenta malaria. However, the effect on adverse morbidity outcomes in the neonate (low birth weight, prematurity, small for gestational age) was either modest or absent [42], suggesting that SP may have some additional effect beyond the antimalarial one.

As mentioned above, mefloquine has been considered for IPTp and has been discarded on the basis of poor tolerability, e.g. dizziness, nausea and vomiting [43]. In addition, mefloquine may increase the risk of HIV mother-to-child transmission, an effect possibly mediated by an unexplained and unexpected rise in maternal viral load [44]. Such an effect may result in some HIV patients being classified as antiretroviral treatment failures [45]. This is a major drawback for the use of mefloquine as IPTp, particularly in sub-Saharan Africa which bear the highest burden of HIV.

In conclusion, although this trial confirmed that available ACTs for the treatment of uncomplicated malaria are safe and efficacious in pregnant women, there is the need to monitor

how they are used across the health system, both public and private, and the quality of case management. The general public and more specifically pregnant women should be informed about the adverse effect of malaria on the mother's and offspring's health, and on the efficacy and safety of ACT. Until now, several studies have collected information on the safety and efficacy of ACT. However, the number of pregnant women with documented safety data after ACT treatment is relatively limited. Although we are confident that ACT can be safely used during pregnancy, the data available is not sufficient to identify rare adverse events. This can be solved by setting up a pharmacovigilance program with a pregnancy registry in which adverse events following the administration of any treatment, including antimalarial drugs, would be registered. Country-wide post-marketing surveillance would require substantial resources and collaboration between funders, policy makers and researchers.

Antimalarial drug resistance is a major threat to malaria control efforts, including the treatment and prevention of malaria during pregnancy. Resistance to artemisinin derivatives has emerged in Cambodia and spread to neighboring countries, although it has not been observed in sub-Saharan Africa yet [46–50]. Similarly, resistance to some of the ACT's partner drugs, e.g. piperaquine, has also emerged and spread in South-East Asia. This is extremely worrying, not only for pregnant women but for the whole population. There are currently some strategies under evaluation, e.g. adding another partner drug (amodiaquine to artemether-lumefantrine or mefloquine to dihydroartemisinin-piperaquine), deployment of multiple first-line treatments (MTF), that may decrease the risk of selecting resistant parasites. These approaches are currently tested in the non-pregnant population but, if shown to be useful, they would need to be tested during pregnancy as well.

Although in the last few years our knowledge on malaria in pregnancy, including its pathogenesis and immunity, has increased substantially, there is still the need for a deeper understanding of the biology of malaria in pregnancy [51].

Another important tool to control and prevent malaria in pregnancy would be the use effective vaccines especially in the pre-erythocytic phase [52]. There have been a number of clinical trials on vaccines globally (WHO. Tables of Malaria vaccine projects globally 2016) [53]. Recently a completed phase three trial on malaria vaccine (RTS, S/AS01 - Mosquirix) has shown to reduce clinical malaria in children by 39% and severe malaria by about 32% [54]. Placental malaria is mediated by the VAR2CSA protein expressed on the infected red blood cells that accumulate in the maternal blood spaces of the placenta. A study has shown improved pregnancy outcomes associated with antibody to VAR2CSA protein [55]. The risk of malaria in pregnancy decreases with increase in parity, therefore a potential vaccine might be one targeting adolescent girls given together with other vaccines like human papilloma virus vaccine [56]. Another study using PAMVAC, a vaccine candidate based on a recombinant fragment of VAR2CSA, has been completed and results show that PAMVAC is safe, well-tolerated and induced functionally active antibodies. Further studies are needed to assess PAMVAC in women before first pregnancy in an endemic area [57]. Another vaccine trial based on the domains of VAR2CSA is expected to completed by end of 2019 (NCT02658253)

WHO has recommended implementation on pilot scale of the RTS, S vaccine in three sSA countries (Malawi, Ghana and Kenya) to provide data on feasibility, safety and mortality impact. The vaccine, if successful could be added to the core WHO preventive, diagnostic and treatment measures. Such a vaccine could also play an important role in preventing malaria in pregnancy and save thousands of lives every year [52].

6.6 References

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