

**Synthesizing the quality of study methodology and
evidence to support guidelines for optimal treatment of
falciparum malaria in pregnancy**



Makoto Saito

Nuffield Department of Medicine / St Edmund Hall

University of Oxford

Student ID: 835559

A thesis submitted in fulfilment of
the requirements for D.Phil.

University of Oxford

Trinity term 2019

Synthesizing the quality of study methodology and evidence to support guidelines for optimal treatment of falciparum malaria in pregnancy

Makoto Saito, St Edmund Hall, University of Oxford

Submitted for the degree of Doctor of Philosophy, Trinity term 2019

Abstract

Pregnant women are particularly susceptible to malaria, which is the leading cause of mortality and morbidity of the mother and fetus in the tropics. The current World Health Organization (WHO) recommended study design for assessing antimalarial efficacy excludes pregnant women, which has resulted in fewer studies on treatment efficacy and greater variability of study designs in pregnant women. Based on limited evidence, WHO has recommended quinine (with clindamycin, if available) for first-trimester and artemisinin-based combination therapy (ACT) for second- and third-trimester women since 2015. The data on the safety of artemisinin-derivatives in the first trimester have been collected, mostly from inadvertent treatments, over two decades, and form reassuring evidence of safety, strikingly different to animal-based reproductive health studies. This thesis aims to summarize the evidence on the efficacy of antimalarials for uncomplicated falciparum malaria in pregnancy and to improve future research in tropical infectious diseases in pregnancy.

Secondary data from prospective studies that assessed antimalarial efficacy by polymerase chain reaction were sought. Individual patient data of 4968 pregnant women (92% of all published work) from 19 studies in ten countries were pooled in a standardized format and analysed. These confirmed that quinine is less effective and less tolerable than ACTs. Quinine is also less suitable for elimination because of a higher risk of gametocyte development. Among ACTs, artemether-lumefantrine (AL) showed a slightly lower efficacy. In the second and third trimesters, the risks of congenital abnormality, miscarriage, stillbirth, preterm birth and small-for-gestational-age were similar among the four major ACTs (namely, AL, artesunate-amodiaquine, artesunate-mefloquine and dihydroartemisinin-piperaquine).

Study designs need to be tailored for pregnant women. It is probably necessary to follow up pregnant women for longer to assess efficacy accurately, but proof can only be obtained by including them alongside non-pregnant reproductive-age women. To assess pregnancy outcomes, precise and reliable estimates of gestational age and longitudinal recording of repetitive malaria episodes through to delivery are essential.

Quinine monotherapy should be replaced with ACTs for treating uncomplicated *Plasmodium falciparum* malaria in pregnant women. For AL, the most commonly used compound, dose optimization studies are needed. Investigations to find safe and effective drugs for pregnant women should be continued as every effort to prevent and reduce recurrence contributes to health of the mother and fetus.

Statement of contribution

This study used three different sources. Firstly, Chapter 3 was based on two systematic literature reviews. I conducted both literature searches including the search, screening and data extraction. Dr Mary Ellen Gilder at the Shoklo Malaria Research Unit (SMRU) helped with the screening as the second reviewer. I conducted the aggregated data meta-analyses.

Secondly, Chapters 4 and 6 used individual patient data (IPD) from published or unpublished secondary data shared with the WorldWide Antimalarial Resistance Network (WWARN) by the principal investigators of the original studies. I was responsible for managing and coordinating this study group within WWARN on the treatment of uncomplicated falciparum malaria in pregnancy, serving as the study group scientific lead. I identified and contacted the principal investigators to ask whether they were willing to join this study group and share their raw data. I checked and cleaned the shared raw data, and the subsequent data standardization was done by data managers at WWARN, according to the variable list that I developed specifically for pregnant women. I cleaned the standardized data for the analyses, drafted the statistical analysis plan after discussion with Dr Rashid Mansoor and conducted all the IPD meta-analyses.

Thirdly, Chapters 5, 7 and 8 used data collected at SMRU on the Thailand-Myanmar border. In all three chapters, I managed the data and their analysis. For Chapter 5, most of the data had been already cleaned by Professor Rose McGready, and I further cleaned them for

analyses. My statistical analyses in Chapter 5 were validated by Dr Mansoor for publication and he provided me some figures in Chapter 5. For Chapter 7, I read the electrocardiograms (ECG) that had already been recorded in a randomized control trial (RCT), which was conducted in 2010–2016. I participated in this RCT as a site clinician and supervised the medical care of the patients enrolled (and also those who were not enrolled but came to one of the SMRU clinics) in 2016. I created a database for the ECG findings and entered the results in 2018. The rest of the clinical and laboratory data were collected and recorded by SMRU staff and I combined and analysed them. For Chapter 8, I extracted clinical information from written medical records, and combined it with the electronic database at SMRU, which had been developed and maintained by Professor McGready and other SMRU researchers. The secondary data of pregnant patients enrolled in the SEAQUAMAT trial were provided courtesy of Professor Arjen Dondorp. At SMRU, I had an opportunity to serve as a site clinician in another RCT on malaria, which is not part of this thesis.

Some chapters are modified from manuscripts that have been published in (or submitted to) peer-reviewed journals. I wrote the first drafts of all these manuscripts. I would like to acknowledge that these manuscripts would not have been in their current form without the insightful comments of the co-authors, particularly Dr Gilder, Professor Nicholas White, Professor François Nosten, Professor Philippe Guérin and Professor McGready.

Published articles

1. Saito M, Mansoor R, Kennon K, McGready R, Nosten F, Guérin PJ, Stepniewska K. The efficacy of artemisinin-based and quinine-based treatments for uncomplicated falciparum malaria in pregnancy: a protocol for systematic review and individual patient data (IPD) meta-analysis. *BMJ Open*. 2019;9:e027503 (Chapter 2 & Chapter 4)
2. Saito M, Gilder ME, McGready R, Nosten F. Antimalarial drugs for treating and preventing malaria in pregnant and lactating women. *Expert Opin Drug Saf*. 2018;17(11):1129-44. (Chapter 3-I)
3. Saito M, Gilder ME, Nosten F, McGready R, Guérin PJ. Systematic literature review and meta-analysis of the efficacy of artemisinin-based and quinine-based treatments for uncomplicated falciparum malaria in pregnancy: methodological challenges. *Malar J*. 2017;16:488. (Chapter 3-II)
4. Saito M, Gilder ME, Nosten F, Guérin PJ, McGready R. Methodology of assessment and reporting of safety in anti-malarial treatment efficacy studies of uncomplicated falciparum malaria in pregnancy: a systematic literature review. *Malar J*. 2017;16:491. (Chapter 3-III)
5. Saito M, Mansoor R, Wiladphaingern J, Paw MK, Pimanpanarak M, Proux S, Guérin PJ, White NJ, Nosten F, McGready R. Optimal duration of follow-up for assessing antimalarial efficacy in pregnancy: a retrospective analysis of a cohort followed up until delivery on the Thailand-Myanmar border. *Open Forum Infect Dis*. 2019;6(7):ofz264. (Chapter 5)

Conference presentation

1. 'Efficacy and safety of artemisinin-based and quinine-based treatments for uncomplicated falciparum malaria during pregnancy: A WWARN IPD meta-analysis' At the 11th European Congress on Tropical Medicine and International Health. (poster presentation, 18 Sep 2019, Liverpool)
2. 'Efficacy of ACTs & quinine treatments for uncomplicated falciparum malaria in pregnancy in Africa.' At the 7th Multilateral Malaria Initiative Pan African Malaria Conference. (oral presentation, 18 Apr 2018, Dakar, Senegal)
3. 'Factors relating to the efficacy of artemisinin-based and quinine-based treatments for uncomplicated falciparum malaria in pregnancy in Asia: a systematic review and individual patient data meta-analysis.' At the Research in Progress, Royal Society of Tropical Medicine and Hygiene. (oral presentation, 5 Dec 2017, London)
4. 'Efficacy of artemisinin-based and quinine-based treatments for uncomplicated

- falciparum malaria in pregnancy in Asia: a systematic review and individual patient data meta-analysis.' At the 66th Annual Meeting of American Society of Tropical Medicine and Hygiene. (oral presentation, 8 Nov 2017, Baltimore)
5. 'Optimal duration of follow-up for assessing efficacy of drugs for uncomplicated falciparum malaria in pregnancy in Asia: a systematic review and individual patient data meta-analysis.' At the 10th European Congress on Tropical Medicine and International Health. (oral presentation, 17 Oct 2017, Antwerp)
 6. 'Malaria in pregnancy and health systems: A study of factors relating to the efficacy of artemisinin-based and quinine-based treatments for uncomplicated falciparum malaria in pregnancy in Asia.' At the Crossing Boundaries 3. (poster with rapid oral presentation, 7 Dec 2017, Oxford)
 7. 'Tackling the global burden of infectious diseases with collaborative network of clinical data sharing - from malaria to neglected/emerging infectious diseases.' At the Japan Society for the Promotion of Science London Research Promotion Conference 2016. (oral presentation, 16 Nov 2016, London)
 8. 'Urgent need to assess treatment efficacy in pregnancy: IPD meta-analyses of uncomplicated *P. falciparum* malaria.' At the Oxford Tropical Network Conference 2016. (oral presentation, 7 Sep 2016, Oxford)

Unrelated first-author articles published during DPhil

1. Saito M, Keereevijit A, San TD, Thein YY, Gilder ME, McGready R. Challenges to primary healthcare services in the management of non-communicable diseases in marginalized populations on the Thailand-Myanmar border: a pilot survey. *Trop Doct.* 2018; 48(4):273-7.
2. Saito M, Hatakeyama S, Hashimoto H, Suzuki T, Jubishi D, et al. Dose-dependent prolongation of prothrombin time by daptomycin in patients receiving warfarin: a prospective *in vivo* clinical study. *Ann Clin Microbiol Antimicrob.* 2017;16(1):27.
3. Saito M and Hatakeyama S. Adult-onset acute rheumatic fever presenting with erythema marginatum. *N Eng J Med* 2016; 375(25): 2480.

Table of Contents

Chapter 1. Introduction to malaria in pregnancy and the scope of the thesis.....	1
1.1 Global burden of malaria in pregnancy.....	2
1.2 Geographical distribution of malaria diseases	4
1.3 Biology and pathogenesis of malaria in pregnancy	5
1.4 Immunity against malaria parasites.....	9
1.5 Long-term impact of malaria in pregnancy on fetus.....	11
1.6 Control of malaria in pregnancy	12
1.7 Antimalarial resistance.....	16
1.8 Treatment of malaria.....	18
1.9 Treatment of falciparum malaria in pregnancy.....	20
1.10 Study aim and objectives	22
1.11 Chapter outline.....	24
Chapter 2. Methods	27
2.1 Introduction.....	28
2.2 Review of literatures	28
Registration	30
2.3 Development of standardized data for IPD meta-analysis.....	30
Rationale for IPD meta-analysis	30
Identification of studies.....	31
Acquisition of data.....	32
Data standardization.....	34
Data management.....	34
Rationale for one-stage IPD meta-analysis.....	35
Assessment of study quality.....	35
Registration	35
2.4 Study settings and acquisition of the data at SMRU.....	35
2.5 Definitions of the terms.....	37
2.6 Statistical analysis.....	40
2.7 Ethics approval.....	40
Chapter 3 Systematic literature reviews.....	41
Chapter 3-I Antimalarial drugs in pregnancy: their use for treating falciparum malaria and safety profiles	43

3-I.1 Introduction.....	44
3-I.2 Quinine.....	45
Treatment in pregnancy	45
Acute adverse reactions	45
Impact on the fetus.....	46
3-I.3 Artemisinin derivatives	47
Treatment in pregnancy	47
Acute adverse reactions	48
Impact on the fetus.....	49
3-I.4 Lumefantrine.....	50
Treatment in pregnancy	50
Acute adverse reactions	51
Impact on the fetus.....	51
3-I.5 Amodiaquine.....	52
Treatment in pregnancy	52
Acute adverse reactions	52
Impact on the fetus.....	53
3-I.6 Mefloquine	54
Treatment in pregnancy	54
Acute adverse reactions	54
Impact on the fetus.....	55
3-I.7 Piperaquine.....	56
Treatment in pregnancy	56
Acute adverse reactions	56
Impact on the fetus.....	56
3-I.8 Atovaquone-proguanil	57
Treatment in pregnancy	57
Acute adverse reactions	58
Impact on the fetus.....	58
3-I.9 Other drugs.....	59
3-I.10 Discussion.....	60
3-I.11 Conclusions.....	62

Chapter 3-II. Systematic literature review and meta-analysis of the efficacy of artemisinin-

based and quinine-based treatments for uncomplicated falciparum malaria in pregnancy: methodological challenges	63
3-II.1 Introduction.....	64
3-II.2 Methods.....	65
3-II.3 Results.....	68
Study drugs	73
Inclusion criteria	75
Assessment of treatment efficacy	76
3-II.4 Discussion	89
3-II.5 Conclusions.....	93
Chapter 3-III. Methodology of assessment and reporting of safety in antimalarial treatment efficacy studies of uncomplicated falciparum malaria in pregnancy: a systematic literature review	95
3-III.1 Introduction.....	96
3-III.2 Methods.....	97
3-III.3 Results.....	98
Obstetric care and characteristics of participants.....	99
Assessment of maternal safety	100
Assessment of fetal safety.....	102
Associated obstetric risk factors for adverse pregnancy outcomes.....	106
3-III.4 Discussion	107
3-III.5 Conclusions.....	111
Chapter 4. Efficacy and tolerability of artemisinin-based and quinine-based treatments for uncomplicated falciparum malaria in pregnancy: a WorldWide Antimalarial Resistance Network individual patient data meta-analysis	113
4.1 Introduction.....	114
4.2 Methods.....	115
Search strategy and inclusion criteria	115
Outcomes and statistical analysis.....	116
4.3 Results.....	118
Study inclusion.....	118
Baseline characteristics	118
PCR-corrected efficacy at day 28, 42, 63	120
Risk factors for PCR-corrected treatment failure	123
Dose-response	127

Parasite clearance	128
Fever clearance	133
Gametocyte development after treatment	134
PCR-uncorrected treatment failure	137
Adverse events	137
4.4 Discussion	139
4.5 Conclusions	146
Chapter 5. Optimal duration of follow-up for assessing antimalarial efficacy in pregnancy: a retrospective analysis of clinical trials on the Thailand-Myanmar border	149
5.1 Introduction	150
5.2 Methods	151
Study settings	151
Inclusion criteria	152
Modelling the distribution of time to recrudescence	152
5.3 Results	154
Baseline characteristics	154
Factors affecting the time to recrudescence	157
Distribution of intervals to recrudescence	158
5.4 Discussion	160
5.5 Conclusions	166
Chapter 6. Pregnancy outcomes and risk of placental malaria following artemisinin-based and quinine-based treatment for uncomplicated falciparum malaria in pregnancy: individual patient data meta-analysis	167
6.1 Introduction	168
6.2 Methods	169
Search strategy and inclusion criteria	169
Definition of outcomes	170
Inclusion criteria at the individual level	170
Statistical analysis	171
Sensitivity analysis	173
6.3 Results	173
Study inclusion	173
Congenital abnormality	174
Miscarriage	174

Stillbirth	175
Preterm birth	180
Small-for-gestational-age.....	187
Placental malaria	194
6.4 Discussion	199
6.5 Conclusions.....	207
Chapter 7. Safe cardiotoxic profile of four antimalarials for pregnant women with falciparum or vivax malaria on the Thailand-Myanmar border.....	209
7.1 Introduction.....	210
7.2 Methods.....	212
Study site and eligibility criteria	212
ECG assessment.....	213
Statistical analysis	214
7.3 Results.....	216
Baseline characteristics	216
Clinical outcomes.....	217
QTcF	221
QTc by population correction	221
Factors associated with QTcP	222
Sensitivity analyses	226
7.4 Discussion	227
7.5 Conclusions.....	231
Chapter 8. Risk factors for adverse maternal and fetal outcomes following severe malaria in Southeast Asia	233
8.1 Introduction.....	234
8.2 Methods.....	238
Study populations.....	238
Data extraction	238
Management of the patients	239
Statistical analysis	241
8.3 Results.....	244
Increasing ANC and declining malaria in pregnancy at SMRU over time..	244
Baseline characteristics of the SMRU cohort	244
Description of the SEAQUAMAT pregnancy cohort.....	249

Maternal mortality.....	249
Factors associated with maternal death.....	250
Pregnancy outcomes	253
Timing of the adverse outcomes	257
8.4 Discussion	257
8.5 Conclusions.....	262
Chapter 9. Discussion.....	263
9.1 Summary of the research and implications	264
9.2 Limitations and suggestion for future work.....	272
9.3 Concluding remarks	277
References	279
Appendices	308

List of Figures

Figure 1-1. Geographical distribution of malaria at country level in 2017	3
Figure 1-2. Consequences of malaria in pregnancy.....	4
Figure 1-3. Geographical distribution of <i>P. falciparum</i> malaria.....	5
Figure 1-4. Lifecycle of falciparum malaria parasites.....	7
Figure 1-5. Potential causal pathways of placental sequestration causing fetal growth restriction.....	8
Figure 1-6. The number of malaria deaths and malaria cases in the world estimated by WHO	14
Figure 1-7. <i>Artemisia annua</i> at the Oxford Botanic Garden, Oxford, the United Kingdom....	17
Figure 1-8. Pharmacokinetics in pregnancy	22
Figure 2-1. Flowchart of the WWARN system from data-sharing to IPD meta-analysis	33
Figure 2-2. Different landscapes along the Thailand-Myanmar border in 2016	36
Figure 3-1. Flowchart of the systematic literature search on treatment efficacy of uncomplicated falciparum malaria in pregnancy.....	69
Figure 3-2. Number of studies on treatment efficacy of malaria in pregnancy per five years (1985-2019).....	73
Figure 3-3. PCR-corrected proportional appropriate adequate clinical and parasitological response (ACPR) for each study	81
Figure 3-4. Meta-analysis of risk of PCR-corrected treatment failure comparing quinine-based treatment and artemisinin-based treatment.....	84
Figure 4-1. Flowchart of inclusion of the eligible studies.....	119
Figure 4-2. Geographical locations of the included studies	120
Figure 4-3. Adjusted odds ratio of developing adverse symptoms after treatment compared with artemether-lumefantrine	138
Figure 5-1. Number of recurrent malaria episodes in pregnant women attending antenatal care on the Thailand-Myanmar border, 1994–2010.....	154
Figure 5-2. Recrudescence-free survival curves in pregnant women treated for uncomplicated falciparum malaria.....	156
Figure 5-3. Adjusted probability of recrudescence over time by treatment in pregnant women	158
Figure 7-1. Comparison between corrected QT interval (QTc) by Fridericia correction method and population correction method	222
Figure 7-2. Difference in adjusted QTc by population correction method from baseline over	

time by treatment	225
Figure 7-3. Adjusted QTc by population correction method over time by treatment in the final model and two sensitivity analysis models	226
Figure 8-1. Number of pregnant women who attended SMRU ANC, malaria cases in pregnancy and severe malaria in pregnancy in refugees (top) and migrants (bottom).....	245
Figure 8-2. Timings of malaria episodes, maternal and fetal outcomes	256

List of Tables

Table 3-1. Pharmacokinetic properties of the five different combinations of artemisinin-based combination therapies recommended by WHO.....	48
Table 3-2. Summary of the methodology of assessing malaria efficacy and its reporting.....	70
Table 3-3. Gametocyte carriage following the quinine-based and artemisinin-based treatments	87
Table 4-1. Baseline characteristic of pregnant women assessed for PCR-corrected efficacy	121
Table 4-2. PCR-corrected efficacy on day 28, 42 and 63 by Kaplan-Meier estimates pooled by random effects model	123
Table 4-3. Risk of PCR-corrected treatment failure for different antimalarial treatments in two periods after treatment.....	124
Table 4-4. Baseline characteristics associated with the risk of PCR-corrected treatment failure	125
Table 4-5. The effect of lumefantrine dose on the risk of PCR-corrected treatment failure for each malaria transmission intensity area	128
Table 4-6. Univariable and multivariable logistic regression of the risk of positive parasitaemia on day 1	129
Table 4-7. Univariable and multivariable logistic regression of the risk of positive parasitaemia on day 2	132
Table 4-8. Proportions of febrile women on day 0 to day 3 after different treatment, pooled by random effects	133
Table 4-9. Proportions of women with gametocytaemia after different treatment on day 1-6 and day 7-31 in women without gametocytaemia on day 0, pooled by random effects	134
Table 4-10. Univariable and multivariable logistic regression of the risk of positive gametocytaemia on day 1–6 among women without gametocytaemia on day 0	135
Table 4-11. Univariable and multivariable logistic regression of the risk of positive gametocytaemia on day 7–31 among women without gametocytaemia on day 0	136
Table 5-1. Baseline characteristics of pregnant women with PCR-confirmed recrudescence of falciparum malaria.....	155
Table 5-2. Univariable and multivariable analyses for the time to recrudescence in pregnant women by log-logistic accelerated failure time models	157
Table 5-3. Predicted cumulative proportions of treatment failure detected by days 28, 42 and 63 after antimalarial treatment in pregnant women.....	159
Table 6-1. Baseline characteristics of pregnant women assessed for stillbirth	177

Table 6-2. Univariable and multivariable analyses on the risk of stillbirth.....	179
Table 6-3. Baseline characteristics of pregnant women who were assessed for moderate to late preterm birth	181
Table 6-4. Univariable and multivariable analyses of the risk of moderate to late preterm birth	185
Table 6-5. Univariable and multivariable analyses of the risk of moderate to late preterm birth after different treatments in different gestational periods of pregnancy.....	186
Table 6-6. Baseline characteristics of pregnant women who were assessed for small-for-gestational-age	188
Table 6-7. Univariable and multivariable analyses of the risk of small-for-gestational-age..	192
Table 6-8. Univariable and multivariable analyses of the risk of small-for-gestational-age after different treatments in different gestational periods of pregnancy	193
Table 6-9. Baseline characteristics of pregnant women who were assessed for placental malaria	195
Table 6-10. Univariable and multivariable analyses of the risk of malaria pigment in the placenta by treatment.....	196
Table 6-11. Univariable and multivariable analyses of the risk of malaria pigment in the placenta by parity in different malaria transmission intensities	197
Table 6-12. Univariable and multivariable analyses of the risk of malaria pigment in the placenta by treatment outcome	198
Table 7-1. Baseline characteristics on day 0 of pregnant women who were assessed by ECG	218
Table 7-2. Baseline characteristics comparing those who were assessed by ECG and those who were not	219
Table 7-3. QT interval (uncorrected and corrected by Fridericia method) for each treatment	220
Table 7-4. Factors associated with QT corrected by population correction (QTcP) by multi-level univariable and multivariable linear regression.....	223
Table 8-1. Definition of the malaria diagnostic groups at SMRU	239
Table 8-2. Treatment for severe malaria, uncomplicated hyperparasitaemia and severe anaemia at SMRU over time	240
Table 8-3. Proportion of patients by severe criterion	246
Table 8-4. Background of pregnant women with the WHO criteria for severe malaria.....	248
Table 8-5. Proportion and pooled risk ratio for maternal death by baseline characteristics and WHO severe criterion.....	251

Table 8-6. Pooled odds ratio for maternal death by continuous baseline characteristics	252
Table 8-7. Risk of maternal death by diagnostic group	253
Table 8-8. Summary of maternal and fetal outcomes in SMRU cohort	255
Table 9-1. Summary of evidence profile comparing artemisinin-based treatment (ABT) and quinine-based treatment (QBT) following GRADE scale.....	266

List of Boxes

Box 2-1. Terms used for searching the efficacy of antimalarials in pregnancy	29
Box 2-2. Terms used for searching the safety of antimalarials in pregnancy.....	29
Box 2-3. WHO definition of treatment response for non-pregnant populations modified for WWARN IPD meta-analysis	39
Box 8-1. WHO definition of severe malaria.....	235
Box 9-1. Summary of the findings in this thesis	265
Box 9-2. Recommendations for reporting antimalarial drug safety in pregnancy in efficacy studies	274
Box 9-3. Recommendations to determine antimalarial efficacy in uncomplicated <i>P. falciparum</i> infection in pregnancy (beyond current WHO standards for non-pregnant patients)	276

Acknowledgements

Academically and personally, my two supervisors gave me ample support, opportunities and continued encouragement. Professor Guérin taught me important lessons in developing collaboration. Professor McGready provided me with a model of how to be a good clinician working for patients and conducting excellent research at the same time.

I would like to thank the assessors of my transfer of status and confirmation of DPhil status. Their comments and suggestions were valuable for forming the whole thesis.

I acknowledge the generous support from the University, namely the Nuffield Department of Medicine, University of Oxford Clarendon Fund and St Edmund Hall through the NDM prize studentship (Clarendon Fund - St Edmund Hall Frederick Brockhues Scholarship - Nuffield Department of Clinical Medicine Prize Studentship). Without this financial support, I could not have started this DPhil project. I am honoured to have been awarded a British Council Japan Association scholarship (2017). My DPhil travel was supported by a travel grant by the Centres for Tropical Medicine and Global Health (2016, Thailand), a travel award by the European Congress on Tropical Medicine and International Health (2017, Antwerp) and WWARN (2017, Baltimore; 2018, Dakar).

I cannot mention all the people I met at SMRU individually, but I am thankful to all of them. I want to specifically thank Dr Gilder at SMRU for her patient and kind support for my work at SMRU. Professor Nosten gave me a precious opportunity to spend fantastic time at SMRU, as well as his insightful academic stimuli.

I would like to thank to Dr Mansoor and Dr Prabin Dahal for their peer support and friendship. I must also acknowledge backup from the WWARN team. The statistics team (Dr Kasia Stepniewska), data management team (particularly Ms Kalynn Kennon), informatics team and communication team were all necessary to conduct the pooled meta-analysis and its dissemination. Outside of WWARN, Professor Stephen Kennedy helped me to build the pregnancy database at WWARN. Dr Jenny Hill helped communication with primary investigators.

I must also take this opportunity to thank the pregnant women enrolled in the studies and the staff working on the original studies.

Finally, I would like to thank my family. My parents supported me financially. I am also profoundly grateful to my wife, Mari, who patiently supported me while she was doing her own PhD. Without her, I could not have survived my DPhil. I have enjoyed our never-ending discussion on the statistics and clinical implication of our projects.

List of abbreviation

AAP: artesunate-atovaquone-proguanil
ABT: artemisinin-based treatments
AC: artesunate with clindamycin
ACT: artemisinin-based combination therapy
ACPR: adequate clinical and parasitological response
AFT: accelerated failure time
AIC: Akaike information criterion
AL: artemether-lumefantrine
ANC: antenatal clinic
AP: atovaquone-proguanil
AS: artesunate monotherapy
ASAQ: artesunate-amodiaquine
ASMQ: artesunate-mefloquine
BMI: body mass index
CDC: the United States Center for Disease Control and Prevention
CI: confidence interval
CSA: chondroitin sulphate A
DP: dihydroartemisinin-piperaquine
ECG: electrocardiogram
EGA: estimated gestational age
HIV: human immunodeficiency virus
HR: hazard ratio
ICD: international classification of diseases (11th revision)
ICTRP: the WHO International Clinical Trials Registry Platform
IPD: individual patient data
IPTp: intermittent preventive treatment in pregnancy
IUGR: intrauterine growth restriction
IV: intravenous
LBW: low birthweight
LILACS: Literatura Latino Americana em Ciências da Saúde
LMIC: low- and middle-income countries
MMR: maternal mortality ratio
OR: odds ratio

PCR: polymerase chain reaction

PfEMP1: *Plasmodium falciparum* erythrocyte membrane protein 1

PK: pharmacokinetic

PTB: preterm birth

QBT: quinine-based treatments

QC: quinine with clindamycin

RCT: randomized control trial

RR: risk ratio

SD: standard deviation

SEAQUAMAT: South East Asian Quinine Artesunate Malaria Trial

SGA: small for gestational age

SMRU: the Shoklo Malaria Research Unit

SP: sulfadoxine-pyrimethamine

WHO: the World Health Organization

WWARN: the WorldWide Antimalarial Resistance Network

Chapter 1. Introduction to malaria in pregnancy and the scope of the thesis

1.1 Global burden of malaria in pregnancy

Pregnancy can be a delightful life event. However, it is also a period of vulnerability for the mother and fetus. An estimated 303,000 mothers died while pregnant in 2015, 99% of which occurred in low- and middle-income countries (LMIC).¹ Infectious diseases are one of the few preventable causes of this mortality, particularly in LMIC. However, research on diseases in pregnancy, particularly in relation to drug use, has been limited.²⁻⁵ This limited evidence has, to date, prevented the prescription of newer, potentially more effective drugs. Old drugs continue to be used in potentially improper regimens derived from the non-pregnant population. Both lead to sustained unnecessary risk for the mother and fetus. The situation for tropical infectious diseases is even worse as the market-driven incentive for the pharmaceutical industry to conduct research in pregnancy is almost nil.^{2,3} There are few evidence-based recommendations for pregnant women.³ Malaria remains one of the most deadly parasitic infections in humans but is easy to treat when diagnosed early and the infection is uncomplicated; this thesis will address the clinical management of treatment of malaria in pregnant women.

Malaria is an infectious disease caused by parasites of the *Plasmodium* species transmitted by female *Anopheles* mosquitos. The previously encouraging decreasing trends of malaria observed over the past decade have now stalled. This was confirmed by the World Health Organization (WHO) report 2018, which noted that an estimated 219 (uncertainty range 203–262) million cases of malaria had led to an estimated 435,000 deaths globally.⁶

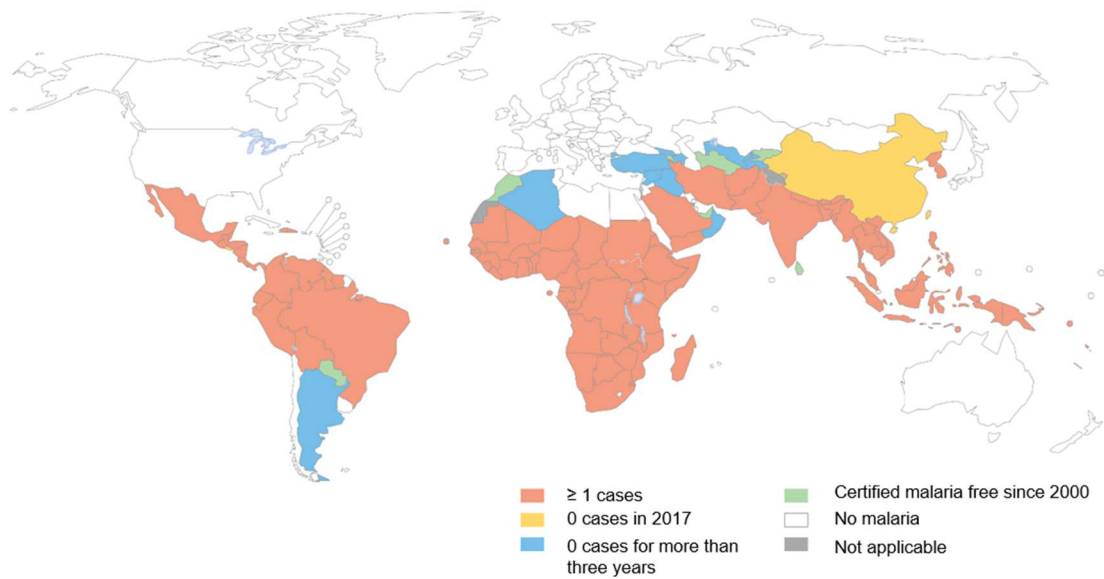


Figure 1-1. Geographical distribution of malaria at country level in 2017

Reprinted from World Malaria Report 2018, World Health Organization, Page 2, Copyright (2018).⁶

Billions of people are at risk of malaria, which is endemic in 90 countries. These are mainly LMIC in the tropics where malaria remains a significant threat, despite decades of extensive efforts to contain it (Figure 1-1).⁶

Malaria during pregnancy is a particular public health challenge. It is estimated that about 60% of all pregnancies worldwide occur in malaria endemic areas, resulting in 125 million pregnant women at risk of malaria every year.⁷ In addition, women are more vulnerable to malaria infection during pregnancy, resulting in disproportionately higher morbidity and mortality for both mothers and infants than the rest of the population.^{8,9} Malaria in pregnancy, regardless of whether it is clinically symptomatic or not, has been reported to be associated with preterm birth (PTB), low birthweight (LBW), small for gestational age (SGA), higher risk of stillbirth/miscarriage and maternal anaemia, all of which lead to a higher risk of perinatal and infant mortality (Figure 1-2).¹⁰⁻¹³ Up to 10–20% of

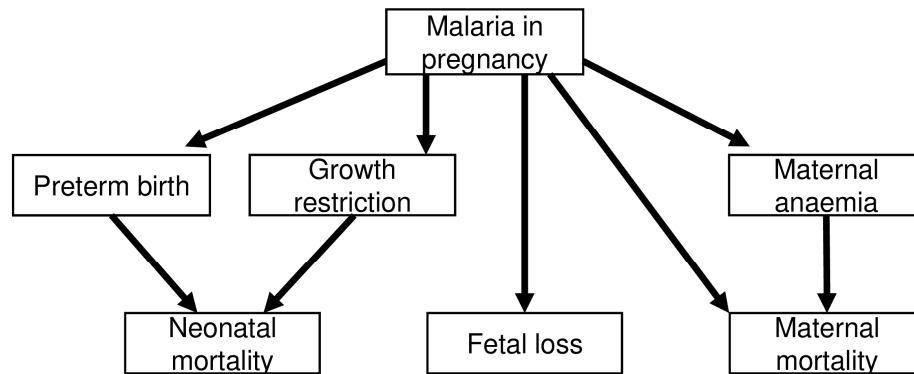


Figure 1-2. Consequences of malaria in pregnancy.

maternal deaths in malaria endemic countries are estimated to be caused by malaria either directly or indirectly.¹⁰ Malaria is estimated to be the cause of 20% of stillbirths¹¹ and a further 20% of LBW¹⁰ in sub-Saharan Africa, resulting in around 200,000 stillbirths and 100,000 infant deaths every year.

1.2 Geographical distribution of malaria diseases

Five *Plasmodium* species infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi*. *P. ovale* is further divided to two subspecies: *P. ovale curtisi* and *P. ovale wallikeri*.

Among the five malaria species that infect humans, almost all deaths are due to *P. falciparum* infections, and most morbidity is attributed to *P. falciparum* and *P. vivax*, depending on the geographical location.⁹

Malaria occurs mostly in sub-Saharan Africa, which contributed 92% of the malaria cases and 93% of the malaria deaths in 2017.⁶ In sub-Saharan Africa, 99.7% of malaria is caused by *P. falciparum*, which contributes most (95%) of the *P. falciparum* malaria cases in

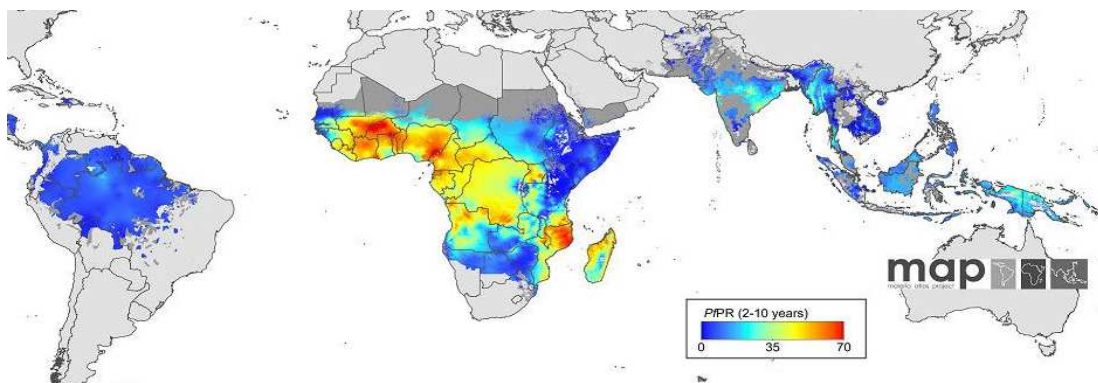


Figure 1-3. Geographical distribution of *P. falciparum* malaria

Estimates of the annual mean *P. falciparum* parasite rate (PfPR) standardized for age 2–10 for 2010 are shown. Modified from Gething PW, *et al. Malar J.* 2011;10(1):378.¹⁴

the world (Figure 1-3).^{6,14}

Globally, 5% of malaria cases and 5% of malaria deaths occur in the Southeast Asia region.⁶ In this region, two-thirds of the cases are *P. falciparum* (63%) and one-third are *P. vivax* (37%).⁶ Human infection by *P. knowlesi* was first and predominantly reported from the Malaysian Kalimantan Island and cases have been sporadically reported in other Southeast Asian countries.¹⁵ In some of these areas, *P. knowlesi* can be underdiagnosed and is in fact as common as *P. falciparum* or *P. vivax*.^{16,17} *P. vivax* is the predominant species (74%) in Latin America, and the top five countries in terms of *P. vivax* globally are: India, Pakistan, Ethiopia, Afghanistan and Indonesia, which together account for 82% of cases.⁶

1.3 Biology and pathogenesis of malaria in pregnancy

Malaria parasites are transmitted by infected female *Anopheles* mosquitos. The lifecycle of malaria involves humans and *Anopheles* mosquitos except for zoonotic *P. knowlesi*, whose primary host is macaque monkeys.¹⁵

One infectious bite contains around 10 infectious parasites,⁹ called sporozoites (Figure 1-4). In the human body, inoculated sporozoites then migrate to the liver through the blood circulatory system. Each sporozoite infects a hepatocyte and starts multiplication (the liver stage). After several multiplication cycles over 5.5–8 days, around 100,000 daughter parasites (merozoites) emerge from the hepatocytes to the blood stream.⁹ The merozoites then infect red blood cells and start asexual multiplication inside the infected erythrocytes (the blood stage). The parasites that infect erythrocytes are called trophozoites. Young trophozoites are particularly described as ring forms based on their morphology. Young ring form trophozoites mature and start asexual reproduction, becoming schizonts. Schizonts rupture and produce new merozoites, restarting the blood-stage asexual reproduction cycle. The number of parasites increases by 6–20 times per asexual cycle.¹⁸ One asexual cycle takes roughly 24 hours for *P. knowlesi*, 48 hours for *P. falciparum*, *P. vivax* and *P. ovale*, and 72 hours for *P. malariae*.⁹

Symptoms of malaria develop when the parasite load in the human reaches approximately 100 million (equivalent to 50 parasites per μL), and at the same time the parasitaemia becomes detectable by microscopy.⁹ Erythrocytes containing mature asexual blood-stage falciparum malaria parasites sequester into small-to-medium-sized blood vessels.¹⁹ The infected erythrocytes bind to endothelial cells as well as other uninfected erythrocytes via *P. falciparum* erythrocyte membrane protein 1 (PfEMP1).^{9,19} PfEMP1 is

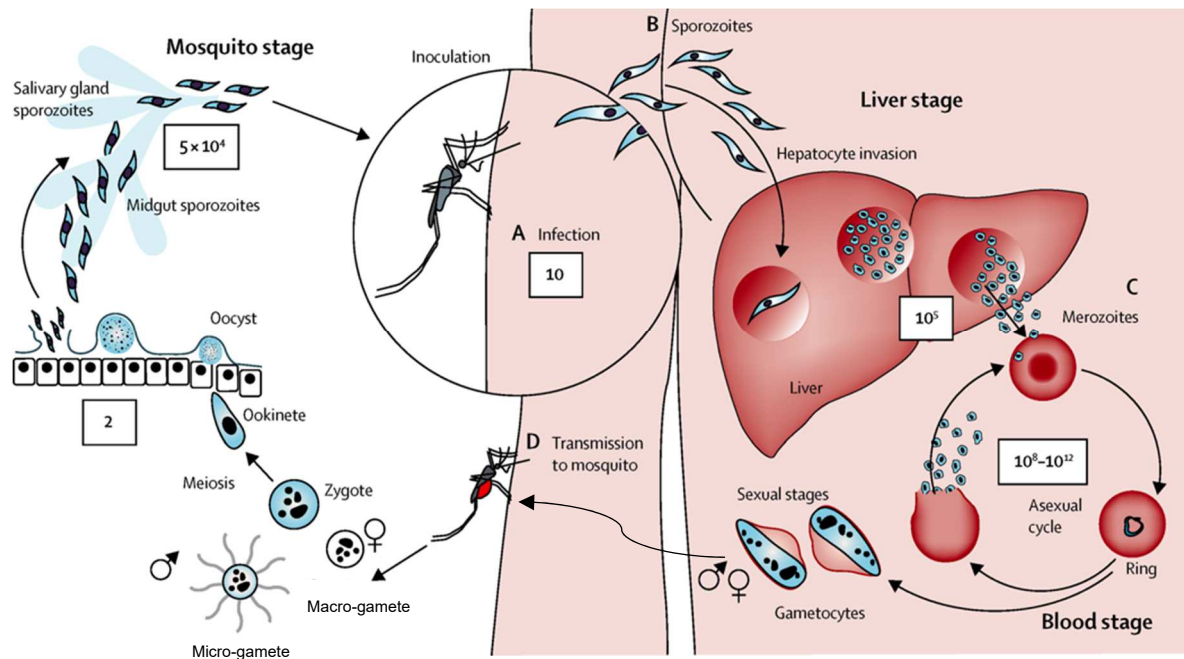


Figure 1-4. Lifecycle of falciparum malaria parasites

Reprinted from The Lancet, Vol. 383 (9918), White NJ, *et al.*, Malaria, 723-35, Copyright (2014), with permission from Elsevier.⁹

coded by a polymorphic gene family called *var*. Each parasite has >50 *var* genes,²⁰ but only one of them is expressed at a time.²¹ These sequestered infected erythrocytes cause microvascular obstruction and endothelial cell injury leading to dysfunction of the affected organs, including lungs, kidneys and brain. In this way, in severe malaria, coma (cerebral malaria), acute kidney injury or acute respiratory failure can occur. Anaemia is caused by accelerated removal of infected and uninfected erythrocytes by the spleen.^{9,22} Intravascular haemolysis and suppressed erythropoiesis also contribute to anaemia to a lesser degree.^{9,23-25}

In pregnant women, sequestration occurs in the placenta. Parasites expressing a specific PfEMP1 variant, VAR2CSA, can sequester in the placenta: VAR2CSA on parasites binds to chondroitin sulphate A (CSA) on the placental cells (syncytiotrophoblasts).²⁶

Sequestration in the placenta and the associated inflammation cause impaired development

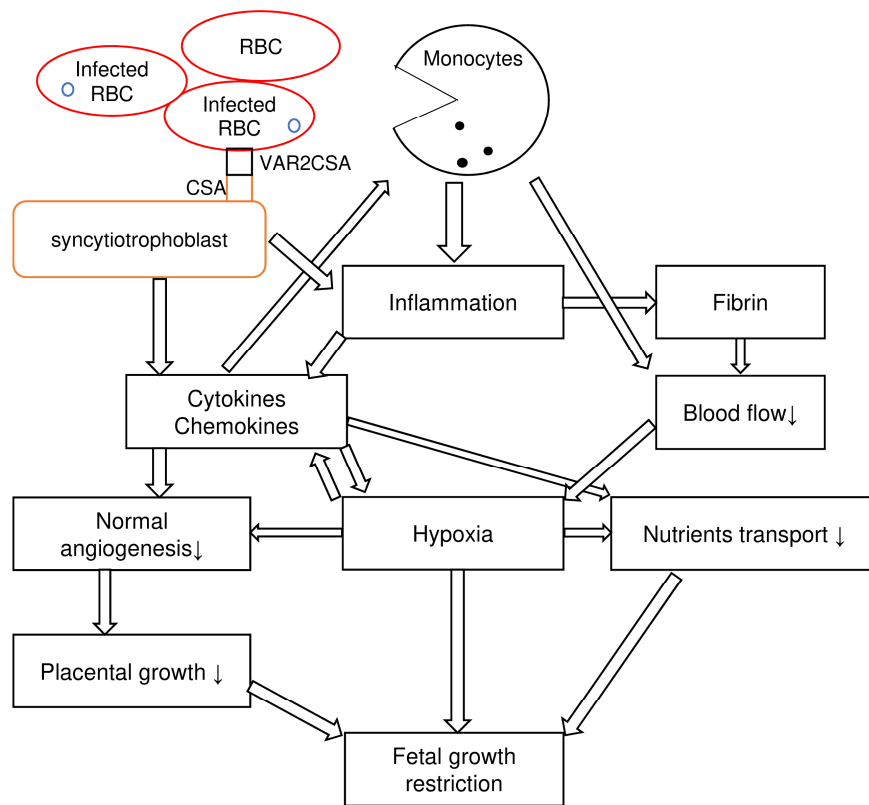


Figure 1-5. Potential causal pathways of placental sequestration causing fetal growth restriction

Blue circle indicates malaria parasites. Black dots in the macrophage indicate malaria pigments. RBC: red blood cell. Based on Brabin BJ, *et al. Placenta*. 2004;25(5):359-78 and Rogerson SJ, *et al. Lancet Infect Dis*. 2007;7(2):105-17.^{26,27}

and dysfunction of placenta, and hypoxia of the fetus, which is hypothesized to result in fetal growth restriction (Figure 1-5).²⁶⁻²⁹ Placental malaria is also reported to be associated with pregnancy-induced hypertension.³⁰ Intrauterine growth restriction (IUGR) occurs regardless of whether the infection is symptomatic or not and each episode of falciparum malaria cumulatively increases the odds of SGA by 1.13 times.³¹ Fetal distress and maternal systemic inflammation caused by falciparum malaria leads to pregnancy loss.^{11,29,32,33} Pregnant women with malaria are more prone to anaemia because of the high background need for micronutrients (i.e. iron and folate) in pregnancy.²⁶

A small portion of the parasites in the blood stage stop the asexual cycle and become sexual forms, called gametocytes. Gametocytes appear in the bloodstream 7–15 days after the appearance of asexual parasites but are not associated with symptoms of malaria in humans.³⁴ The transmission to mosquitos occurs when a female mosquito takes a blood meal containing gametocytes. In the midgut of the infected mosquitos, male and female gametocytes transform into micro- and macro-gametes, respectively. These gametes fuse and become a zygote, which develops into an ookinete. After penetrating the gut wall of the infected mosquito, an ookinete becomes an oocyst. One oocyst produces around 50,000 sporozoites in the midgut.⁹ The sporozoites migrate to the salivary glands of the mosquito and can then be inoculated into another host the next time the female mosquito needs to feed.

In *P. vivax* and *P. ovale*, some sporozoites become dormant hypnozoites in hepatocytes, causing relapses a long time after inoculation, sometimes triggered by other febrile diseases.^{35,36}

1.4 Immunity against malaria parasites

Both cellular and humoral immunity act against malaria infection. Acquired immunity against malaria is species-specific and mainly mediated through humoral immunity (i.e. IgG antibodies to PfEMP1).³⁷ This acquired immunity is primarily considered to be strain-specific (i.e. PfEMP1-specific), but strain-transcending immunity also seems to be present.^{37,38} Immunity level affects the parasite growth rate (the density of parasitaemia and the length of time to recurrence)^{39,40}, clinical pictures (the risk of symptomatic malaria and

severity)^{37,38,40,41} and treatment outcomes (parasite clearance rate and treatment success).^{40,42,43}

This immunity is believed to be acquired by constant exposure to malaria parasites over years.^{37,38} Although sterilizing immunity is not achievable, in holoendemic areas in sub-Saharan Africa, the majority of infected adults are immune: asymptomatic with low parasitaemia (premunity).^{37,38} A lack (or underdevelopment) of this acquired immunity means that infants and young children are most vulnerable to symptomatic malaria (clinical malaria) in highly endemic areas.³⁸ On the other hand, in lower endemic areas, acquired immunity is lower and symptomatic infection can occur at any age.⁹ In areas of declining malaria, immunity can decrease: increased symptomatic malaria and an age-shift towards older patients have been reported.⁴⁴

Pregnant women, particularly primigravid women, are also more susceptible to malaria than non-pregnant adults.^{26,38} This is due to the modulated general immunity level in pregnancy⁴⁵⁻⁴⁸ and the lack of immunity in women of low gravidity against the parasites that infect placenta.^{49,50} IgG antibodies against VAR2CSA inhibit the cyto-adhesion of the parasites, thus reducing the risk of placental infection.⁴⁹ CSA is only expressed on the placental syncytiotrophoblasts, thus VAR2CSA is only selectively expressed in parasites that infect pregnant women,⁵¹ meaning that immunity against this specific strain of *P. falciparum* expressing VAR2CSA is believed to be acquired only during pregnancy.^{26,49} Therefore, primigravid women are considered to be more susceptible than multigravid women.^{50,52} This

immunity against parasites with VAR2CSA is called pregnancy-specific (or parity-dependent) immunity. Pregnancy-specific immunity level has been reported to be associated with treatment outcome,⁵³ and the risks of maternal anaemia, placental infection and adverse fetal outcomes.⁵³⁻⁵⁶ Declining malaria can lower the pregnancy-specific immunity against malaria as well.⁵⁷ This pregnancy-specific immunity remains relevant to *P. falciparum* infection, not to the pathogenesis of *P. vivax* infection.⁵⁸

1.5 Long-term impact of malaria in pregnancy on fetus

Placental malaria is reported to decrease the transplacental transfer of maternal antibodies to the fetus.^{59,60} In-utero exposure to malaria parasite antigens is shown to affect the long-term development of the immune system of the fetus;⁶⁰⁻⁶² malaria in pregnancy is associated with a higher risk of malaria infection in the fetus, presumably because of the sensitization to the parasite antigens in utero.^{60,63} Haemolysis of red blood cells sensitized to malaria antigens is also hypothesized to cause anaemia in infants and in later life.^{62,64} Malaria in pregnancy also increases the risks of other infections during the early year of life,^{65,66} including an increased risk of mother to child human immunodeficiency virus (HIV) transmission.^{60,67}

In addition to the immunological impact, malaria in pregnancy leads to IUGR, which is related to higher risks of long-term morbidities as well as short-term mortality.^{68,69} For example, the mortality in the first year of life was almost twice in babies with LBW in sub-Saharan Africa.⁷⁰ Moreover, IUGR is known to be associated with a higher risk of

cardiovascular disease and type 2 diabetes in later adult life.^{71,72} Neurocognitive development can be delayed in IUGR infants,^{73,74} and some mental disorders have been shown to be associated with IUGR.⁷¹ PTB due to malaria is much more likely than IUGR to result in developmental delay and higher neonatal and post-neonatal mortality,⁶⁹ especially in low resource settings where special care baby units remain a rarity. Malaria in pregnancy thus causes an increase in the short- and long-term risks of both communicable and non-communicable diseases of the fetus.

1.6 Control of malaria in pregnancy

A set of control and preventive measures have been deployed in varying degrees in different settings and have decreased the burden of malaria in the last decade.⁶ WHO's three pillars of malaria control have included: prompt diagnosis with parasitological confirmation and treatment with artemisinin-based combination therapy (ACT); long-lasting insecticide-treated bed-net use; and indoor residual spraying.^{9,75} The first pillar is aimed at malaria parasites and the last two are intended to control vectors (i.e. mosquitos). These two vector controls are particularly effective in sub-Saharan Africa where the main vectors feed largely on humans (anthropophilic), indoors (endophagic) at night¹⁹ but less successful in Southeast Asia where the vectors are more diverse and some are zoophilic and exophagic.⁷⁶⁻⁷⁹

There are two other options for controlling malaria parasites: chemoprophylaxis and vaccination. Chemoprophylaxis can be used for targeted populations, such as infants and pregnant women. Intermittent preventive (presumptive) treatment in pregnancy (IPTp) and in

infant, and seasonal malaria chemoprevention in children are deployed in highly endemic countries in sub-Saharan Africa. In areas of pre-elimination of malaria, on the other hand, mass drug administration is considered a control measure and results in some areas have shown considerable success.⁸⁰⁻⁸² Vaccine is a potential option to reduce infection, severity or transmission, and several vaccines are under development.⁸³

In pregnancy, IPTp and early detection by screening are currently deployed. In highly endemic countries in sub-Saharan Africa, IPT with sulfadoxine-pyrimethamine (SP) is recommended to be given to all pregnant women at every antenatal care visit from as early as possible in the second trimester until delivery, at least one month apart.⁸⁴⁻⁸⁶ IPT-SP, three times or more in a pregnancy, given as a package together with other antenatal care was reported to be associated with reduced risk of LBW and maternal anaemia.^{87,88} However, implementation in sub-Saharan Africa has been challenged by low uptake in many countries.^{6,89-93} Moreover, SP is contraindicated in the first trimester, thus IPTp-SP policy cannot prevent malaria infection in the first trimester when the women and fetus are most vulnerable.^{31,94-98}

Early detection and treatment by screening peripheral parasitaemia in pregnancy (particularly at the first antenatal care visit) is employed outside of Africa (i.e. in Asia and Latin America) to minimize the adverse impact of asymptomatic parasitaemia as one of the only alternatives.^{12,99-101} With lower transmission, the risk-benefit of large scale IPTp is not in favour of deployment and no alternative drug to SP is recommended in areas with high

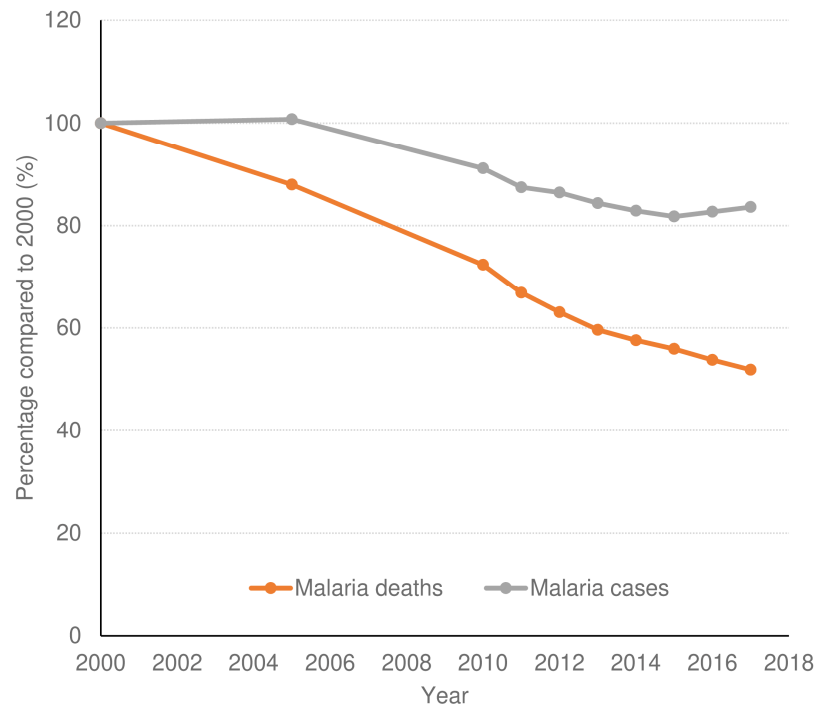


Figure 1-6. The number of malaria deaths and malaria cases in the world estimated by WHO

Figures for 2000 and 2005 are derived from the World Malaria Report 2016 and figures for 2010—2018 are derived from the World Malaria Report 2018.^{6,110}

resistance.¹²

Vaccines specifically targeting the VAR2CSA antigen to prevent placental malaria are under development.¹⁰² The preconception period is likely the best time to provide this vaccine, enabling it to protect women from malaria infection during the first trimester.¹⁰³ The main problems of vaccine are a lack of protective longevity, the need for repeated doses to provide protection for the duration of reproductive age, and failure to develop/co-develop a vaccine for *P. vivax*.¹⁰²

These key strategic interventions are, however, threatened by the emergence and spread of antimalarial resistance in malaria parasites¹⁰⁴⁻¹⁰⁷ and insecticide resistance in mosquitos.¹⁰⁸ A growing number of conflicts worldwide has resulted in international and

internal displacement of people, particularly on country borders. This hampers control and elimination efforts (either by government or non-governmental organizations) and creates a more challenging and complicated situation, together with a high risk of reigniting malaria.^{78,109} Although there was a dramatic decrease in malaria mortality after 2000,¹¹⁰ both mortality and the number of malaria cases decreased very slowly after 2010 and have stalled since 2014 (Figure 1-6).^{6,110}

IPT and seasonal malaria chemoprevention are hampered by the spread of SP-resistant parasites throughout the African continent. IPTp-SP showed a reduced efficacy in the presence of (quintuple or) sextuple mutations of parasites of dihydrofolate reductase (*Pf dhfr*) and dihydropteroate synthetase (*Pf dhps*), which show resistance to SP.^{87,88,111} IPT, which is intermittent preventive ‘treatment’, depends on efficacy of the drug used.^{88,112-114} the objective of IPTp is primarily to clear peripheral and placental malaria, and secondly to prevent new infection.^{88,112-115} Other commonly used outcomes (e.g. LBW or maternal anaemia) are surrogate outcomes, which can be affected by many other confounding factors, including better antenatal care or effects on other bacteria.^{88,112,114,116,117} There has been criticism that a drug that was dropped from the treatment armamentarium for malaria continues to be recommended for use in pregnancy, an immunologically compromised population.^{88,114}

The appearance of artemisinin resistance in the Greater sub-Mekong region (described below) is the biggest threat to frequent screening and treatment strategies.

Continuous efforts in malaria research, especially monitoring treatment efficacy, are

thus vital to eliminate the disease. Ever since the thalidomide tragedy, there has been a fear of treating pregnant women with drugs and a lack of willingness from the pharmaceutical industry to include them in research.²⁻⁴ This is in stark contrast to the situation in non-pregnant patients: treatment of malaria in Asia changed rapidly to newer lifesaving antimalarials, although little was known of their safety in the 1990s.^{118,119} Only in recent years, after decades of neglect and specific exclusion from research on malaria treatment, has the importance of including pregnant women, a vulnerable group, garnered more attention.^{3,8,112}

1.7 Antimalarial resistance

The exciting history of the development of new antimalarials has also driven the dismal history of emerging antimalarial resistance, as it is always associated with increased mortality. Quinine is derived from the cinchona herb, which was brought from Peru to Europe in the 17th century.¹¹⁹ In the 1930s, chloroquine was developed as a semi-synthesized antimalarial based on quinine and replaced quinine in the late 1940s.¹²⁰ Although resistance to chloroquine emerged in the late 1950s,¹²¹ it was widely used as the antimalarial for all species until the 1970s in Asia when resistance became predominant. In Africa, chloroquine was used until the late 1990s.¹²² SP is a combination of two anti-folates and was introduced in the 1960s for chloroquine-resistant malaria but it was not used as the first-line treatment for a long time: its resistance started to be seen in the 1960s¹²² and became prevalent globally.¹²³ Mefloquine was developed in the 1980s and replaced chloroquine and SP monotherapy in the



Figure 1-7. *Artemisia annua* at the Oxford Botanic Garden, Oxford, the United Kingdom (Taken in August 2018.)

mid-1980s but resistance when used as monotherapy emerged very rapidly. By the 1980s, mefloquine resistance had already been observed.¹²⁰ Drug resistance is considered to be the major cause of the global increase in the burden of malaria in the 1990s, which contrasts starkly with the contemporary decreasing trend of other major infectious diseases.^{124,125}

A dramatic change occurred in the 1990s with the use of artemisinin derivatives. Traditional Chinese medicine has used *Artemisia annua* (Figure 1-7) for treatment of fever but the active ingredient artemisinin was extracted by re-evaluation of the herbal remedy in 1971.¹¹⁹ Artemisinin is effective against parasites that are resistant to other antimalarials.¹¹⁹ To prevent the emergence of resistance to this key component, artemisinin is combined with antimalarials with different actions and longer half-life.¹²⁶ This combination is called ACT.

Direct observation and pathological confirmation of malaria (rather than using antimalarials for all febrile patients) are also encouraged for ensuring efficacy and preventing resistance.

Despite these efforts, however, in 2006/2007, it was first reported in West Cambodia that falciparum parasites had become less sensitive to artemisinin.¹²⁷⁻¹³⁰ This reduced sensitivity was only observed in the young ring form, not in trophozoites or shizonts.¹³¹ This ‘resistance’ was clinically manifested as slow parasite clearance,¹⁰⁶ with higher treatment failure reported when the parasite was not cleared in three days.⁹ Later, this artemisinin resistance was confirmed to be associated with genetic mutations of the *kelch13* gene (mutations in the propeller region of K-13 protein) of falciparum parasites.^{132,133} This mutation is clinically confirmed to be associated with a higher risk of treatment failure.¹³⁴ Since the first report in mid-2000s, this genotype has spread widely in the Greater sub-Mekong region in the 2010s.^{107,135,136} In this area, administering both artesunate and quinine has been proposed for treatment of severe cases¹³⁷ and the presence of these resistant parasites has bolstered efforts towards malaria elimination.^{80,138,139}

1.8 Treatment of malaria

Treatment of malaria needs to be tailored based on the malaria species, the local sensitivity and the severity of the disease. For falciparum malaria, mortality has dramatically improved since the implementation of artemisinin derivatives.¹⁹ Artemisinin derivatives are the current key antimalarial for both severe and uncomplicated falciparum malaria.

Severe malaria is the cause of most malaria mortality. It is predominantly caused by

P. falciparum and less frequently by *P. vivax* and *P. knowlesi*.¹⁹ For severe malaria, intravenous artesunate is the first line treatment for all patients, including pregnant women in all trimesters.¹⁴⁰ If not available, intramuscular artemether (or intravenous quinine if neither artesunate nor artemether is available) can be an alternative option.¹⁴⁰ Once oral intake is possible after at least 24 hours of parenteral treatment, a full course of ACT as in the treatment of uncomplicated malaria is recommended.¹⁴⁰

For uncomplicated falciparum malaria (and knowlesi malaria), the currently recommended treatment is ACT, an oral combination therapy of artemisinin derivatives with a partner drug. When patients are diagnosed and treated promptly with effective drugs, the mortality from uncomplicated falciparum malaria is roughly 0.1%.⁹ Uncomplicated malaria by vivax, malariae or ovale is treated with chloroquine except in some areas in the Western Pacific and Southeast Asia (i.e. Papua New Guinea, Indonesia, Vanuatu, Solomon Islands, and Cambodia), and in the Horn of Africa (i.e. Sudan and Somalia) where vivax has become resistant to chloroquine.^{6,140,141}

Artemisinin derivatives kill both circulating intra-erythrocytic young ring form parasites and sequestered matured asexual parasites,^{19,142} which leads to a rapid reduction of parasite biomass as well as reduced pathology from parasite sequestration.¹¹⁹ In addition, artemisinins kill sexual parasites (gametocytes), reducing the risk of transmission after treatment.¹⁴³ Quinine, on the other hand, only kills the sequestered matured asexual parasites,^{19,142} and therefore the killing speed is slower than artemisinins. Artemisinin

derivatives have a shorter elimination half-life of several hours.¹⁴⁰ Partner drugs currently used with ACTs have a longer elimination half-life of several days to weeks, kill the remaining parasites and offer a certain level of prophylactic effect after the end of treatment (post-treatment prophylaxis).

Treatment efficacy is conventionally assessed by the clearance of malaria in the peripheral blood. Highly drug-resistant parasites cannot be cleared from blood following treatment (early treatment failure). Parasites that have a lower degree of drug resistance can be reduced in number below the detection level following treatment but recur later manifested as detectable parasitaemia with or without clinical symptoms.⁴⁰ For *P. falciparum*, this recurrence of the same strain of the initial parasites (recrudescence) is confirmed by polymerase chain reaction (PCR) and regarded as late treatment failure defined by the WHO guidelines for assessing antimalarial efficacy.¹⁴⁴

In the latest guideline (2015) for the treatment of uncomplicated falciparum malaria, WHO recommends five ACT combinations: artemether-lumefantrine (AL), artesunate + amodiaquine (ASAQ), artesunate + mefloquine (ASMQ), artesunate + SP (ASSP) and dihydroartemisinin-piperaquine (DP), depending on the local sensitivity of the partner drug.¹⁴⁰

1.9 Treatment of falciparum malaria in pregnancy

In pregnancy, ACT is recommended only during the second and third trimesters.¹⁴⁰ In the first trimester, quinine (with clindamycin if available) is the recommended first-line drug.¹⁴⁰

Although studies on the efficacy of antimalarials have been conducted extensively, pregnant women have been systematically excluded from the majority of them in the past, mainly because of concerns about the safety of artemisinin derivatives for the fetus. In rats, rabbits and monkeys, artemisinin derivatives used in early pregnancy are reported to be associated with higher risk of fetal loss (embryotoxicity) and congenital abnormality (teratogenicity).¹⁴⁵⁻¹⁴⁹ The safety of antimalarials during human pregnancy had not been assessed systematically. The safety assessment of antimalarials during pregnancy requires follow-up of participants until delivery and beyond to assess the development of children, which makes it complicated and costly. Together with the paucity of reliable background data on pregnancy outcomes in the target population in LMIC, safety assessment is very challenging.¹⁵⁰

Because of the lack of clinical evidence, quinine (with clindamycin) is recommended as the first-line treatment for uncomplicated falciparum malaria in the first trimester,¹⁴⁰ although evidence for the safety of quinine in the first trimester is also limited.¹⁵¹ In addition, quinine is poorly tolerated by both pregnant women and non-pregnant individuals.¹⁵² The adverse symptoms caused by quinine are collectively called cinchonism, which includes tinnitus, hearing loss, nausea, abdominal pain and blurred vision.¹¹⁸

Recently, however, safety data from prospective observational cohort studies have been accumulated. The risks of stillbirth or congenital abnormality among those who were treated by artemisinin derivatives in the first trimester were no different from those who were

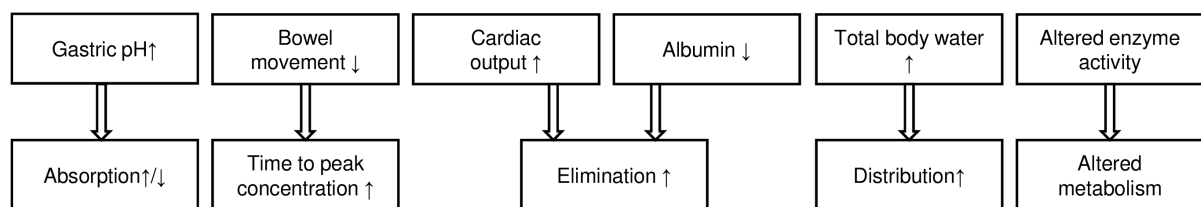


Figure 1-8. Pharmacokinetics in pregnancy

Based on Costantine MM. *Front Pharmacol.* 2014;5:65. and Pariente G, et al. *PLoS Med.* 2016;13(11):e1002160.^{158, 159}

treated with quinine.^{32,33,153-155} Based on this accumulated evidence on assuring fetal safety, the United States Center for Disease Control and Prevention (CDC) changed its policy in 2018: AL is now suggested as an option in the first trimester.¹⁵⁶ The WHO evidence review group concluded that the WHO treatment guideline should recommend ACTs in the first trimester in the next edition.¹⁵⁷ Collective evidence for treatment efficacy now needs to be gathered.

For assessing treatment efficacy, simple extrapolation of the results from the studies in the non-pregnant populations may not be necessarily directly applicable because of the physiological changes in pregnancy: different drug metabolism and distribution due to pregnancy (Figure 1-8);^{150,158,159} altered immunity level;²⁶ and the sequestration of parasites to placenta.²⁶ Taken together, study designs to assess efficacy may need to be tailored specifically for pregnant women. Treatment outcomes, including both efficacy and safety, need to be assessed specifically in pregnancy.

1.10 Study aim and objectives

The aim of this thesis is to improve the clinical management and the quality of clinical studies in the future on falciparum malaria in pregnancy by summarising the currently

available evidence on the treatment of *P. falciparum* malaria during pregnancy.

The objectives of this thesis include

- To identify the knowledge gap
- To assemble and synthesize the currently available evidence on antimalarial efficacy in pregnancy for uncomplicated falciparum malaria
- To explore the optimal duration of follow-up to assess antimalarial efficacy in pregnancy for future studies
- To describe the existing available evidence on the safety of antimalarials used for treating falciparum malaria in pregnancy
- To describe complicated malaria in pregnancy on the Thailand-Myanmar border
- To propose a way to assess efficacy and safety of antimalarials in pregnancy in treatment efficacy studies

Our hypotheses are:

- Artemisinin-based treatment is better than quinine-based therapy in the clearance of parasites for pregnant women
- The optimal duration of follow-up of pregnant women is longer than the current recommendation
- Better treatments are related to better pregnancy outcomes
- Currently used ACTs are safe for treating pregnant women

1.11 Chapter outline

Chapter 2 summarizes the overall description of methodology used in this thesis. The process of data acquisition is described here, and details of the statistical analysis are explained in each chapter.

Chapter 3 comprises the literature review. Firstly, the use and the safety of antimalarials in pregnancy are summarized (Chapter 3-I). Secondly, the knowledge gap in the treatment of uncomplicated falciparum malaria in pregnancy is identified by conducting a systematic literature review. This systematic review revealed overall limited evidence on antimalarial treatment in pregnancy (Chapter 3-II) and the variability of methodology assessing and reporting the efficacy and safety outcomes (Chapter 3-III).

Chapter 4 and 5 are about efficacy of antimalarial treatment for pregnant women. Chapter 4 attempts an individual patient data (IPD) meta-analysis on treatment efficacy, based on the systematic literature review in Chapter 3-II. IPD meta-analyses were conducted using data shared by primary researchers with the WorldWide Antimalarial Resistance Network (WWARN).

Chapter 5 describes the modelling of the time to treatment failures for exploring the optimal duration of follow-up to assess antimalarial efficacy in pregnancy, using the data collected at the Shoklo Malaria Research Unit (SMRU) where pregnant women were followed up until delivery with PCR used for recurrences of falciparum malaria.

Chapters 6–8 describe safety of antimalarial treatment for pregnant women with

uncomplicated falciparum malaria (Chapters 6 and 7) or complicated falciparum malaria (Chapter 8). Chapter 6 is an IPD meta-analysis on the pregnancy outcomes after different treatment, using the same pooled data as Chapter 4.

Chapter 7 focuses on one particular safety issue: cardiotoxicity of antimalarials in pregnancy using the data from SMRU on the Thailand-Myanmar border. The impact of four different antimalarials on QT/QTc interval measured by electrocardiogram (ECG) was analysed to confirm the safety of them in pregnant women.

Chapter 8 is the only chapter on complicated (severe) falciparum malaria in pregnancy. Retrospective descriptive analysis was conducted on maternal and fetal outcomes using routinely collected clinical data at SMRU, combined with the data of pregnant women enrolled in a cornerstone randomized control trial (RCT) of severe malaria in Southeast Asia (South East Asian Quinine Artesunate Malaria Trial [SEAQUAMAT]).

Chapter 9 discusses the currently available evidence on the treatment of malaria in pregnancy based on the findings in the preceding chapters. Considering the lack of standard methodology and lack of evidence, a set of recommendations was made for conducting antimalarial treatment efficacy studies in pregnancy in the future.

Chapter 2. Methods

* Part of this chapter has been published in a peer-reviewed journal and is freely available as follows under the Creative Commons Attribution licence. Saito M, Mansoor R, Kennon K, McGready R, Nosten F, Guérin PJ, Stepniewska K. The efficacy of artemisinin-based and quinine-based treatments for uncomplicated falciparum malaria in pregnancy: a protocol for systematic review and individual patient data (IPD) meta-analysis. *BMJ Open*. 2019;9:e027503.

2.1 Introduction

This chapter explains the methodologies used in this study. Firstly, the methodologies of two systematic literature reviews (Chapter 3) are described. The IPD meta-analyses described in Chapters 4 and 6 are also based on one of the systematic reviews. Secondly, the acquisition of data is explained. The study used two different sources of data: pooled data of published or unpublished studies (Chapters 4 and 6); and the data collected at SMRU (Chapters 5, 7 and 8). These are described separately. As different statistical methods were used for each research question, detailed descriptions of the methods will be given in each chapter.

2.2 Review of literatures

Chapter 3 describes two systematic literature reviews that were conducted. Firstly, the efficacy of the currently recommended antimalarials, i.e. quinine-based and artemisinin-based treatments, for uncomplicated falciparum malaria in pregnancy was reviewed. This review was conducted using seven databases (MEDLINE, Embase, Global Health, Cochrane Library, Scopus, Web of Science and Literatura Latino Americana em Ciências da Saúde [LILACS]) and two clinical trial registries (the WHO International Clinical Trials Registry Platform [ICTRP] and ClinicalTrials.gov). The search terms are listed in Box 2-1. Briefly, it consists of three components: malaria or the name of antimalarials; pregnancy; and study type. Two researchers independently assessed the eligibility of the articles, and any discrepancy was solved by re-checking and discussion. The initial literature search was

conducted on 5 July 2016 and was finally updated on 26 April 2019.

Secondly, the safety profile of antimalarials in pregnancy was reviewed. This second review was conducted using three databases (MEDLINE, Embase, Global Health) by one person on 28 April 2018. The search terms are listed in Box 2-2. Briefly, it consists of four components: the names of antimalarials; pregnancy; safety; and human subjects.

Box 2-1. Terms used for searching the efficacy of antimalarials in pregnancy

1. (malaria or Plasmodium).m_titl.
 2. "pregnan*".m_titl.
 3. (treatment or therap* or antimalaria* or anti-malaria* or artemisinin* or artesunate* or dihydroartemisinin* or artemether* or chloroquine* or mefloquine* or amodiaquine* or quinine* or piperazine* or lumefantrine* or atovaquone* or proguanil* or sulfadoxine* or pyrimethamine* or azithromycin* or clindamycin*).ab,kf,kw,ti.
 4. (trial or cohort or prospective or follow or intervention or random* or pharmaco*).ab,kf,kw,ti.
 5. (efficac* or success or failure or recrudescence or recurren* or reappear* or response or outcome or effect* or clearance or cure or dose or dosage or surviv*).ab,kf,kw,ti.
- Only condition 1 and 2 were used for ClinicalTrials.gov, 1, 2 and 3 for ICTRP and LILACS.

Box 2-2. Terms used for searching the safety of antimalarials in pregnancy

1. "pregnan*".m_titl.
2. (*artemisinin* or *artesunate* or *dihydroartemisinin* or *artemether* or *chloroquine* or *mefloquine* or *amodiaquine* or *quinine* or *piperazine* or *lumefantrine* or *atovaquone* or *proguanil* or *sulfadoxine* or *pyrimethamine* or *clindamycin* or *primaquine* or *pyronaridine*).ab,kf,kw,ti.
3. (safe* or pharmaco*).ab,kf,kw,ti.
4. human subject

Registration

The systematic literature review on the efficacy of antimalarials for uncomplicated falciparum malaria in pregnancy is registered on PROSPERO (CRD42017054808).

2.3 Development of standardized data for IPD meta-analysis

Rationale for IPD meta-analysis

Conventional meta-analysis uses aggregated data (e.g. summarized results), which are estimated across all participants in a study. Aggregated data can be extracted from published manuscripts. IPD meta-analysis, on the other hand, uses data recorded for each patient, which are obtained from the primary investigators. IPD meta-analysis has several advantages over conventional aggregated data meta-analyses. Using IPD, it is possible to adjust for confounding factors (and any imbalance of the baseline characteristics between studies) and to standardize data across studies, which make those with variable study designs more comparable.¹⁶⁰ IPD meta-analysis also increases statistical power, which is another advantage, especially when the number of studies is small.^{161,162} In aggregated data meta-analysis including network meta-analysis, only RCTs can be included, whereas in IPD meta-analysis, single-arm studies can be included. Outcomes that were not reported in the original studies can be assessed in IPD meta-analysis, which makes it more suitable for answering study questions on neglected or understudied populations, such as pregnant women.

In this thesis, an aggregated data meta-analysis was firstly attempted and is described

in Chapter 3-II. Based on the findings, IPD meta-analyses were conducted and are described in Chapters 4 and 6. The acquisition of the data for the IPD meta-analyses is described next.

Identification of studies

Eligible studies were identified based on the systematic literature review described above. Studies were included if they met the following criteria.

Types of studies

- Prospective clinical efficacy studies with a minimum 28-day active follow-up
- Both interventional and observational cohort studies regardless of the number of treatment arms (i.e. comparative or single arm)
- Genotyping to distinguish recrudescence and reinfection by PCR

Types of participants

- Pregnant women in any trimester
- Parasitologically confirmed *P. falciparum* parasitaemia
- Either asymptomatic or symptomatic

Types of intervention/exposure and controls

- Treated with artemisinin-based or quinine-based treatments

Types of outcomes

- Clinical and parasitological efficacy
- Acute adverse symptoms

- Pregnancy outcomes

The following studies were excluded.

- No active follow-up within 28 days after treatment (e.g. studies that followed up only at delivery will be excluded)
- Studies with ≤ 10 eligible pregnant women
- Studies conducted in non-malaria endemic countries (e.g. returned travellers)

Acquisition of data

Principal investigators of the published and unpublished studies identified by the systematic literature review were invited to share their IPD with WWARN. At least three emails were sent to the corresponding authors asking whether they were willing to join this study group on treatment of uncomplicated falciparum malaria in pregnancy within WWARN (WWARN malaria in pregnancy treatment efficacy study group).¹⁶³ Further efforts were made to meet the principal investigators face-to-face at international conferences in 2017–2018. The attempts to acquire data were started in August 2016 and continued until November 2018.

WWARN has developed a seamless system from data-sharing to IPD meta-analyses (Figure 2-1). Through a secure web-based platform developed by WWARN, IPD are uploaded after agreeing with the terms and conditions of the submission.¹⁶⁴ Original raw data are stored in a secure server hosted by the University of Oxford and data are fully anonymized.

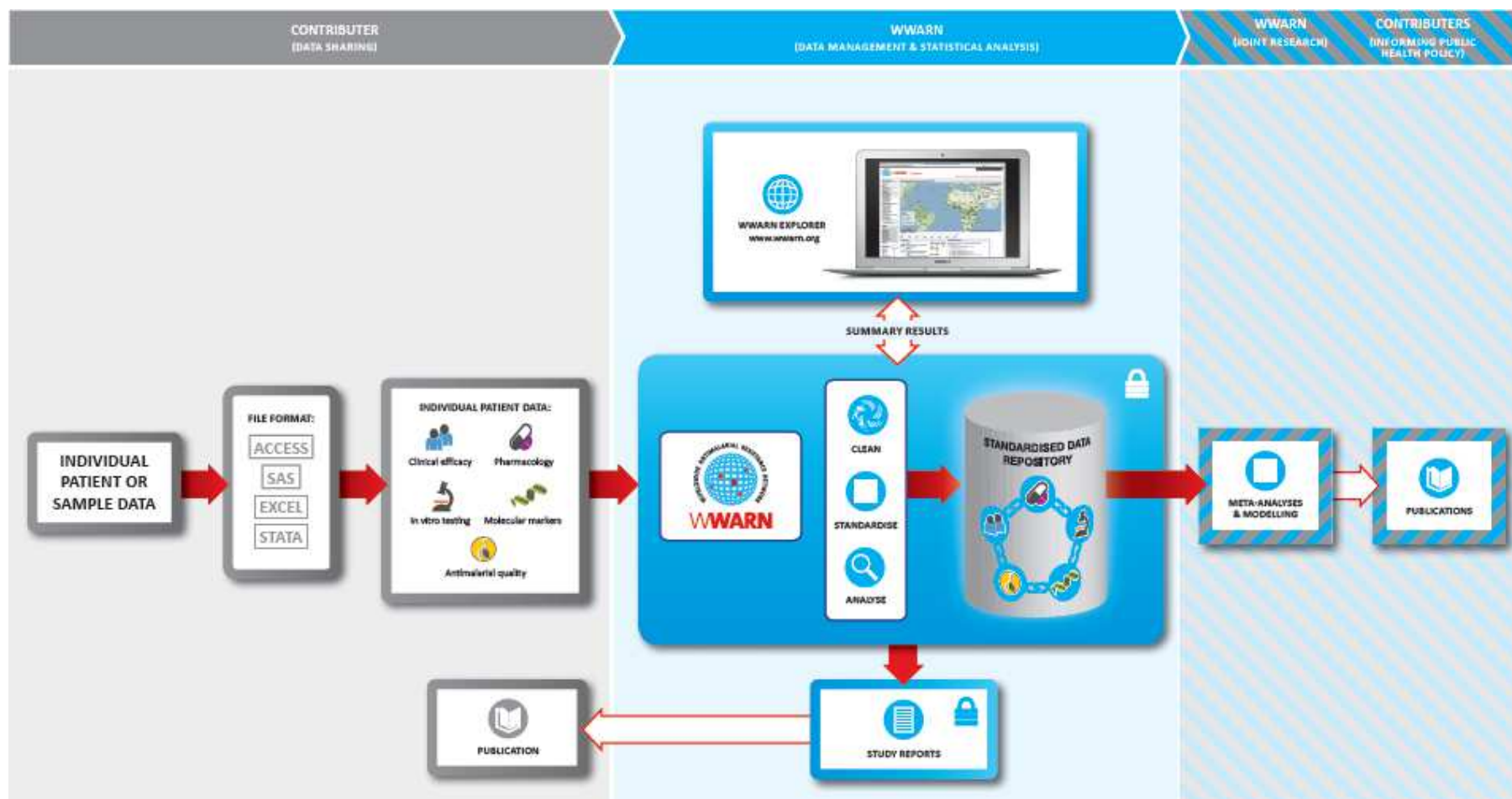


Figure 2-1. Flowchart of the WWARN system from data-sharing to IPD meta-analysis (Figure provided courtesy of WWARN.)

Data standardization

A list of variables needed for the planned analyses was made (Appendix 2-1) in order to harmonize the IPD that had been collected and recorded in different ways. This list was developed based on the variables assessed specifically for malaria in pregnancy at SMRU and on the case report form of the INTERGROWTH-21st project (courtesy of Professor Stephen Kennedy, University of Oxford).¹⁶⁵ The aim of the INTERGROWTH-21st project was to produce international standards for anthropometric measurements of newborns and was conducted globally, including malaria endemic countries. These two sources assure the practicality of data collection in resource-limited settings. The list was then reviewed by an external expert on malaria in pregnancy and an obstetrician.

Data management

Raw data were curated in a standardized format using the WWARN Clinical Module data management and statistical analysis plan to facilitate one-stage IPD meta-analyses.¹⁶⁶ After checking the raw data, any queries on the availability of data, ambiguities in the variables or potential errors were solved by asking the data contributors. After the initial check and cleaning, the WWARN data management team conducted the data standardization, according to the variable list described above. The standardized dataset was traceable to the raw data by a combination of unique patient (or episode) ID and study ID. This standardized dataset was used for the analyses.

Rationale for one-stage IPD meta-analysis

A process described as one-stage IPD meta-analysis was conducted; all data extracted from each study were pooled and this pooled dataset was used for the statistical analyses.^{160,161,167} One stage IPD meta-analysis increases statistical power thus making it more suitable than the two-stage IPD meta-analysis if the number of studies is small.¹⁶⁷ Patient-level interaction or sub-group analysis, and heterogeneity across studies can be managed with one-stage IPD meta-analysis.^{167,168}

Assessment of study quality

Risk of bias was assessed by the Cochrane tool for risk of bias (for RCTs) and the Newcastle-Ottawa scale (for non-comparative cohort studies). The quality of evidence summarized across studies was assessed using the GRADE system (Chapter 9).¹⁶⁹

Registration

The IPD meta-analysis is registered on PROSPERO (CRD42018104013).

2.4 Study settings and acquisition of the data at SMRU

SMRU has been providing primary health care and antenatal care in rural areas at the north-western border of Thailand since 1986. The area covered by the SMRU clinics stretches for over 100 km along the border (Figure 2-2) with low (or unstable) transmission of *P. falciparum* and *P. vivax* malaria.^{78,170,171} The majority of the patients are of Karen ethnicity,



Figure 2-2. Different landscapes along the Thailand-Myanmar border in 2016

Left: A refugee village in a forested hilly area. Right top: Plantation agriculture where many migrants work. Right bottom: Viewing the Myanmar side of the Moei river.

either refugees or migrants from Myanmar, who have been dislocated over >30 years because of the chronic conflict on this border.⁷⁸ Pregnant women are followed up every week at antenatal clinics (ANCs) with malaria smear screening and treatment if positive. This weekly screening continued until 2007 for refugees and 2010 for migrants.³² Since 2007, screening among refugees has been conducted every week for three weeks from the first antenatal visit; weekly screening is then continued only for those who were positive in any of the initial three screenings. Since 2010, screening among migrants has been conducted every two weeks. Trained microscopists at the clinics assess blood smears (Giemsa stained thick and thin blood smears) with quality control by the SMRU central microscopy laboratory in Mae Sot, Thailand. Chemoprophylaxis against falciparum malaria was not adopted in this settings

because there was no drugs proven to be safe and effective as a prophylaxis in this area^{172,173} and also IPTp is not recommended in low transmission settings.⁸⁶

In this setting, around one quarter of pregnant women had at least one falciparum malaria during their pregnancy in the early 2000s, with a declining trend of morbidity and mortality owing to the deployment of early detection and ACT treatment for non-pregnant populations which started in the mid-1990s.^{78,170} To estimate the gestational age (estimated gestational age [EGA]) of the fetus, last menstrual period (all years), symphysis-pubis fundal height (1986–1994), Dubowitz assessment (1992–2002) and ultrasound (2001–present) have been used.³² Details of the original studies and specific study settings will be described in each chapter.

The data collected at SMRU were used for analyses described in Chapters 5, 7 and 8. In all chapters, only data that had already been collected by the researchers at SMRU were used. Some data were extracted from written medical records for this project and then combined with electronic databases, which had been built by the researchers and data managers at SMRU. Data were fully anonymized, and no confidential information was included in the datasets used for analyses.

2.5 Definitions of the terms

Uncomplicated malaria was defined as malaria infection without features of severe malaria, as defined by WHO,¹⁴⁰ which will be discussed in detail in Chapter 8.

For recurrences of parasitaemia after treatment, PCR is used to differentiate

recrudescence of the original strain responsible for the infection (treatment failure) from reinfection from a novel parasite strain.¹⁴⁴ Adequate clinical and parasitological response (ACPR) is used as the definition for treatment success according to the WHO guideline¹⁷⁴ for non-pregnant populations (Box 2-3).

In IPD meta-analyses, malaria transmission for each study site was classified into three categories based on *P. falciparum* malaria for children two to ten years of age (PfPR₂₋₁₀) obtained from the Malaria Atlas Project for specific location and year of study:^{14,175} low (PfPR₂₋₁₀ <0.10); medium (PfPR₂₋₁₀ 0.10–0.40); and high (PfPR₂₋₁₀ >0.40) malaria transmission.

Pregnancy was divided into trimesters: the first trimester is defined as less than 14 weeks (13 6/7 weeks), the second from 14 weeks (14 0/7 weeks) to 27 weeks (27 6/7 weeks), and the third from 28 weeks (28 0/7 weeks) until delivery. The postpartum period starts at delivery and lasts until 42 days (6 weeks).¹⁷⁶ For the analysis, pregnancy outcomes are defined as follows. Miscarriage is defined as fetal loss before 28 weeks of gestation. Stillbirth is defined as fetal loss at 28 weeks or more of gestation.^{177,178} Preterm is defined as babies born alive before 37 weeks of gestation:¹⁷⁹ very preterm as 28 to 32 weeks and moderate to late preterm as 32 to 37 weeks.¹⁸⁰ Low birthweight is defined as birthweight of less than 2,500 g, regardless of gestational age unless otherwise specified.¹⁷⁹ Small for gestational age is defined as birthweights of smaller than the 10th percentile^{181,182} of the standard growth curve derived from the INTERGROWTH-21st international standard.¹⁶⁵ Anaemia was defined for

Box 2-3. WHO definition of treatment response for non-pregnant populations modified for WWARN IPD meta-analysis

Early treatment failure (ETF)

- danger signs or severe malaria on day 1, 2 or 3, in the presence of parasitaemia;
- parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature;
- parasitaemia on day 3 with axillary temperature $>37.5^{\circ}\text{C}$; and
- parasitaemia on day 3 $\geq 25\%$ of count on day 0.

Late clinical failure (LCF)

- danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and the last day of follow-up in patients who did not previously meet any of the criteria of early treatment failure; and
- presence of parasitaemia on any day between day 4 and the last day of follow-up with axillary temperature $>37.5^{\circ}\text{C}$ in patients who did not previously meet any of the criteria of early treatment failure.

Late parasitological failure (LPF)

- presence of parasitaemia on any day between day 7 and the last day of follow-up with axillary temperature $<37.5^{\circ}\text{C}$ in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure.

Early late treatment failure (ELTF)

- Falciparum parasitaemia, found on day 4 to day 7 inclusive in patients who did not previously meet any of the criteria for early treatment failure, late clinical failure or late parasitological failure.

Adequate clinical and parasitological response (ACPR)

- absence of parasitaemia on the last day of follow-up, irrespective of axillary temperature, in patients who did not previously meet any of the criteria above.

* A modification was made to accommodate the different length of follow-up: WHO guidelines define day 28 or 42 as the end of follow-up. ELTF was added since patients with positive parasitaemia without fever during day 4 to 7 could not be categorized by the WHO definition.

Modified and reprinted from Methods for surveillance of antimalarial drug efficacy, World Health Organization, Page 7, Copyright (2009).¹⁷⁴

pregnant women as: haemoglobin <11 g/dL or haematocrit $<30\%$ for anaemia; and

haemoglobin <7 g/dL or haematocrit $<20\%$ for severe anaemia.¹⁸³

2.6 Statistical analysis

Statistical analysis was conducted using Stata 15.1 MP (StataCorp, Texas, USA) in all chapters. R (R Foundation for Statistical Computing, Vienna, Austria) in Chapters 4 and 5, and Microsoft Excel (2016) in Chapters 1, 3 and 8 were used to produce some figures.

2.7 Ethics approval

For Chapters 4 and 6, ethics approval for the IPD meta-analyses using anonymized secondary data at WWARN is waived by the Oxford Tropical Research Ethics Committee. The official letter regarding this decision is shown in Appendix 2-2. Each study included in the IPD meta-analyses has received local ethics approval.

For Chapters 5 and 8, ethics approval for retrospective analysis of anonymized clinical records at SMRU was granted by the Oxford Tropical Research Ethics Committee (OxTREC 28-09) and the Tak Community Advisory Board (TCAB-4/1/2015) (Appendices 2-3, 2-4). In Chapter 7, secondary data that had been collected in an RCT (OxTREC 45-09, Mahidol University Tropical Medicine 2009-065-01, SMRU 0905) were used.

Chapter 3. Systematic literature reviews

Chapter 3-I Antimalarial drugs in pregnancy: their use for treating falciparum malaria and safety profiles

Part of this chapter has been published in a peer-reviewed journal and is freely available as follows under the Creative Commons Attribution licence. Saito M, Gilder ME, McGready R, Nosten F. Antimalarial drugs for treating and preventing malaria in pregnant and lactating women. *Expert Opin Drug Safety*. 2018;17(11):1129-44.

3-I.1 Introduction

Pregnant women are more vulnerable to malaria, leading to unfavourable impacts on both mother and fetus even if asymptomatic.^{10,12,184} Antimalarials are used for preventing and treating malaria infection in pregnant women. In both situations, the risk/benefit of toxicity of these drugs and clinical benefits need to be considered. This is particularly complicated in pregnancy as the long-term consequences of drug exposures in pregnancy and fetal outcomes need to be weighed up with potential under- or over-dosing due to the altered pharmacokinetics in pregnancy.

Embryotoxicity and teratogenicity are of special concern for any new drugs. For malaria, artemisinin derivatives are the current key antimalarial for non-pregnant patients. Since their introduction in the 1990s, a dramatic decrease in the mortality as well as global prevalence of the disease has been witnessed.¹⁹ However, artemisinin is shown to be embryotoxic in animal studies.^{145,185} As a consequence, quinine, an old drug with inferior efficacy and poor adherence, has continued to be recommended for women in the first trimester by WHO.¹⁴⁰ There is recently, however, growing evidence on the safety of artemisinin in pregnancy including the first trimester.^{32,33}

In this chapter, the use and safety profiles of the currently used antimalarials in pregnancy, primarily in the context of treatment of falciparum malaria, is reviewed. A literature search was performed using three databases (MEDLINE, Embase and Global health) on human subjects as described in Chapter 2. Treatment efficacy of these antimalarials

will be described in the following chapters.

3-I.2 Quinine

Treatment in pregnancy

Quinine is given as treatment (10 mg/kg/dose three times a day for seven days) with or without clindamycin. Quinine should be administered under full supervision because of poor adherence: about half of the patients treated with unsupervised quinine are estimated to fail treatment.¹⁵² Increase of oral quinine dose may be needed in pregnancy.^{186,187}

The efficacy of quinine can be greatly improved by adding clindamycin,¹⁸⁸ although other disadvantages of quinine cannot be overcome (discussed in Chapters 3-II and 4).¹⁸⁹ Quinine with clindamycin, therefore, can be an option when artemisinin cannot be used (e.g. allergy), but special care (e.g. full supervision, and close clinical and parasitological monitoring with hospitalization) should be considered.

Acute adverse reactions

The adverse symptoms of quinine are collectively called cinchonism, which affects almost all patients.¹¹⁸ This leads to poor adherence particularly among the pregnant women in the first trimester when morning sickness peaks. The actual adherence without supervision is thought to be around five days.¹⁵² The prevalence of tinnitus in pregnant patients on quinine is reported to be from 35% to 85% in >450 patients assessed in six studies.^{188,190-195} A meta-analysis reported that the risks of tinnitus (pooled risk ratio [RR] 4.70, 95% CI 1.20 to 18.39,

five RCTs), vomiting (pooled RR 2.01, 95%CI 1.23 to 3.30, five RCTs) and dizziness (pooled RR 1.51, 95% CI 1.02 to 2.25, three RCTs) were higher than those of artemisinin-based treatment (see Chapter 3-II).¹⁸⁹ Nausea and anorexia are also more common with quinine than AL in an open-label RCT.¹⁹² The risk of quinine-related hypoglycaemia is higher in pregnant women than the general population, particularly in cases with severe malaria^{196,197} and when compared with other antimalarials.¹⁹⁸ In a study in Uganda, QTc prolongation (QTc by Fridericia >440 ms) was observed on day 2 in 1% (2/149) of the patients.¹⁹²

To reduce the side effects, under-dosing treatment by prescribing twice daily dosing or only five days is potentially harmful and likely to induce resistance.^{152,193}

Impact on the fetus

Though historically used as an abortifacient, quinine is regarded as safe in the first trimester at therapeutic doses¹⁴⁰ and there was no evidence of an oxytocic effect on uterine contractions following intravenous quinine for severe malaria.¹⁹⁶ Studies on uninfected non-primate mammals showed embryotoxicity of quinine at doses equivalent to therapeutic doses for humans,¹⁹⁹ but there has been no clear evidence of fetal toxicity in humans when it is used for treatment.

A recent meta-analysis of five cohorts^{32,153,155,200-203} including 947 pregnant women who were exposed to quinine in the first trimester reported higher risk of miscarriage (adjusted hazard ratio [aHR] 1.48, 95% CI 1.18 to 1.86, p<0.001), but there was no difference

in the risk of stillbirth (aHR 1.35, 95% CI 0.69 to 2.65, $p=0.39$) and congenital abnormality (1.2% , 95% CI 0.6 to 2.4%) than those who were not exposed to any antimalarials.³³ The increased risk of miscarriage was considered to be related to malaria infection itself and was not different from those who were treated with artemisinin derivatives.³³

Further two observational cohort studies in Papua New Guinea and Sudan reported the risk of miscarriage after quinine exposure in the first trimester was 2.6% (1/38)²⁰⁴ and 3.8% (1/26)²⁰⁵, respectively. The latter study reported no detectable congenital abnormalities up to 1 year ($n=26$).²⁰⁵ Surveillance of Michigan Medicaid recipients between 1985 and 1992 reported that the prevalence of major congenital abnormality among those who were exposed to quinine during the first trimester was 5.7% (2/35).²⁰⁶

In the second and third trimester, meta-analyses showed the risks of stillbirth and congenital abnormality are not different between quinine-based treatments and artemisinin-based treatments (see below).^{189,207}

3-1.3 Artemisinin derivatives

Treatment in pregnancy

Artemisinin derivatives are the most effective antimalarials. For uncomplicated malaria, an artemisinin component, namely artesunate, artemether or dihydroartemisinin is given for three days (target dose of artesunate: 4 mg/kg/day) combined with drugs with a longer half-life (partner drugs) (Table 3-1).¹⁴⁰ This is called artemisinin-based combination

Table 3-1. Pharmacokinetic properties of the five different combinations of artemisinin-based combination therapies recommended by WHO¹⁴⁰

Antimalarial	Half-life of artemisinin derivative	Hal-life of partner drug
Artemether-lumefantrine	0.86–5.16 hours	~3 days
Artesunate-mefloquine	54 minutes 48–150 minutes‡	≤3 weeks
Dihydroartemisinin-piperaquine	0.85–1.40 hours	13.5–28 days
Artesunate-amodiaquine	54 minutes 48–150 minutes‡	4–10 days*
Artesunate-sulfadoxine-pyrimethamine	54 minutes 48–150 minutes‡	4.1–10.9 days (sulfadoxine) 60–450 hours (pyrimethamine)

Derived from the Guidelines for the treatment of malaria (2015), WHO.

‡ half-life for active metabolite, dihydroartemisinin() or desethylamodiaquine(‡)

therapy (ACT). For severe malaria in any trimester of pregnancy, intravenous artesunate is the drug of choice.¹⁴⁰ Monotherapy of artemisinin is discouraged because of the risk of developing resistance to this important antimalarial.¹⁴⁰ Pharmacokinetic (PK) studies suggest an increase of artemisinin doses in pregnancy may be needed.²⁰⁸⁻²¹⁵

Acute adverse reactions

Artemisinin derivatives are very well tolerated in the general population without any serious adverse drug reactions.¹¹⁸ Delayed haemolysis after artemisinin treatment has been infrequently reported especially in non-immune travellers and in individuals with severe malaria: an estimated 13% (95% CI 9 to 18%) of severe malaria cases receiving artesunate experienced haemolysis.²¹⁶ Neurotoxicity was extensively assessed in the 1990s and no evidence of neurotoxicity in humans was found after artemisinin treatment.¹¹⁸ Allergy to artemisinins is estimated to affect 1 in 2833 patients (95% CI 1 in 1362 to 6944),²¹⁷ and has also been reported in pregnancy.^{188,218,219}

Impact on the fetus

Safety of artemisinin derivatives in the first trimester of pregnancy has been a concern because of embryotoxicity and teratogenicity (particularly musculoskeletal and cardiovascular defects) of artemisinin derivatives shown in animals, including one study on primates.¹⁸⁵

A recent meta-analysis of five cohorts^{32,153,155,200-203} analysed 671 pregnant women who were exposed to artemisinin derivatives in the first trimester.³³ The risk of miscarriage (aHR 0.73, 95% CI 0.44 to 1.21, p=0.23), stillbirth (aHR 0.29, 95% CI 0.08 to 1.02, p=0.053) and congenital abnormalities (1.5%, 95%CI 0.6 to 3.5%) were not different from those who were exposed to quinine in the first trimester,³³ even when the exposure was restricted to the embryo-sensitive period (6–12 gestational weeks).³³ This meta-analysis of observational data suggests a non-statistically significant protection from early pregnancy loss with artemisinins compared with quinine treatment of malaria. Proof would require randomization in the first trimester, a trial that is unlikely to ever take place. This trend may be due to the better clinical and parasitological efficacy of artemisinin, as a falciparum malaria episode in the first trimester itself is associated with a 1.61-fold increase in the risk of miscarriage.³²

Pregnancy outcomes have been described for an additional 259 women with first trimester artemisinin exposures.^{204,220-224} In one report from Timika, west Papua, 11 women received ACTs and 10 received intravenous artesunate (for severe malaria) in the first trimester.²⁰⁴ The prevalence of miscarriage was 63% (7/11) of those who received an ACT but

0 in those who received intravenous artesunate. No explanation was given for why these women received ACTs against the local treatment policy, and congenital abnormalities were not assessed. The other studies did not report apparent excesses of adverse pregnancy outcomes in exposed women. Further 63 women in two studies were exposed to artemisinin in the first trimester, but no pregnancy outcomes were reported.^{225,226}

In the second and third trimester, a meta-analysis of five RCTs reported that the risk of stillbirth (pooled RR 1.04, 95% CI 0.42 to 2.60) and congenital abnormality (pooled RR 1.29, 95% CI 0.40 to 4.17) after artemisinin-based treatment were not different from those who were treated with quinine-based treatment.¹⁸⁹ A meta-analysis of observational cohort studies reported no difference between the risks of miscarriage (pooled RR 1.13, 95% CI 0.77 to 1.66, three studies), stillbirth (pooled RR 1.10, 95% CI 0.79 to 1.54, four studies) and congenital abnormalities (pooled RR 0.79, 95% CI 0.37 to 1.67, three studies) in women exposed to artemisinins and those not exposed to any antimalarials.²⁰⁷

3-I.4 Lumefantrine

Treatment in pregnancy

Lumefantrine is available as a fixed dose combination with artemether (480 mg lumefantrine plus 80 mg of artemether twice daily for three days) for treatment of uncomplicated falciparum malaria, recommended for second and third trimester pregnant women by WHO. US CDC has recently changed their recommendation and listed AL as an option for the first trimester women if neither mefloquine nor quinine with clindamycin is

available.¹⁵⁶

Lumefantrine blood concentration on day 7 was lower than non-pregnant populations in four cohorts,^{187,192,208,227-232} but was not different in one study.²³³ Dose optimization by extending the length of treatment and/or increasing the dose was suggested by mathematical modelling,^{227,232,234} and assessed in two unpublished studies.^{235,236}

Acute adverse reactions

AL is generally well tolerated compared with other ACTs in pregnant women.^{237,238} Early vomiting was rare and observed in <0.4²⁰⁰ to 0.8% (1/125) patients.²¹⁹ Abdominal pain (28%, 43/152), headache (17%, 26/152), flu-like symptoms (16%, 25/152) were relatively common in an RCT in Uganda, but only headache was significantly more common with AL than quinine.¹⁹² Abnormal biochemistries are rare in pregnancy.^{192,219,233,238,239} Two studies assessing cardiotoxicity in pregnancy found no increase with AL.^{192,208,219}

Impact on the fetus

Of all ACTs, AL has the largest number of documented first trimester exposures. A recent meta-analysis of five cohorts^{32,153,155,200-203} included 511 pregnant women who were exposed to AL in the first trimester, and no increased risk of miscarriage or congenital abnormality was reported (see above).³³ Another observational cohort study reported three women exposed to AL in the first trimester without miscarriage or congenital abnormality.²²⁰

In the second and third trimester, the prevalence of stillbirth (1.9%, 16/856), PTB

(10.2%), LBW (17.2%) and congenital abnormality (2.0%, 17/832) were not different among ACTs in a multicentre RCT in Africa.²³⁷ In another two RCTs including around 250 patients in total, the prevalence of miscarriage, stillbirth, PTB, LBW and congenital abnormality did not differ between quinine¹⁹² or artesunate monotherapy.²¹⁹

3-I.5 Amodiaquine

Treatment in pregnancy

Amodiaquine is used as a treatment for uncomplicated falciparum malaria in combination with artesunate. ASAQ is available either as fixed-dose or non-fixed dose combination, with amodiaquine of 10 mg base/kg/day given once daily for three days. ASAQ is recommended in the second and third trimester by WHO.¹⁴⁰ Limited data from 24 pregnant women in second and third trimester indicated there were no clinically meaningful pharmacokinetic changes in pregnancy.^{240,241}

Acute adverse reactions

In pregnancy, ASAQ is generally tolerated but associated with a higher risk of adverse symptoms than AL^{237,238,242} or DP^{237,243}. Anorexia, nausea, vomiting, dizziness and weakness were more common than AL^{237,238,242} or DP^{237,243} and were more common in pregnant than in post-partum women.²⁴⁰ Weakness was particularly more frequent than other antimalarials.^{237,238,242-245} Insomnia, behavioural change or hallucination were also reported.²³⁷ Prolonged use of amodiaquine (as prophylaxis) is reported to be associated with an increased

risk of agranulocytosis and hepatitis,¹¹⁸ although clinically significant haematopoietic or hepatic toxicity is not reported after its short-term use as treatment in non-pregnant populations¹¹⁸ as well as in pregnant women.^{237,238,240,244,246-248} Hypotension was reported in 1.5% of the patients, which is more frequent than with other ACTs.²³⁷ No QTc prolongation (>500 msec) was observed in 83 pregnant women.²⁴⁷

Impact on the fetus

Although its use in the first trimester has not been well documented, amodiaquine is considered safe in pregnancy as it is structurally similar to chloroquine,¹⁵¹ which is used widely in pregnancy either as antimalarial or antirheumatic. Three studies reported birth outcomes with amodiaquine treatment of malaria before the 20th gestational week, in the first all eleven women gave full-term live-births without congenital abnormalities¹⁵³ and of a further nine included, seven gave birth to full term lie-births, one miscarried and one left before outcome was available²⁴⁰. Three women were treated with ASAQ and one of them miscarried.²⁰⁴

In the second and third trimester, the prevalence of stillbirth (2.1%, 17/815), PTB (3.4%), LBW (15.5%) and congenital abnormality (1%, 8/776) were not different from other ACTs in an RCT.²³⁷ In other RCTs assessing the pregnancy outcomes of around 150 women in total, the risk of pregnancy loss was not different from those for AL²⁴² or DP²⁴³.

3-I.6 Mefloquine

Treatment in pregnancy

Mefloquine is used as a treatment for uncomplicated falciparum malaria in combination with artesunate. ASMQ is available either as fixed-dose (440 mg mefloquine hydrochloride plus 200 mg artesunate once daily for three days) or non-fixed dose combination (mefloquine 25 mg/kg in total given for a course). ASMQ is recommended in the second and third trimester by WHO.¹⁴⁰

Pharmacokinetic properties of mefloquine in pregnancy are not consistently reported across the literature.²⁴⁹⁻²⁵² Further studies are needed to assess whether dose optimization is necessary for pregnant women.

Acute adverse reactions

In pregnancy, ASMQ is generally tolerated for treatment of acute malaria episodes but associated with a higher risk of adverse symptoms than AL or DP²³⁷ but lower than quinine.^{190,198,253} An increased risk of anorexia, nausea, vomiting, dizziness and weakness compared with DP or AL was reported.²³⁷ Early vomiting is frequent when mefloquine is given as a single dose,²⁵⁴ but this risk is mitigated by divided dosing, given after artesunate.^{118,190} Early vomiting was observed in 9% (5/55) after the administration of fixed-dose combination.²⁵² Other distressing but uncommon adverse symptoms include behavioural change (1/850) and insomnia (2.5%, 21/850).²³⁷ There were no cases with clinically

significant haematological or biochemical abnormality reported.^{237,252,255-257} Mefloquine is not regarded as cardiotoxic¹¹⁸ and there was no cardiotoxicity recorded in a study assessing ECG of 60 pregnant women who received weekly mefloquine prophylaxis.²⁵⁸

Impact on the fetus

Currently, US CDC recommends mefloquine monotherapy for the first trimester.¹⁵⁶ Although early clinical studies and animal studies suggested an increased risk of fetal loss after mefloquine exposure in pregnancy,^{199,206,258,259} subsequent clinical studies in pregnancy are reassuring. Post-marketing surveys among travellers in non-endemic countries who were exposed to mefloquine found no increased risk of pregnancy loss (10.3%, 112/1090) or congenital malformation (4.4%, 43/978) compared with the background population level even used in the first trimester.²⁶⁰⁻²⁶² At the Thailand-Myanmar border, the risk of miscarriage after the first-trimester exposure to mefloquine (8%, 2/23) was not different from that after quinine (11%, 92/750).³²

In the second and third trimester, in a multi-centre RCT in Africa, the prevalence of stillbirth (2.8%, 23/821), PTB (7.7%), LBW (15.2%) and congenital abnormality (1.7%, 13/780) after ASMQ were not different from other ACTs.²³⁷ Another five studies assessed an additional 363 pregnant women in total (including 13 in the first trimester) exposed to mefloquine reported three miscarriages, three stillbirths and two congenital abnormalities.^{190,254,255,257,263,264}

Neuropsychiatric side effects, which have plagued military malaria prophylaxis programs and limit mefloquine use in severe malaria, appear to be rare with uncomplicated malaria treatment. However, neurodevelopment in exposed fetuses has not been studied.

3-I.7 Piperaquine

Treatment in pregnancy

Piperaquine is only available as a fixed-dose combination with either dihydroartemisinin (960 mg piperaquine plus 120 mg dihydroartemisinin once daily for three days) or artemisinin (750 mg piperaquine plus artemisinin 125 mg once daily for two days). DP is recommended as a treatment for the second or third trimester by WHO.¹⁴⁰ Pregnancy is associated with a shorter terminal elimination half-life.^{214,215,265-267} However, the impact of pregnancy on the area under the curve is inconsistent: increased^{214,266} or decreased.²⁶⁷

Acute adverse reactions

DP is very well tolerated^{214,237,243,266-269} and adverse symptoms were fewer than ASAQ^{237,243} and ASMQ.²³⁷ Early vomiting was observed in 0.8% (2/245) of the pregnant women who received DP as treatment and 0.2% (4/1725) of the episodes given as IPTp.²⁶⁹ QT prolongation was reported, but no associated clinical cardiotoxicity was reported.^{267,270-272}

Impact on the fetus

Reproductive toxicity of piperaquine was not shown in rats and rabbits.^{199,273} The

clinical use of DP in the first trimester has not been well documented.³³ A small number of women in the observational cohort in west Papua miscarried after the treatment of DP in their first trimester (63%, 5/8), which was higher than quinine (2.6%, 1/38).²⁰⁴ It was not clear why these patients were treated with DP against the local health policy. Another three women who failed quinine treatment were treated with DP in the first trimester;²⁶⁸ among them, there were no stillbirths or congenital abnormalities.²⁶⁸

In the second and third trimester, the prevalence of stillbirth (2.7%, 22/818), PTB (9.5%), LBW (14.1%), and congenital abnormality (0.8%, 6/767) were not different among ACTs in an RCT.²³⁷ In two cohort studies that include >100 patients, the risks of stillbirth after the exposure to DP mainly in the second and third trimester were <3%.^{204,243} One RCT reported the risk of LBW was higher than ASAQ (13.2% [12/91] versus 4.2% [3/72]; p=0.05), but the authors concluded this could have been a chance finding.²⁴³ The average birthweight was not different between two groups in the study.

3-I.8 Atovaquone-proguanil

Treatment in pregnancy

Atovaquone-proguanil (AP) can be used as a prophylaxis in areas of chloroquine/mefloquine resistance and can be used as a treatment with or without artesunate,²⁷⁴ although it is currently not on the list of ACTs recommended by WHO.¹⁴⁰ Pharmacologically, increasing the dose of AP may be considered in pregnancy.²⁷⁵⁻²⁷⁸

The use of this drug in endemic countries was restricted because the resistance of

atovaquone can be easily acquired by single point mutations of *cyt b* gene,⁹ although a recent study indicates that parasites with this mutation cannot survive in mosquitos, and thus the resistance cannot be transmitted.²⁷⁹

Acute adverse reactions

AP is very well tolerated in non-pregnant populations¹¹⁸ and is regarded to be safe for pregnant women.^{9,140,151,206} In a total of 126 pregnant women treated with AP,^{194,275,276,280} there were few adverse symptoms that could have been due to malaria itself²⁷⁶ and whose risks were similar to those after artesunate alone.²⁸⁰ No cardiotoxicity was reported in 24 pregnant patients assessed.²⁷⁵

Impact on the fetus

No teratogenic or embryotoxic effects were shown in rats and rabbits.²⁸¹ However, a theoretical concern on its use in the first trimester remains as cycloguanil, the active metabolite of proguanil, is an anti-folate. Co-administration of folic acid supplements at least during the first trimester should thus be considered.²⁰⁶

A recent meta-analysis concluded that the risks of adverse pregnancy outcomes following exposures to AP in pregnancy are similar to those in background populations.²⁸² In total 485 women were reported to be exposed to AP in the first trimester either as treatment or prophylaxis.^{280,283-286} In a post-marketing surveillance in 1997–2017, 198 women were reported to the pharmaceutical company that they took AP during the pregnancy or breast-

feeding, of which 158 were in the first trimester.²⁸⁶ While the proportion of miscarriage of 19.4% (37/191) was comparable to the general population, increased proportion of congenital abnormality was reported (22.8%, 31/136).²⁸⁶ This can be interpreted with caution as this surveillance is based on passive reporting, thus more likely to be biased toward higher risk of adverse outcomes. In a cohort of Danish travellers receiving AP as prophylaxis in their first trimester, the prevalence of congenital abnormality was 1.3% (2/149) and the risk was not increased in comparison to that of the background population (2.5%, 13,993 / 570,728, odds ratio 0.55, 95% CI 0.14 to 2.21).²⁸³ Another case series in Europe including 165 women exposed to AP either as prophylaxis or treatment in the first trimester reported 13% (12/165) of miscarriage and 4% (4/162) of congenital malformation.²⁸⁴ The other 13 women exposed to AP in the first trimester delivered live-born babies without congenital abnormality.^{32,280,285}

After AP use as treatment in the second and third trimester, one study reported two congenital abnormalities (5.9%, 2/34).¹⁹⁴ In the other three studies including at maximum 70 women in total, no congenital abnormalities were documented.^{275,276,280} No pregnancy loss was documented in around 100 women.^{194,275,276,280}

3-I.9 Other drugs

Clindamycin is used as treatment (10 mg/kg/dose, three times a day for seven days) in combination with either quinine or artesunate. Although clindamycin is not easily available in many malaria endemic countries, it should always be considered when quinine treatment is given to improve the low efficacy of quinine monotherapy (Chapters 3-II and 4).¹⁸⁹

Clindamycin is one of the few antibiotics that are generally regarded as safe in pregnancy, including the first trimester.^{140,199,206} Surveillance of Michigan Medicaid recipients between 1985 and 1992 reported that the prevalence of major congenital abnormality among those who were exposed to clindamycin during the first trimester was 4.8% (31/647).²⁰⁶

SP is no longer used for treating falciparum malaria as a partner drug of ACT in most of the world because of the widespread resistance.²⁸⁷ Despite the resistance, SP (1,500 mg of sulfadoxine and 75 mg of pyrimethamine given as a single dose) is recommended for use by WHO in sub-Saharan Africa for IPTp.⁸⁷

3-I.10 Discussion

Malaria in pregnancy leads to severe and fatal outcomes for both mother and fetus. Safe, effective and affordable antimalarials are needed for treating and preventing malaria in pregnancy. In both purposes, toxicity of these drugs and potential acquired clinical benefits need to be balanced. The safety of a drug is usually dose-dependent and relates to the frequency of administration. In pregnancy, the safety assessment is further complicated by potential impacts on the unborn or breastfed child, and the altered pharmacokinetics in pregnancy. The variability of safety assessment and reporting in studies of antimalarials in pregnancy studies make it difficult to draw a summary conclusion.²⁸⁸

Over the last decade, evidence on the safety of artemisinin derivatives in pregnancy including the first trimester has been gathered, debunking fears based on animal studies that lead to the systematic use of antiquated antimalarials for pregnant women. Use of artemisinin

derivatives in any trimester does not appear to be associated with increased risk of miscarriage, stillbirth or congenital abnormality compared with those who are treated with quinine or the background population level.^{32,33,207} Artemisinin-based therapy is more efficacious and is well-tolerated in pregnancy.¹⁸⁹ Given the benefits to both mother and fetus of effective maternal treatment, ACTs should be considered first-line for treating all pregnant women with uncomplicated falciparum malaria. Ongoing systematic monitoring for adverse effects should be strengthened and continued.

Because of the altered immunity and pharmacokinetics in pregnancy in addition to the sequestration of parasites to placenta, simple extrapolation of the results from non-pregnant population may not be necessarily valid. Although it has the safest profile among ACTs, the efficacy of AL can be lower possibly because of the suboptimal dosing for pregnant women. Dose optimization, usually by increasing the dosage, can be required for some antimalarials in pregnancy, and these alternate regimens require further evaluation of treatment efficacy and safety in pregnant women and their offspring. Newer drugs, such as pyronaridine or naphthoquine, require investigation of reproductive toxicity without delay.

In the setting of malaria elimination, drug efficacy can be attenuated because of declining immunity, and this can theoretically be exacerbated by the relative immune suppression of pregnancy, resulting in the urgent need for antimalarials in the development pathway that are efficacious and safe in pregnancy. The efficacy of the currently used antimalarials described here will be assessed in the following chapters of this thesis.

3-I.11 Conclusions

Recent evidence shows that use of artemisinin derivatives and the first-line partner drugs in any trimester does not appear to be associated with increased risk of miscarriage, stillbirth or congenital abnormality compared with those who are treated with quinine or the background population level. However, because of the rarity of the adverse events, such as congenital abnormality, monitoring of the safety should be continued. Most of the evidence comes from AL, thus more research is needed for other combinations of ACT.

Based on these reassuring safety profiles, the efficacy of antimalarials will be assessed in the following chapters in this thesis.

Chapter 3-II. Systematic literature review and meta-analysis of the efficacy of artemisinin-based and quinine-based treatments for uncomplicated falciparum malaria in pregnancy: methodological challenges

Part of this chapter has been published in a peer-reviewed journal and is freely available as follows under the Creative Commons Attribution licence. Information included in this chapter was updated after the publication. Saito M, Gilder ME, Nosten F, McGready R, Guérin PJ. Systematic literature review and meta-analysis of the efficacy of artemisinin-based and quinine-based treatments for uncomplicated falciparum malaria in pregnancy: methodological challenges. *Malar J.* 2017;16:488.

3-II.1 Introduction

Approximately 60% of all pregnancies worldwide take place in malaria endemic areas, leading to 125 million pregnant women at risk of malaria every year.⁷ Malaria in pregnancy, regardless of whether it is clinically symptomatic or not, has been reported to be associated with a higher risk of PTB, lower birthweight for gestational age, miscarriage, stillbirth and maternal anaemia.^{10,154,289,290} These adverse outcomes lead to a higher risk of perinatal mortality and maternal mortality in areas with low or declining malaria prevalence than in high transmission areas because of lower levels of premunition.^{12,57}

To mitigate these adverse effects, effective treatments need to be clearly identified for pregnant women. However, several factors have limited the available evidence on antimalarials efficacy during pregnancy. Pregnant women are usually excluded from RCTs of new antimalarials mainly because of concerns about the safety for the fetus. Safety concerns were particularly critical for artemisinin derivatives, as fetal resorption was observed in animal studies.^{145-149,185} Quinine-based treatment (QBT) is still recommended as the first-line treatment for uncomplicated falciparum malaria in the first trimester,¹⁴⁰ despite limited clinical data on its safety in the first trimester.^{151,291} Quinine's poor side effect profile and long treatment course of 5–7 days make it an undesirable choice for patients.^{151,152,291} Recently, as was described in Chapter 3-I, data from prospective observational cohort studies suggest that the risks of stillbirth or congenital abnormality with artemisinin use in the first trimester did not increase their risks compared with quinine,^{32,33,153-155} bringing the question of comparative

efficacy into centre stage.

Efficacy of antimalarial drugs for treating uncomplicated malaria in non-pregnant patients has traditionally been assessed over a fixed follow-up period set by the current WHO recommendations at 28–42 days.¹⁷⁴ However, this fixed period does not accommodate the pregnant condition as the placenta may become or remain parasitized (placental sequestration) after treatment completion.¹¹² There are currently no standard guidelines on parasitological efficacy studies in pregnancy.⁸

With this in mind, this systematic literature review aims to update the currently available efficacy data of artemisinin-based treatments (ABT) and QBT from both observational and interventional cohort studies in all trimesters with uncomplicated falciparum malaria. Methodological challenges will also be summarized to improve future efficacy studies in pregnancy.

3-II.2 Methods

A systematic literature review following PRISMA statement²⁹² was conducted to identify studies measuring the efficacy of ABT or QBT in pregnant women with parasitologically confirmed uncomplicated falciparum malaria, regardless of trimester or clinical symptoms. Seven databases (MEDLINE, Embase, Global Health, Cochrane Library, Scopus, Web of Science, and LILACS) and two clinical trial registries (ICTRP and ClinicalTrials.gov) were used. This review is registered to PROSPERO (CRD42017054808), and the search terms and conditions are available in Box 2-1.

Both interventional and observational cohort studies were included. Studies without any active follow-up in the first 28 days were excluded. Studies with fewer than ten pregnant women were excluded, as they could not be included in further meta-analyses. Systematic reviews on the antimalarial treatment in pregnancy^{151,293-297} were checked for any other possible missing articles that should be included.

After screening, the following data were extracted: demographic information of study (year, country, study design, study drugs and eligibility criteria), availability of outcome assessment (clinical outcomes, parasitological outcomes) and the methodology of assessment of variables (definition of treatment success and statistical method). Information was sought from published articles, clinical trial registry and protocols if available. Missing information was supplemented by personal correspondence to authors of the original studies if possible. This review describes the methodology of assessment and reporting and summarizes the reported efficacy results. Comparisons were made only if the same or similar assessment methods were used.

WHO recommends that treatment failure and completion of follow-up without treatment failure (adequate clinical and parasitological response [ACPR]) be used as efficacy endpoints in non-pregnant populations, and that results be expressed as the proportion of ACPR or the cumulative success rate using Kaplan-Meier survival analysis. WHO advises patients with PCR-confirmed reinfection with *P. falciparum*, infection with other malaria species, or loss to follow-up to be censored on the day of these events in the survival analysis

and to be excluded from the proportional ACPR analysis.¹⁷⁴ Patients without PCR results or with indeterminate PCR results are to be excluded from both PCR-corrected survival analysis and PCR-corrected proportional ACPR analysis.

For the comparability across studies, PCR-corrected proportional ACPR was recalculated using the WHO guideline for non-pregnant populations,¹⁷⁴ with the exception of cases with non-falciparum malaria infection that could not be excluded without detailed individual patient data. When the number of patients with ACPR was unavailable, it was estimated from the presented results and included for reference. PCR-corrected ACPR of 90% was used as the cut-off value to judge whether the treatment was satisfactory or not.¹⁴⁰ The 95% confidence interval (CI) for the proportion was calculated by the Wilson method.²⁹⁸ Random effects meta-analyses using aggregated results from RCTs were conducted if there were more than two RCTs. Heterogeneity was assessed using I^2 .²⁹⁹ If there was no treatment failure, the continuity correction was made by adding 0.5 for both treatment success group and failure group. For assessing publication bias, funnel plots of the proportions were drawn with log odds for the x-axis and study size (number of patients in each treatment arm) for the y-axis.³⁰⁰ Asymmetry was judged visually, as formal statistical tests have not been developed for this method.³⁰⁰ For odds ratios, Egger's test was used for checking the asymmetry of funnel plots.³⁰¹

3-II.3 Results

A total of 49 study cohorts assessing treatment efficacy for uncomplicated falciparum malaria in pregnancy were identified (Figure 3-1) evaluating at least 7,549 episodes of parasitologically confirmed uncomplicated falciparum. Studies based on the same cohort were considered as a single study. Forty-two studies were published, six presented at conferences, and one registered on a public trials database but not yet published. Twenty-seven studies (55%) were from sub-Saharan Africa, 19 (39%) from Southeast Asia, two (4%) from Latin America and one (2%) from India (Figure 3-2). As of April 2019, only one study was ongoing (not completed), and all other unpublished registered trials assessing antimalarial efficacy in pregnancy were either completed (n=1) or withdrawn (n=3): two trials (NCT00331708, NCT01082731) were terminated because of slow recruitment, and the other (NCT01082718) was withdrawn before enrolment.

The study designs comprised 23 RCTs comparing two or more treatment regimens,^{152,188,190,192-194,198,219,235-239,242,243,247,255-257,263,302-305} ten PK studies including clinical outcome assessment,^{211,213,214,230-233,252,266,275,306} six single arm interventional studies^{205,221,246,248,307,308} and ten observational cohort studies^{191,201,218,253,268,280,309-313} (Table 3-2 and Appendix 3-1). Blinding varied with one double-blind study²⁴² and one study using placebo but with unspecified blinding¹⁵² (Appendix 3-2, 3-3). In eight studies, assessors and laboratory staff (n=3)^{192,219,305} or only laboratory staff (e.g. microscopists) (n=5)^{236,237,243,247,303} were blinded.

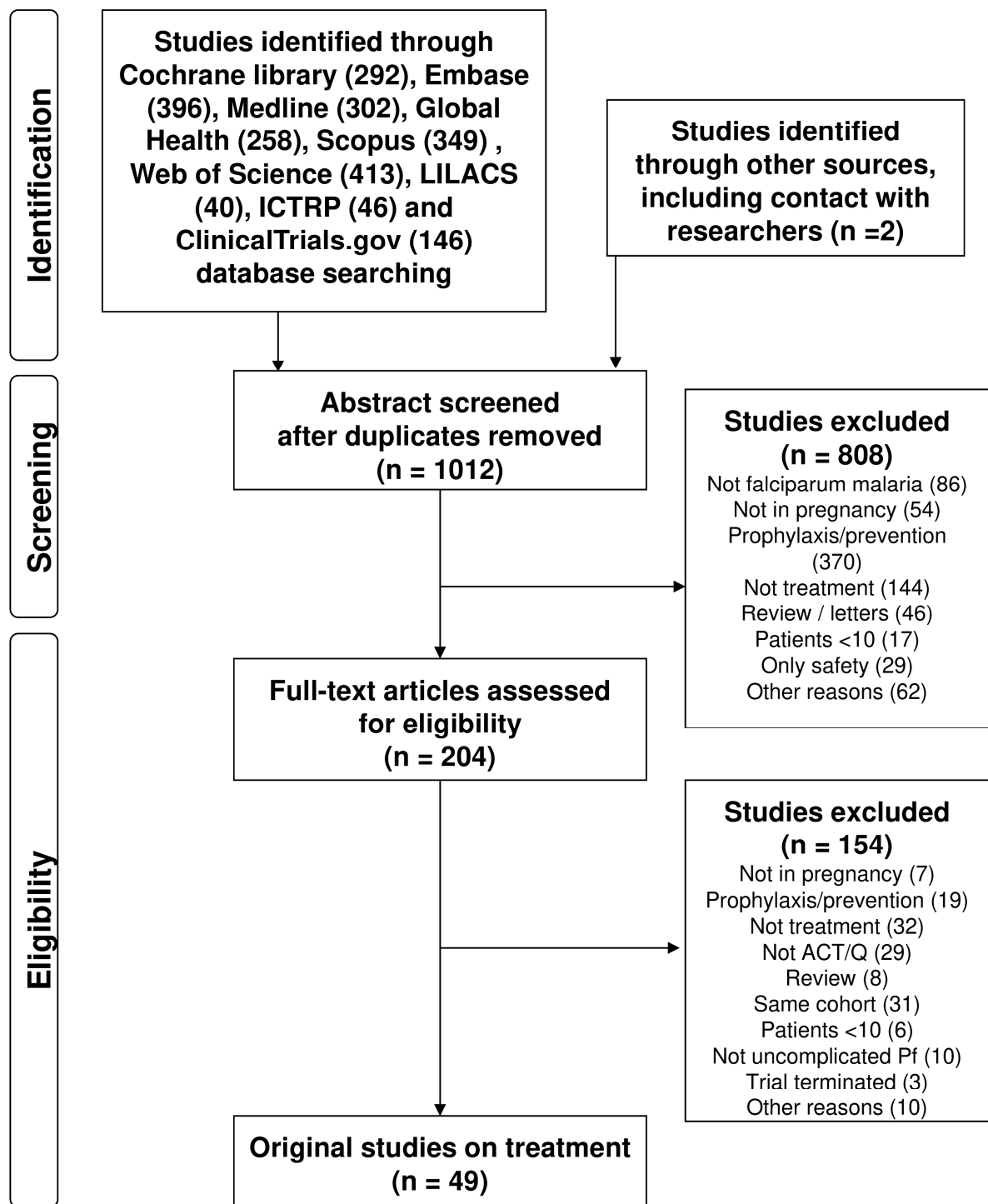


Figure 3-1. Flowchart of the systematic literature search on treatment efficacy of uncomplicated falciparum malaria in pregnancy

Table 3-2. Summary of the methodology of assessing malaria efficacy and its reporting

Name [reference]	Design	Efficacy endpoint (day)	Malaria until delivery	Retreatment (previous drug)	Symptom	Supervision	PCR for recurrence	Efficacy reported	Statistical method for summary	Adjustment of summary result by PCR
Naing, 1988 [302]	RCT	7	INA	Not excluded	Symptomatic	Hospitalised	No	Parasite clearance time	Mean	No PCR
Harinasuta, 1990 [263]	RCT	7	INA	No	INA	INA	No	Parasite clearance	Number	No PCR
Nosten, 1993 [152]	RCT	28	Not followed	Not excluded	Both*	Full	No	Failure	Proportion	No PCR
Sowunmi, 1998 [264]	RCT	14 for AM 28 for AM- MQ	INA	Yes (CHQ/SP)	Symptomatic	Full	No	Cure	Proportion	No PCR
Bounyasong, 2001 [198]	RCT	42	Weekly	No	Symptomatic	Full	No recurrence	Failure	Number	No recurrence
McGready, 2000 [190]	RCT	Delivery or 63	Weekly	Not excluded	Both*	Full	<i>msp-1, msp-2, glurp</i>	Cure	Cumulative success	Corrected only
McGready, 2001a [188]	RCT	42	Weekly	Not excluded	Both*	Full	<i>msp-1, msp-2, glurp</i>	Cure	Cumulative success	Corrected only
McGready, 2005 [194]	RCT	Delivery or 63	Weekly	No	Both*	Full	<i>msp-1, msp-2, glurp</i>	Cure	Cumulative success	Corrected only
Adam, 2004a [193]	RCT	28	INA	Yes (CHQ)	Symptomatic	Full	<i>msp-1, msp-2, glurp</i>	Failure	Proportion	Corrected only
Kalilani, 2007 [303]	RCT	28	Interval not specified	No	Both*	Full	<i>msp-1</i>	Failure	Proportion Survival analysis for comparison	Both corrected and uncorrected
McGready, 2008 [219]	RCT	Delivery or 42	Weekly	28% recrudescence (Q, AS, DP, ASMQ)	Both*	Full	<i>msp-1, msp-2, glurp</i>	Cure	Cumulative success	Corrected only
Mutabingwa, 2009 [247]	RCT	28	At delivery and 42 days postpartum	No	Both*	Full	<i>msp-2</i>	Failure	Proportion	Both corrected and uncorrected
Kaye, 2008 [239]	RCT	28	Not followed	No	Symptomatic	First dose	No late failure	Failure	Proportion	No late failure
Piola, 2010 [192]	RCT	Delivery or 42	Weekly	Not excluded	Both*	Full	<i>msp-1, msp-2, glurp</i>	Cure Failure	Proportion Log-rank test for comparison	Both corrected and uncorrected
Carmona- Fonseca, 2013 [256]	RCT	28	Not followed	No	Symptomatic	INA	No recurrence	Cure	Proportion	No recurrence
D'Alessandro, 2016 [237, 304]	RCT	63	Only at delivery	No	Both*	Full	<i>msp-1, msp-2, glurp</i>	Cure	Proportion Survival analysis for comparison	Both corrected and uncorrected

Table 3-2 continued.

Name [reference]	Design	Efficacy endpoint (day)	Malaria until delivery	Retreatment (previous drug)	Symptom	Supervision	PCR for recurrence	Efficacy reported	Statistical method for summary	Adjustment of summary result by PCR
Osarfo, 2017 [243]	RCT	42	Only at delivery	No	Asymptomatic	First dose	<i>msp-1, msp-2, glurp</i> (Failed)	Cure	Proportion	Uncorrected only
Onyamboko, 2015 [235]	RCT	42	INA	No	INA	Full	INA	Cure	INA	Uncorrected only
Ukah, 2015 [242]	RCT	28	Not followed	No	Symptomatic	INA	No	Cure	Proportion Log-rank test for comparison	No PCR
Iribhogbe, 2017a [238]	RCT	28	Not followed	No	Symptomatic	INA	No	Failure	Proportion	No PCR
Anvikar, 2018 [257]	RCT	63	Only at delivery	No	Both*	Full	<i>msp-1, msp-2, glurp</i>	Cure Failure	Proportion Kaplan-Meier curve	Both corrected and uncorrected
NCT01054248 [236]	RCT	Delivery or 63	Weekly	Not excluded	Both*	Full	Yes	Cure	Cumulative success	Both corrected and uncorrected
NCT02909712 [305]	RCT	28	INA	No	Asymptomatic	Full	Yes	Cure	Proportion	Both
McGready, 2003a [275]	PK study	Delivery or 42	Weekly	Yes (Q)	Both*	Full	<i>msp-1, msp-2, glurp</i>	Cure	Proportion	No recurrence
Adam, 2012 [266]	PK study	63	Weekly (some)	No	INA	Full	Yes	Cure Failure	Number	Corrected only
Onyamboko, 2011 [213]	PK study	28	INA	No	Asymptomatic	Full	No	Failure	Number	No PCR
McGready, 2012 [211]	PK study	Delivery or 63	Weekly	Not excluded	Both*	Full	<i>msp-1, msp-2, glurp</i>	Cure	Cumulative success	Both corrected and uncorrected
Rijken, 2011 [214]	PK study	Delivery or 63	Weekly	Two pregnant or non- pregnant women were self-treated (CHQ)	Symptomatic	Full	<i>msp-1, msp-2, glurp</i>	Cure	Cumulative success	Both corrected and uncorrected
Valea, 2014 [252]	PK study	63	Passively	No	Both*	Full	<i>msp-1, msp-2, glurp</i>	Cure	Proportion	Both corrected and uncorrected
Juma, 2014 [306]	PK study	28	INA	No	Symptomatic	Full	INA	INA	INA	INA
Mosha, 2014 [232]	PK study	42	Not followed	No	Symptomatic	Full	<i>msp-2</i>	Cure Failure	Proportion	Uncorrected only
Nyunt, 2016 [233]	PK study	42	Not followed	No	Symptomatic	INA	<i>msp-1, msp-2</i> and 4 microsatellites	Cure Failure	Proportion	Uncorrected only
Mutagonda, 2017 [230, 231]	PK study	28	Not followed	No	INA	First dose and last dose	<i>msp-2</i>	Cure Failure	Proportion	Both adjusted and unadjusted

Table 3-2 continued.

Name [reference]	Design	Efficacy endpoint (day)	Malaria until delivery	Retreatment (previous drug)	Symptom	Supervision	PCR for recurrence	Efficacy reported	Statistical method for summary	Adjustment of summary result by PCR
Adam, 2004b [205]	single-arm	28	Every two weeks	Yes (CHQ)	Symptomatic	Full	No	Failure	Number	No PCR
Adam, 2004c [221]	single-arm	28	Every two weeks	Yes (CHQ and Q)	Symptomatic	Full	No recurrence	Cure	Proportion	No recurrence
Adegnika, 2005 [307]	single-arm	56	Only at delivery	No	Both*	First dose	No	Cure	Proportion	No PCR
Adam, 2006 [308]	single-arm	28	Every two weeks	No	Symptomatic	Full	No recurrence	Cure	Proportion	No recurrence
Ndiaye, 2011 [246]	single-arm	42	Only at delivery	No	Symptomatic	Full	No recurrence	Cure	Number	No recurrence
Iribhogbe, 2017b [248]	single-arm	28	Not followed	No	Symptomatic	INA	No	Failure	Proportion	No PCR
McGready, 1998a [309]	cohort	42	Weekly	68% retreatment (Q/MQ)	Both*	Full	No	Failure	Cumulative failure	No PCR
McGready, 1998b [253]	cohort	28	Weekly	45% retreatment (Q/MQ)	Both*	INA	No	Failure	Proportion	No PCR
McGready, 2001b [218]	cohort	42	Weekly	58% retreatment (Q/MQ)	Both*	Full	No	Failure	Proportion Cumulative failure	No PCR
Laochan, 2015 [310]	Collation of studies	Delivery	Weekly	Some	Both*	INA	<i>msp-1, msp-2, glurp</i>	Time to recrudescence	geometric mean	Corrected only
McGready, 2002 [189]	cohort	28	Weekly	Some	Both*	Recorded	No	Failure	Proportion	No PCR
McGready, 2003b [278]	cohort	42	Weekly	Yes (Q/QC/AS/AC)	Both*	Full	<i>msp-1, msp-2, glurp</i>	Cure	Proportion	No recurrence
Villegas, 2005 [311]	cohort	INA	INA	INA	Symptomatic	Full	INA	Cure	INA	INA
Rijken, 2008 [268]	cohort	63	Weekly	Yes (Q/QC/AS/AC)	Both*	Full	Yes	Cure	Cumulative success	Corrected only
Rulisa, 2012 [201, 314]	cohort / PK	56	INA	Some	Symptomatic	Only subset in PK study	No	Cure Failure	Proportion	No PCR
Kalilani, 2013 [312, 313]	cohort	Delivery	Every four weeks	Not excluded	Both*	INA	6 microsatellite markers	Failure	Proportion	Both corrected and uncorrected

AS: artesunate, AC: artesunate + clindamycin, CHQ: chloroquine, ITT: intention-to-treat analysis, MQ: mefloquine, N: number of pregnant women included in the study, INA: information not available, PCR: polymerase chain reaction, PK: pharmacokinetic study, PP: per-protocol analysis, Q: quinine, QC: quinine-clindamycin, RCT: randomized controlled trial, SP: sulfadoxine-pyrimethamine.

Efficacy endpoint is the duration of follow-up for the primary endpoint. If pregnant women were followed up for malaria after the primary endpoint, the schedule is indicated in 'Malaria until delivery'.

Name(s) of molecular markers are listed if they were specified.

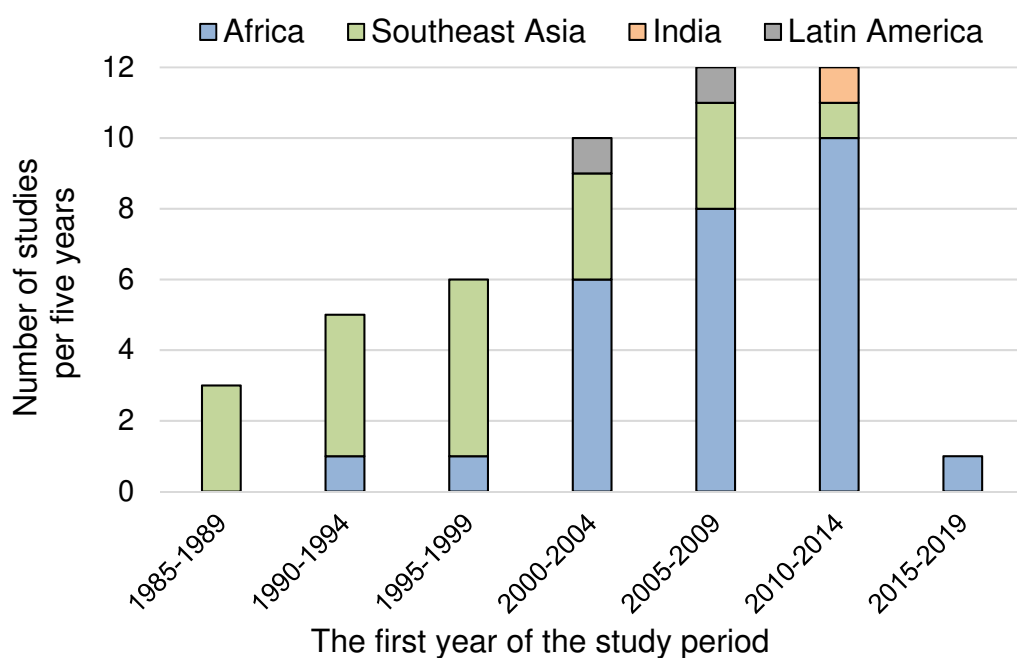


Figure 3-2. Number of studies on treatment efficacy of malaria in pregnancy per five years (1985-2019)

The first year of study period was used for categorization.

Study drugs

Fourteen studies included women treated with QBT, 41 studies included ABT, and six studies included both. Altogether, 6,514 and 1,035 episodes were treated with ABT or QBT, respectively.

Quinine was administered at 30 mg/kg/day for seven days in nine studies (64%, 9/14). The other five studies gave similar dosing with slight variations: one study administered for seven or more days depending on the clinical condition;¹⁹⁸ one study started with intravenous quinine until oral therapy was tolerated;³⁰² and one study compared standard dose with lower dose (20 mg/kg/day) for seven days;¹⁹³ one study administered 30 mg/kg/day for five days;¹⁵² one study gave 1,800 mg/day for seven days.²⁶³ Artesunate was given at 10–

16 mg/kg/course (600–900 mg/course) over 3–7 days except in two PK studies: one PK study administered 200 mg of artesunate once;²¹³ and the other PK study administered total 28 mg/kg over seven days (one day intravenously and six days orally).²¹¹ ASAQ was given as a fixed dose combination in four studies (57%, 4/7) and as a non-fixed dose combination with 30 mg/kg of amodiaquine in three studies (43%, 3/7). ASMQ was given as a fixed dose combination in four studies (36%, 4/11) and as a non-fixed dose combination with 25 mg/kg of mefloquine in six studies (55%, 6/11). Non-fixed dose combination of an unknown dose was assumed to be used in one study.³¹¹ AL was administered for three days (88%, 14/16) except two studies: one for four days²³⁶ and the other for five days.²³⁵ DP was given as fixed-dose combination in all seven studies.

The treatment administration was supervised fully in 33 studies and partially in 6 studies (Table 3-2).^{191,201,230,239,243,307} The treatment administration was not clearly reported in ten studies.^{233,238,242,246,248,253,256,263,310,312} In 16 studies with AL, eight studies co-administered fat (i.e. milk) to increase the bioavailability^{153,192,219,230,233,235,236,314} and one study recommended patients to take high fat food.²³⁷ The other seven studies with AL did not specify the concurrent fat intake.

Five RCTs compared QBT and ABT but only two of them used the same ABT (non-fixed dose combination ASMQ).^{188,190,192,194,198} Ten RCTs compared different combinations of ABT (Appendix 3-4).^{219,235-238,242,243,255-257} Two RCTs compared different regimens of QBT,^{152,193} two compared quinine versus non-ABTs^{263,302} and four compared ABT versus non-

ABT (e.g. SP or chlorproguanil-dapsone).^{239,247,303,305}

Inclusion criteria

The baseline characteristics of patients are summarized in Appendix 3-5.

Asymptomatic patients were included in 57% (13/23) of RCTs and 50% (13/26) of non-RCTs.

Three of them included only asymptomatic women.^{213,243,305} Fifteen studies (31%, 15/48)

intentionally included patients who failed previous treatment, but only three of them were

RCTs.^{193,219,255}

Overall, twelve studies (25%, 12/48) included first trimester women, describing at

least 599 parasitologically confirmed first-trimester malaria episodes treated with ABT

(n=108) or QBT (n=491). Only one RCT intentionally included first trimester women; it was

published in 1990 and compared quinine versus mefloquine.²⁶³

In 27 studies from Africa, three studies gave two doses of IPTp-SP,^{233,242,252} and one study gave three doses.³¹² One study reported the percentage of women who received IPTp.²⁰¹

IPTp-SP was not administered during the whole pregnancy in one study¹⁹² and during the

study period in two other studies.^{237,243} In one study, enrolled patients systematically received

the study drug four weeks after the initial treatment instead of IPTp-SP.³⁰³ In other 18 studies,

information was not provided.

Assessment of treatment efficacy

Duration of follow-up

The primary endpoint for assessing antimalarial efficacy varied: less than 28 days (n=2), day 28 (n=19), day 42 (n=10), day 56 (n=2), day 63 (n=5) and until delivery (n=10) (Table 3-2). The duration of follow-up was unclear for one conference abstract.³¹¹ After the primary endpoint of 28–63 days, a further 14 studies continued assessing parasitaemia until delivery but with different schedules and five other studies did not continue but assessed parasitaemia only at delivery.

Methodology of reporting efficacy

For handling treatment failure, majority of the studies (96%, 47/49) followed WHO standards for non-pregnant populations, except two studies that excluded cases who developed severe malaria after the treatment (i.e. early treatment failure) from the final result.^{190,239}

Twenty-seven studies (55%, 27/49) showed only the proportional ACPR or the number of patients with ACPR (or failure). Ten studies derived cumulative success (or failure) by survival analysis instead of or in addition to proportional ACPR.^{188,190,194,211,214,218,219,236,268,309} Six other studies used survival analysis to compare the different groups, but the proportional ACPR was presented as the point estimates.^{192,231,237,242,257,303} Two studies did not report ACPR but reported time to parasite

clearance based on daily parasitaemia measurements³⁰² or time to recurrence.³¹⁰ Information was not available from five unpublished studies.

PCR was used in 25 studies (51%, 25/49) for differentiating recurrences (Table 3-2), although in one of the studies PCR results were considered not to be reliable because of technical problems.²⁴³ Fourteen studies (29%, 14/49) used three standard molecular markers (i.e. *msp-1*, *msp-2* and *glurp*). Three of the studies adopted sequential genotyping strategy,^{192,237,252} while the remaining ten studies ran all three markers for all recurrences. Four studies (8%, 4/48) used one of the three markers.^{231,232,247,303} Two studies (4%, 2/48) used six microsatellite markers.^{233,313} Two published studies and two registered trials (8%, 4/49) did not specify the details of molecular markers.^{236,266,268,305} An additional six studies (12%, 6/49) did not observe any late treatment failure,^{198,221,239,246,256,308} so a total of 30 (61%, 30/49) studies contained adequate information to obtain PCR-corrected results.

There was substantial heterogeneity in how reinfections and indeterminate PCR results were handled in deriving proportional ACPR. WHO recommends excluding these cases from proportional ACPR calculations in non-pregnant populations.¹⁷⁴ Reinfection was excluded except in three studies in which reinfections were included as treatment success.^{237,257,303} One study did not clearly report whether there were any reinfections.¹⁹³ Two studies excluded indeterminate outcomes from the calculation,^{192,237} and one of them also presented results with imputation for indeterminate cases for reference.²³⁷ Two studies using survival analysis regarded indeterminate results as recrudescence as the worst-case

scenario.^{190,268} One study did not explain how they dealt with missing PCR results.²⁴⁷ For proportional ACPR, no studies clearly excluded patients infected with other species of malaria before recurrence of falciparum, as recommended by the WHO non-pregnant guideline. Only one study presented results of a model that censored patients infected with *P. vivax* before recurrence of *P. falciparum*, and this result was provided as additional information and not the primary analysis (that did not account for *P. vivax* infection).²¹⁹

Fever and parasite clearance

Fever was assessed daily or more frequently in 28 studies (57%, 28/49) and time to fever clearance was reported in 12 studies (24%, 12/49). Prevalence of clearance of fever at fixed points (on day 2, 3 or 4) was reported in other 12 studies (24%, 12/49). Blood smear was assessed for parasitaemia daily or more frequently at least for the first three days in 32 studies (65%, 32/49) and time to parasite clearance was reported in 16 studies (33%, 16/49). The proportion of women who cleared parasitaemia at fixed points (i.e. by 12 hours, on day 1, 2 or 3) was reported in another 15 studies (31%, 15/49).

The variability of reporting parasite and fever clearance limited comparisons across studies. In five RCTs comparing ABT and QBT, three and five RCTs reported the comparison of fever clearance and parasite clearance, respectively. One RCT reported that the mean time to fever clearance was significantly shorter by ASMQ than quinine (4.47 days vs 8.04 days, $p<0.001$), although this time was from the appearance of fever according to the patients' history rather than from the treatment.¹⁹⁸ Another two studies did not report a difference in the

proportion of febrile patients or the proportion of fever clearance by day 2 or 3.^{192,194} On the other hand, all five RCTs reported faster parasite clearance by ABT than by QBT. The mean or median time to parasite clearance was shorter by ABT than by quinine in two RCTs.^{194,198} Similarly, the proportion of parasite clearance by 48 hours^{188,190} and negative parasitaemia on day 2¹⁹² were higher with ABT than QBT, although this difference was not observed on day 3.¹⁹² Between different ACTs, lower parasite clearance on day 1 was reported with AL than ASAQ, ASMQ and DP, although this was not significant on day 2.²³⁷ Other two RCTs also showed no difference in parasite clearance on day 3 between AL and ASAQ.^{238,242} The median or mean time to fever and parasite clearance were not different between artesunate monotherapy and AL,²¹⁹ and between intramuscular artemether with or without mefloquine, respectively.²⁵⁵

Summary of reported ACPR

PCR-corrected treatment failure of $\geq 10\%$ at any time points was observed in 3 (9%, 3/32) ABT arms and 3 (43%, 3/7) QBT arms (Figure 3-3). On day 28, two study arms had PCR-corrected treatment failure of $\geq 10\%$. One of them used a lower dose quinine (10 mg/kg twice daily rather than thrice daily).¹⁹³ In the other study that reported the low efficacy of AL, 33% of the enrolled patients were under retreatment, having failed previous treatment.²¹⁹ If retreatments were excluded, the cumulative success of AL on day 42 was $>90\%$.²¹⁹ Other study arms with PCR-corrected treatment failure of $\geq 10\%$ were observed on day 63 (one artesunate monotherapy arm²¹¹ and one quinine monotherapy arm¹⁹⁰) and until delivery (one

quinine arm¹⁹⁴ and one AL arm³¹³). The funnel plots for PCR-corrected ACPR on ABT and QBT did not show apparent asymmetry (Appendix 3-6).

PCR-uncorrected ACPR was available in 14 QBT arms and 49 ABT arms (Appendix 3-7). PCR-uncorrected ACPR at different time points was available in some studies, and if it was not reported on day 28 but was >80% on day 42, 63 or at delivery, it was assumed to be >80% on day 28. Although different levels of the risk of reinfection depending on the time and location complicate comparison between studies, at least 50% (7/14) of QBT arms showed <80% protection within 28 days while at least 80% (39/49) of ABT arms showed >80% protection within 28 days. Two ABT arms reporting $\leq 80\%$ protection within 28 days were both conducted in high endemic areas and used AL or ASSP.^{248,303} PCR-uncorrected ACPR on day 28 was not available but it was $\leq 80\%$ after day 28 in other eight arms from six studies.^{211,218,219,237,268,309} ABT went on to provide protection in >80% of patients at 42 days in at least 75% (24/32) of the study arms that followed for 42 days or longer.

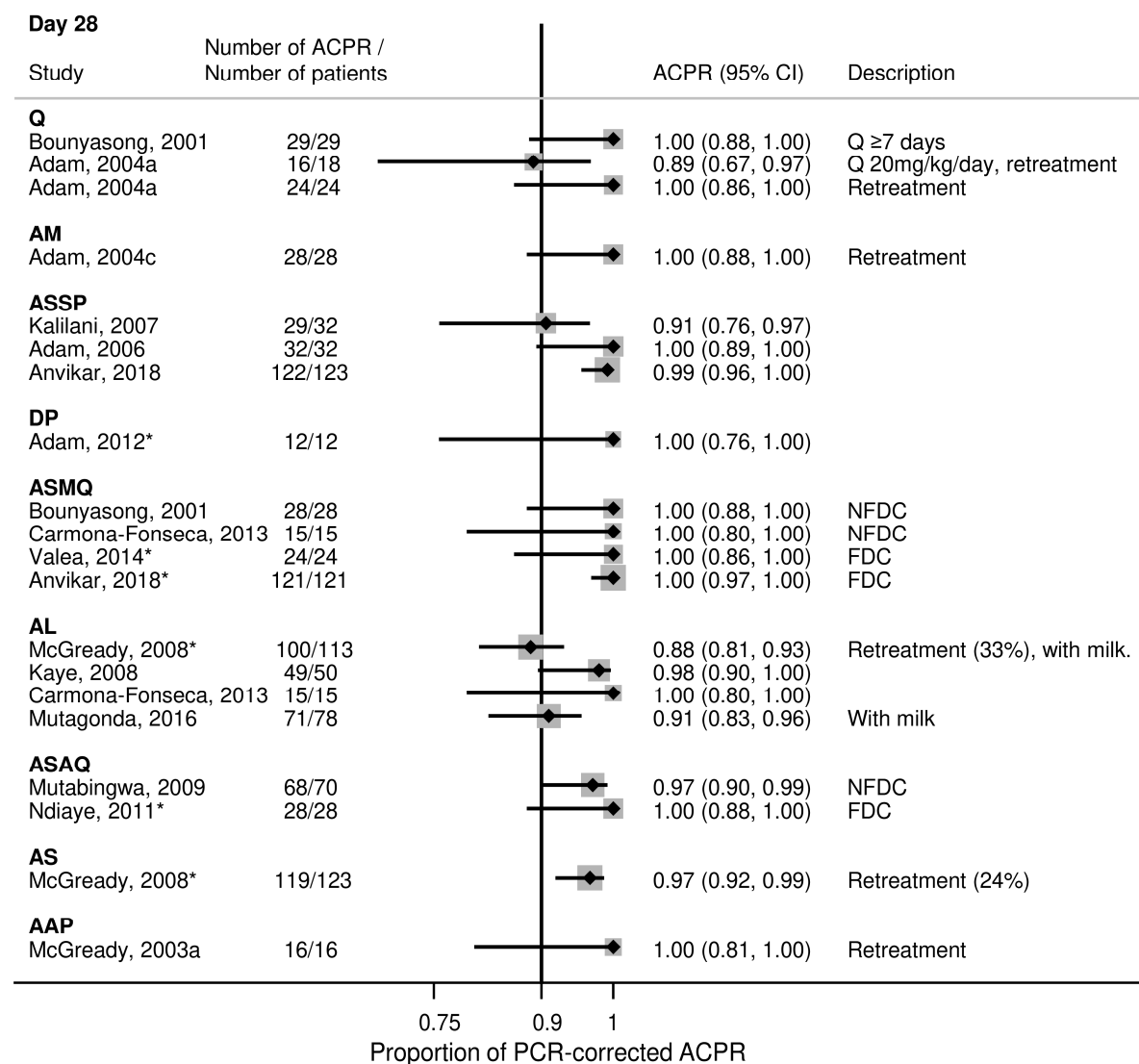


Figure 3-3. PCR-corrected proportional appropriate adequate clinical and parasitological response (ACPR) for each study

ACPR are shown by treatment group and the duration of follow-up (i.e. day 28, 42, 63 and at delivery) with 95% confidence intervals. *: ACPR at later follow-up day is available. AAP: artesunate + atovaquone-proguanil, AC: artesunate + clindamycin, AL: artemether-lumefantrine, AM: artemether, AS: artesunate, AQ: amodiaquine, DP: dihydroartemisinin-piperaquine, FDC: fixed dose combination, MQ: mefloquine, NFDC: non-fixed dose combination, Q: quinine, QC: quinine + clindamycin, SP: sulfadoxine-pyrimethamine.

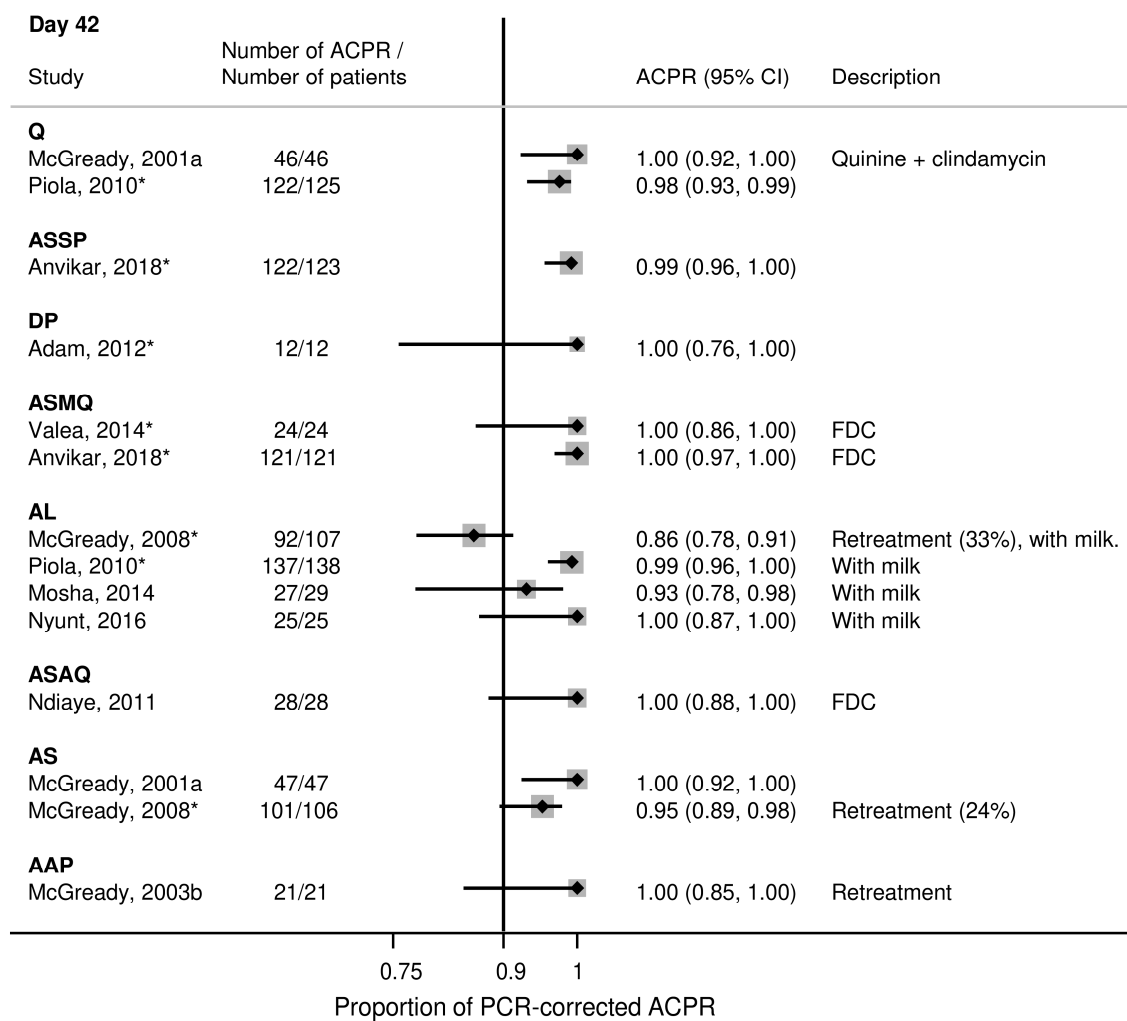


Figure 3-3 continued.

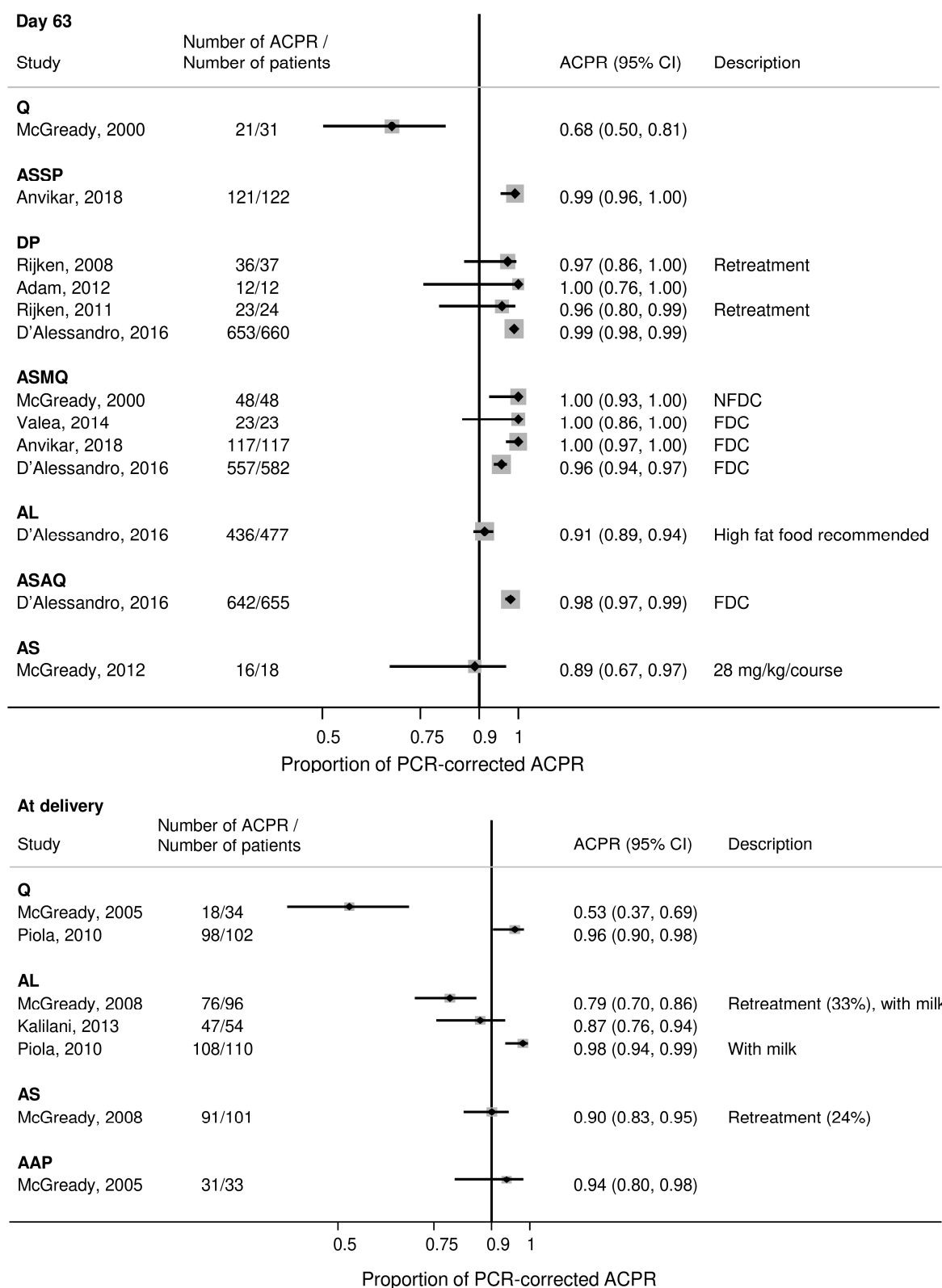


Figure 3-3 continued.

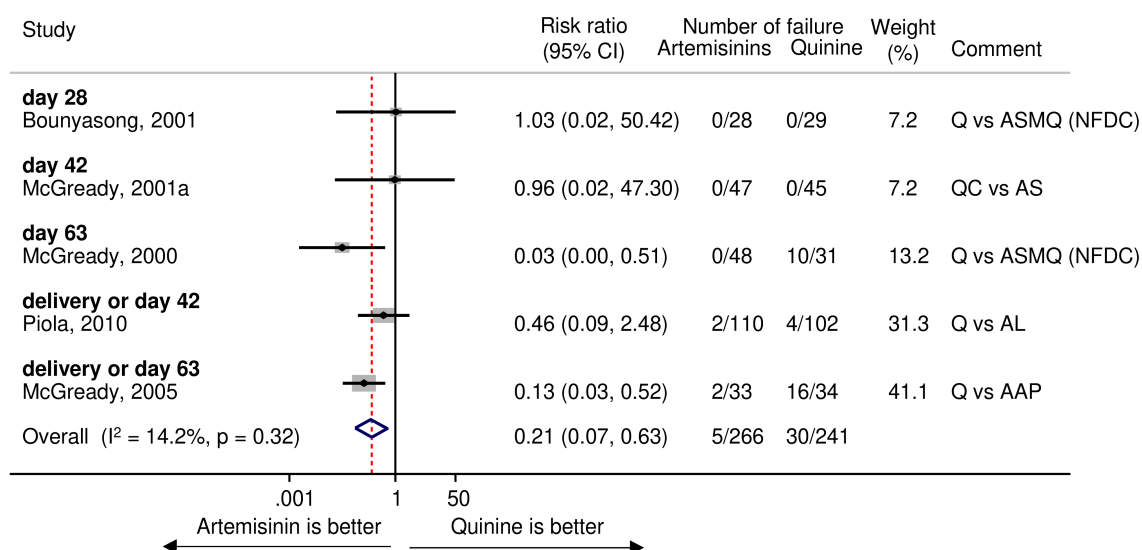


Figure 3-4. Meta-analysis of risk of PCR-corrected treatment failure comparing quinine-based treatment and artemisinin-based treatment

The outcome of longest duration of follow-up was used. Random effects model was used for meta-analysis. Continuity correction was made for two studies without treatment failure by adding 0.5. AAP: artesunate-atovaquone-proguanil, AL: artemether-lumefantrine, AS: artesunate monotherapy, ASMQ: artesunate-mefloquine, CI: confidence interval, Q: quinine, QC: quinine-clindamycin, NFDC: non-fixed dose combination.

PCR-corrected ACPR comparing different treatments

Overall, the risk of treatment failure in the five RCTs available for meta-analysis was significantly lower in patients treated with ABT than QBT (risk ratio [RR] 0.21, 95% confidence interval [CI] 0.07 to 0.63) (Figure 3-4), although the compared treatments and methodologies differed. This increased risk of failure with QBT was prominent when the patients were followed up longer. There was no evidence for asymmetry of the funnel plot suggesting publication bias ($p=0.7$) (Appendix 3-8). None of these five RCTs included pregnant women in the first trimester.

The limited number of available studies with PCR-corrected outcome comparing different ABTs precluded aggregated data meta-analysis. One RCT reported that PCR-

corrected ACPR on day 63 was lower for AL than DP, ASAQ and ASMQ, although the absolute difference was <5%.²³⁷ Lower efficacy of AL than artesunate monotherapy (AS) for seven days was also reported when used for treating recrudescence infections in pregnancy.²¹⁹

Time to recrudescence

Recrudescence in pregnancy occurred after day 28 or 42, the fixed follow-up periods recommended by WHO for non-pregnant populations. In one study, one-third of the recrudescence following AL or artesunate monotherapy occurred after day 42,²¹⁹ and four studies reported recrudescence after 100 days with a maximum of 138 days.^{192,194,303,310} These long intervals were reported from both sub-Saharan Africa and Southeast Asia.

Risk factors for recrudescence in pregnancy

Baseline characteristics were assessed as risk factors for parasite clearance (n=1),³⁰² recrudescence (n=2)^{232,304} and recurrence (n=3).^{230,243,307} One study reported gestation (early second trimester) to be associated with faster parasite clearance than late second trimester but not fever clearance.³⁰² Gravidity was not associated with parasite or fever clearance.³⁰² For recrudescence, higher baseline parasitaemia and younger maternal age were associated with higher risk in one study,³⁰⁴ while the other study did not find significant association with parasitaemia.²³² Gestational age (n=2),^{232,304} gravidity (n=1),³⁰⁴ haemoglobin concentration (n=1),³⁰⁴ body mass index (n=1)²³² and symptomatic infection (n=1)³⁰⁴ were not significant risk factors for recrudescence. For recurrence, higher baseline parasitaemia,³⁰⁷ younger age³⁰⁷

and lower haemoglobin concentration²³⁰ each were associated with higher risk in one of the studies, while the other two studies did not show the associations with parasitaemia,^{230,243} age^{230,243} or baseline haemoglobin concentration.^{243,307} Lower body weight was shown to be associated with higher risk of recurrence after quinine monotherapy.³⁰⁷ Gestational age (n=3),^{230,243,307} gravidity (n=3),^{230,243,307} parity (n=1)³⁰⁷ and body temperature (n=1)³⁰⁷ were not associated with the risk of recurrence.

The risk of recrudescence was higher when the drugs were used for retreatment (artemisinins²¹⁸ and quinine²⁵³) or recrudescence (AL)²¹⁹ than for novel infection.

Summary of gametocyte carriage

Gametocytes were assessed in 17 studies (35%, 17/49). ABT was more effective in clearing and also suppressing the development of gametocytaemia than non-ABT. AL showed better gametocyte clearance on day 2 and day 7 than quinine.¹⁹² The proportion of gametocytaemia development after treatment was lower in artesunate-atovaquone-proguanil (AAP) than in quinine (5% and 29%, respectively)¹⁹⁴ and the prevalence of gametocytes on day 14 was lower in ASAQ than in SP (5% and 31%, respectively).²⁴⁷

Gametocyte carriage defined by the number of weeks with positive gametocytaemia divided by the total number of weeks of follow-up was 2–26.8/1000 person-week in ABT and 39–92.7/1000 person-week in QBT in studies conducted on the Thailand-Myanmar border (Table 3-3). This difference was significant in three RCTs directly comparing ABT and

Table 3-3. Gametocyte carriage following the quinine-based and artemisinin-based treatments

Study	Treatment	Subgroup	Gametocyte carriage (person-week/1000)
McGready, 1998b	Q	Retreatment	92.7 (95%CI 57.4–128)
McGready, 1998b	Q	Primary treatment	79.8 (95%CI 51.5–108.1)
McGready, 2000	Q		46.9 (95%CI 26–78)
McGready, 2002	Q		42.5 (95%CI 27.8–62.1)
McGready, 2005	Q		49 (95%CI 26–89)
McGready, 2001a	QC		39 (95%CI 21–66)
McGready, 2001b	Artemisinins	Retreatment	26.8 (95%CI 18.5–37.4)
Rijken, 2008	DP		11.7 (95%CI 3.0–36.7)
McGready, 2001b	Artemisinins	Primary treatment	11.4 (95%CI 5.5–20.8)
McGready, 2008	AS		10 (95%CI 4–21)
McGready, 2005	AAP		6 (95%CI 1–25)
McGready, 2001a	AS		3 (95%CI 0–19)
McGready, 2000	ASMQ		2.3 (95%CI 0–11)
McGready, 2008	AL		2 (95%CI 0–10)

AAP: artesunate-atovaquone-proguanil, AL: artemether-lumefantrine, AS: artesunate, CI: confidence interval, DP: dihydroartemisinin-piperazine, MQ: mefloquine, Q: quinine, QC: quinine-clindamycin.

QBT.^{188,190,194} Gametocyte carriage was not different between the different ABT in two

RCTs.^{219,237}

Placental malaria

Placental malaria was assessed in 15 studies (31%, 15/49) in various ways (Appendix 3-9). Placental histopathology is the current best practice and was examined in at least six studies,^{192,198,236,237,303,312} but the reporting and interpretation of the results varied with at least four different definitions.³¹⁵⁻³¹⁸ The prevalence of placental malaria after treatment ranged from <10%^{192,214,219,280} to 44.8%,³⁰³ and was not different among different ABTs^{237,243} or between AL and quinine.¹⁹²

Congenital malaria

The peripheral blood of the newborn was assessed for malaria in six studies, of which three assessed all newborns systematically at delivery and reported the results.^{192,219,255}

Another two studies reported congenital malaria as a possible reason for neonatal deaths but did not report whether they tested for congenital malaria systematically.^{190,194} One further study assessed congenital malaria but did not report the results.²⁵² No studies specified further parasitological monitoring of infants for congenital malaria diagnosed after the perinatal period.

Treatment interruption and acute adverse events

Treatment interruption before the completion of the treatment caused by adverse reaction or withdrawal of consent was reported in six out of 32 studies with full supervision (19%, 6/32). Two RCTs reported the proportion of patients whose treatment was interrupted during ABT and QBT. The proportion of treatment interruption was 0% (0/64) with AS for seven days and 3% (2/67) with QC for seven days;¹⁸⁸ and 0% (0/152) with AL for three days and 3% (4/152) with quinine monotherapy for seven days.¹⁹² Treatment disruption during QBT for seven days was 18% (9/51) in another study.¹⁹³ Treatment disruption during ABT was reported in three other studies: 0.8% (1/128 with AS for seven days),²¹⁹ 0% (0/125 with AL),²¹⁹ 3% (1/35 with AL)²³² and 3% (1/30 with ASAQ)²⁴⁶.

To compare the acute adverse events of ABT and QBT, the results from five RCTs directly comparing them were reviewed. Meta-analysis of five RCTs comparing ABT versus QBT revealed that hypoglycaemia (RR 6.67, 95% CI 2.27 to 20.15, one RCT), tinnitus (pooled RR 4.70, 95% CI 1.20 to 18.39, five RCTs), vomiting (pooled RR 2.01, 95% CI 1.23 to 3.30, five RCTs) and dizziness (pooled RR 1.51, 95% CI 1.02 to 2.25, three RCTs) were

more frequently reported in QBT than in ABT. Tinnitus was reported in 35–80% of the women treated with QBT^{188,190-194,198,253} and in 0–30% of the women treated with ABT^{188,190,192,194,218,219,243,248,280} except one study reporting 64% for ASMQ.¹⁹⁸

3-II.4 Discussion

This review revealed the variability of study design, drugs, treatment regimens for the same drug, inclusion criteria, determination of parasitological efficacy, follow-up duration and detection of parasites in mother, placenta and newborn at delivery. The design of studies to assess the efficacy of antimalarials during pregnancy is not standardized, and investigators are using varied adapted versions of the protocol for the non-pregnant populations currently recommended by WHO.¹⁷⁴

The efficacy of ABT was generally satisfactory with ACPR of >90%, although aggregated efficacy results are limited by heterogeneous methods as well as differences in patient immunity, background, symptoms, time and study site. If aggregated results are used, only a maximum of two RCTs was available for each comparison. Slightly lower efficacy of AL than other ABTs was reported in two studies,^{219,237} and this may be explained by differences in the pharmacokinetics and pharmacodynamics of lumefantrine in pregnant women.^{187,192,208,219,228-230,233} On the Thailand-Myanmar border, the proportion of PCR-corrected ACPR of AL was markedly low in a study of pregnant women (<85%)²¹⁹ and not comparable to that (>95%) reported from studies in non-pregnant populations.^{319,320} Monitoring of efficacy in pregnancy remains a useful assessment in its own right. Optimal

dosing for pregnant women should be sought. Extending treatment course can also be an option to improve the efficacy.³²¹ The results of two studies assessing longer duration and higher doses of AL^{235,236} are awaited.

The available data implied QBT has two major but anticipated drawbacks compared with ABT: lower treatment efficacy and lower adherence. In at least 50% (7/14) of the study arms, one in five pregnant women or more suffered another episode of malaria parasitaemia (either recrudescence of reinfection) within 28 days after treatment by quinine. Although these studies were conducted mostly in Southeast Asia, the risk of recurrence of malaria is expected to be even higher in the high endemic areas in sub-Saharan Africa considering the short half-life of quinine. This finding is important as repetitive malaria infections during pregnancy increase adverse effects of miscarriage, SGA and PTB cumulatively.^{31,32} Adherence to a 7-day treatment with QBT outside of a clinical trial context is difficult for pregnant women because of the common side effect of tinnitus, which is particularly intolerable in the first four months of pregnancy when morning sickness peaks.¹⁵² Adding clindamycin to quinine might provide satisfactory efficacy equivalent to ABT, but it will not overcome the poor adherence.

Several components of efficacy evaluation should be standardized for future studies. Duration of the follow-up period needs to be optimized for pregnant women considering physiological variations related to pregnancy:^{112,151,190,310} placenta sequestration;²⁶ different drug metabolism and distribution;^{150,158} and altered immunity profile.²⁶ The current WHO recommendation of follow-up for 28–42 days¹⁷⁴ is based on the fact that the proportion of

recrudescence after 28–42 days is negligible in the non-pregnant populations.³²² However, recrudescence in pregnancy was relatively commonly observed after 42 days, demonstrating the need for prolonged follow-up in pregnancy studies. In the small number of studies that have collected relevant data, recrudescence even after 100 days has been reported in pregnant women both in Africa^{192,303,323} and Southeast Asia.^{194,310} Optimal duration of follow-up in pregnancy will be explored in Chapter 5. The duration of follow-up after delivery also needs to be considered when delivery occurs before the end of the recommended follow-up.

Two problems were encountered with proportional ACPR for pregnancy studies. The lack of a standard led to inconsistent handling of reinfections in proportional ACPR calculations making comparisons across studies difficult. In non-pregnant populations, WHO recommends excluding those with reinfection or indeterminate PCR results from the calculation of PCR-corrected proportional ACPR.¹⁷⁴ However, higher proportions of reinfection and lost-to-follow-up are anticipated if longer follow-up is recommended for pregnant women. If proportional ACPR is calculated, much information will be lost. Survival analysis with censorship of these cases is likely to be a preferable approach for pregnancy studies.³²⁴

Data on efficacy in the first trimester are limited to information from observational studies, precluding comparison of the outcomes using aggregated results. In addition, the published results were rarely presented separately by trimester, and the methods and quality of gestational age assessment were limited.³²⁵

Finally, and importantly, it needs to be emphasized that pregnant women may experience several episodes of malaria during pregnancy. The relationship between placental malaria, which is a direct consequence of malaria infection, and treatment outcomes for each malaria episode is not fully understood and may be heavily biased by gestational age at infection.³²⁶ In this context, PCR-uncorrected ACPR, in which the half-life of drugs will play a key role, becomes an important indicator for choosing treatment options especially in high endemic settings.³⁰⁴ Efficacy cannot be discussed separately from pregnancy outcomes as drugs may affect the fetus. Ideally, the safety of drugs should be assessed and reported within efficacy studies, using a standard that will be reviewed in the next chapter.²⁸⁸ Inclusion rather than exclusion of pregnant women into routine therapeutic efficacy studies could be a key change to address the paucity of data in the future when drugs have a clean teratogenicity profile. This would also permit comparison of efficacy and drug levels in pregnant and non-pregnant females. Recommendations specific to the determination of antimalarial efficacy of uncomplicated *P. falciparum* infection in pregnancy beyond the WHO guideline for non-pregnant populations are summarized in Chapter 9.

The assessment of antimalarial efficacy presented here is limited by the paucity of data, the heterogeneity of studies, and the constraints of aggregated data meta-analysis. With no new RCTs on efficacy in pregnancy notified in trial registries, the treatment episodes in the cohort of studies described here will be the only data available in the near future to describe the efficacy of antimalarials in pregnancy. Because of the paucity of studies, different

methodologies and backgrounds (regional malaria transmission level, seasonality of infection) of patients, conventional meta-analysis using aggregated data will not allow an optimal assessment of the findings. Reviews attempting aggregated data meta-analysis, including the analyses in this chapter, should be interpreted with caution considering the different endpoints and drugs used. In addition, RCTs comparing ABT and QBT were mostly conducted in Southeast Asia. Though the conclusion of the comparison between ABT and QBT is unlikely to change considering the large magnitude of the effect, assessing smaller differences between ABTs would be valuable and cannot be effectively done by aggregated data meta-analysis. Dose-response is also not able to be assessed in aggregated data meta-analysis. IPD meta-analyses offer a better methodological approach to summarize the currently available data, integrating the data from non-comparative studies and assessing outcomes that are not reported in the original studies such as dose-response. An IPD meta-analysis was conducted to overcome these methodological difficulties and will be presented in Chapters 4 and 6.

3-II.5 Conclusions

Although this and other reviews of ABTs and QBTs for malaria in pregnancy suggest ABTs are superior, further aggregated meta-analysis was hampered by inconsistencies in measurement and reporting. A standard framework for antimalarial efficacy studies in pregnancy is warranted and will be a foundation for research with more comparable and reliable outputs.

Chapter 3-III. Methodology of assessment and reporting of safety in antimalarial treatment efficacy studies of uncomplicated falciparum malaria in pregnancy: a systematic literature review

Part of this chapter has been published in a peer-reviewed journal and is freely available as follows under the Creative Commons Attribution licence. Information included in this chapter was updated after the publication. Saito M, Gilder ME, Nosten F, Guérin PJ, McGready R. Methodology of assessment and reporting of safety in anti-malarial treatment efficacy studies of uncomplicated falciparum malaria in pregnancy: a systematic literature review. *Malar J.* 2017;16:491.

3-III.1 Introduction

Safety reporting of new antimalarials in pregnancy has not kept pace with release of new drugs in the face of resistance for the non-pregnant populations. Artemisinins are a particular case in point, as rodent and monkey studies consistently reported fetal resorption (embryotoxicity) and congenital abnormalities (teratogenicity). Fetal resorption by artemisinins was considered to be mediated by the depletion of embryonic erythroblasts.¹⁴⁵ For fetal resorption, an added concern was that the level at which adverse effects were seen was very close to the therapeutic range used in humans.¹⁴⁵ The ‘no observed’ adverse effect level for artemisinin was at 4 mg/kg/day in monkeys although these adverse events were reported at much longer gestational exposure times than typically given for treatment of malaria (e.g. 12–30 gestational days in monkey studies versus 3–7 days for malaria treatment).^{145-149,185} Similarly, reticulocytes were reported to be decreased in humans at therapeutic doses, even though human reticulocytes were shown to be less sensitive to artemisinin than embryonic erythroblasts in animals.¹⁴⁵ Major congenital abnormalities were observed in the cynomolgus monkey studies including skeletal (e.g. shortening of the long bones) and cardiovascular malformations.¹⁴⁹ These concerning findings in animal studies emphasize the importance of assessment and reporting of fetal loss, infant cardiac assessment, and infant length following treatment in humans.

Safety of antimalarials in non-pregnant participants suggested by WHO or the Clinical Data Interchange Standards Consortium includes assessment of symptoms, complete

blood count, blood biochemistry, urinalysis, electrocardiogram (ECG) and serious adverse events (deaths, hospitalizations and disability).^{174,327} In addition to these general assessments, specific safety outcomes such as miscarriage, stillbirth, congenital abnormality, PTB and SGA are important following antimalarial treatment in pregnancy. The safety of antimalarials during human pregnancy has been assessed across continents and in different types of clinical studies.^{33,150,151,185,207,293,295,328}

The prospective nature of efficacy studies provides a framework for capturing safety data, although the detailed assessment and reporting of safety may not be the main interest. Pathological confirmation of the infection parameters, particularly the parasitological confirmation of malaria parasites, provides an advantage in that characteristics of the disease can be considered in addition to drug treatment. While animal studies raised important concerns, they may have overestimated the risk for humans^{32,33,199} so information from every available source should be utilized to clarify clinical safety of antimalarial drugs in pregnancy.

This chapter reviewed the methodology of safety assessment and reporting in studies assessing efficacy of ABT or QBT in pregnancy, with a view to developing a guideline for systematic use in future pregnancy studies.

3-III.2 Methods

The systematic literature review on the efficacy studies in pregnancy was conducted as described in the previous chapter. After screening, the following data were extracted: demographic information of study (year, country, study design, study drugs and eligibility

criteria), reporting of outcome assessment and definition (pregnancy outcomes and other safety outcomes), reporting of risk factors for the pregnancy outcomes, and the methodology of assessment of variables (method of estimating gestational age and anthropometric measurements). Maternal death, laboratory changes including maternal anaemia, fetal loss, preterm birth, anthropometric measurements, congenital abnormality and neonatal death were reviewed.

Definitions for miscarriage (spontaneous abortion), stillbirth, PTB, LBW, neonatal death were summarized across the studies.

3-III.3 Results

A total of 49 studies assessing treatment efficacy of ABT or QBT for uncomplicated falciparum malaria in pregnancy were retrieved (Appendix 3-10). Altogether, 7,372 women with confirmed falciparum malaria were enrolled in those trials, 6,408 and 964 participants were treated with ABT or QBT, respectively. Forty-two were published, six presented at conferences and one registered but not yet published.

There were 23 RCTs comparing two or more treatment regimens,^{152,188,190,192-194,198,219,235-239,242,243,247,255-257,263,302-305} ten PK studies including clinical outcome assessment,^{211,213,214,230-233,252,266,275,306} six single arm interventional studies^{205,221,246,248,307,308} and ten observational cohort studies^{191,201,218,253,268,280,309-313} (Appendix 3-10).

Obstetric care and characteristics of participants

Gestational age

Thirty-three studies (67%, 33/49) reported estimated gestational at the time of the malaria episode in weeks (n=32) or in months (n=1).²⁴⁷ Nine studies (18%, 9/49) did not report gestational age but reported the trimester of the malaria episode.^{231,237,239,252,256,263,302,309,312} One RCT did not report gestational age or trimester,²⁵⁵ and information was not available from six unpublished studies.^{235,236,246,305,306,311}

Thirty-four studies (69%, 34/49) reported the method for estimating gestational age (Appendix 3-10). Among them, ultrasound was used for at least some women in 21 studies (43%, 21/49). Newborn examination for gestational age estimation was used in another 12 studies (24%, 12/49) using the Dubowitz (18%, 9/49) or Ballard score (6%, 3/49). Last menstrual period and symphysis fundal height were the only methods of estimating gestational age in two studies (4%, 2/49). Quality control of gestational age estimation was mentioned in two studies.^{214,237}

Parity and gravidity

Seventeen studies (35%, 17/49) reported parity, nine (18%, 9/49) reported gravidity and 14 (29%, 14/49) reported both (Appendix 3-10). Only three published studies (6%, 3/49) did not report either. Information was not available from six unpublished studies.^{235,236,246,305,306,311}

Antenatal follow-up after malaria efficacy assessment

Thirty-eight studies (78%, 38/49) followed participants until pregnancy outcome (Appendix 3-10). Ten studies did not specify the follow-up of participants after the primary endpoint of efficacy (e.g. day 28–63).

Assessment of maternal safety

Maternal deaths

Maternal deaths were reported in 14 out of 48 published or presented studies, including four studies with no maternal deaths (29%, 14/48).^{190,192,194,201,218,219,221,237,243,247,256,257,302,308}

Maternal anaemia and other laboratory investigations

Forty (82%, 40/49) studies reported haematological assessment at least once during the study. Of them, 20 studies (50%, 20/40) used haematocrit,^{152,188,190,191,194,198,211,213,214,218,219,236,253,255,268,275,280,309,310} 18 studies (45%, 18/40) used haemoglobin^{192,193,205,221,232,233,237,243,246,247,252,256,257,266,303,307,308,312} and two studies (5%, 2/40) used both.^{238,248} Four published studies did not report haematological assessment,^{201,231,239,302} and information was not available in five unpublished studies.^{235,263,305,306,311} Twenty-five studies (51%, 25/49) continued assessing maternal anaemia after the primary endpoint of efficacy (e.g. day 28–63) regularly or only at delivery.^{188,190-192,194,198,205,211,214,218,219,221,236,237,243,246,252,253,268,275,280,303,308,309,312} Seventeen

(35%, 17/49) studies specified the use of haematinics: 15 provided iron and folic acid;^{190-192,194,211,214,218,219,236,237,247,253,268,303,312} and two did not explain the details.^{239,252}

Summary of the assessment of non-obstetric acute safety

Vital signs other than body temperature were assessed in 19 studies (39%, 19/49), and only two studies reported the results after treatment.^{237,252} Five studies (10%, 5/49) assessed maternal neurological examination given early safety concerns for artemisinin derivatives,^{190,198,221,308,309} and three studies reported the results.^{198,221,309}

Forty studies (82% ,40/49) reported symptoms after treatment. Seven studies (14%, 7/49) did not report symptoms,^{221,230,232,302,307,310,312} and no information was available from two conference abstracts.^{235,306}

Biochemistry was assessed in 20 studies (41%, 20/49), and blood glucose was assessed in six studies.^{193,198,246,255,263,275} White blood cell count was assessed in 19 studies (40%, 19/49). One study reported having assessed routine blood tests without specification.³⁰² Urinalysis was assessed in six studies (12%, 6/49) but none of them reported the results.^{192,221,247,256,302,308} One study reported urine colour.²³⁹

ECG was assessed in four RCTs (17%, 4/23) and one PK study (10%, 1/10).^{192,219,247,275,305} ECG was performed at baseline in all five studies. Three studies assessed one time after drug administration (on day 2,¹⁹² 1 hour after the last dose,²¹⁹ and on day 3²⁴⁷) One study assessed four times after drug administration: 1 hours after each dose and 24 hours after the last dose.²⁷⁵ One study was designed specifically to assess cardiotoxicity of DP:

Standard 12-lead ECG was assessed on day 0, day 2 and day 7; and Holter ECG monitoring started on day 2.³⁰⁵

Assessment of fetal safety

Fetal loss

Fetal loss was assessed in 35 studies (92%, 35/38) out of the 38 that followed through to pregnancy outcome (Appendix 3-11) and not reported in three published studies.^{252,307,310} An additional two studies (20%, 2/10) out of ten studies without specified follow-up past the efficacy endpoint still reported pregnancy loss during the study period.^{152,232}

Miscarriage was defined in 12 studies (34%, 12/35). Miscarriage was defined as fetal loss before 28 weeks' gestation in eleven studies.^{191,194,205,218,221,243,253,257,266,303,309} One study defined miscarriage as fetal loss <20 weeks and intrauterine fetal death as fetal loss >20 weeks.¹⁹² Six studies did not define the term but reported miscarriage.^{190,201,219,237,242,302}

Stillbirth was defined in eight studies (23%, 8/35).^{191,192,194,218,243,257,303,309} Seven studies used the cut-off of 28 weeks gestation or more, and one study used 21 weeks.¹⁹² Nine studies did not give a definition but reported stillbirth.^{188,201,219,235,237,242,247,309,312}

Preterm birth

Eleven studies reported the mean or median EGA at delivery (Appendix 3-11).^{188,190-192,194,198,211,218,219,246,253,275,280,303,309,312} Five studies only included singletons for the summary

calculation.^{190,191,194,218,219} Twenty-seven studies (71%, 27/38) assessed PTB or prematurity of the newborn. Ten studies (26%, 10/38) did not report whether they assessed PTB or not,^{198,213,214,242,247,252,253,307,309,310} and information was not available from one unpublished studies.²³⁵

PTB was defined in 16 studies (59%, 16/27). All of them used the cut-off of <37 weeks' gestation. Two used >28 weeks as the lower boundary,^{205,266} and one study differentiated those born <28 weeks as severe prematurity.¹⁹² Six of them defined PTB but did not report the results.^{188,190,191,205,218,303} Eight studies did not define but did reported PTB.^{201,211,221,246,257,263,280,302} In two studies, all deliveries were full-term.^{255,308} One unpublished study plans to assess PTB.²³⁶

Anthropometric assessment of newborns

Birthweight was assessed in 31 studies (82%, 31/38) (Appendix 3-12). LBW was reported in 20 studies (65%, 20/31). LBW was defined in 19 studies (61%, 19/31) but one of them did not report the result.³⁰⁹ Birthweight of less than 2,500 g was used as the cut-off regardless of the EGA at delivery with an exception of one study that included 2,500 g as LBW.²¹³ Two studies did not define LBW but reported the proportion.^{188,190} Five studies reported birthweight related to EGA at delivery (i.e. SGA comparing with the WHO growth curve³¹², body weight of term babies only^{192,201,219} or proportion of LBW stratified by PTB²⁵⁷). Only singleton births were included in the calculation of the average birthweight in eight studies,^{188,190,191,194,218,219,237,268} and women with multiple gestations were excluded from

the study in four studies.^{235,243,247,303} All women delivered singletons in four additional studies.^{211,213,275,280} Gender of the newborns, which affects birthweight, was only reported to be assessed in seven studies (18%, 7/38)^{194,211,236,257,266,275,312} and was used for body weight assessment in one of them³¹².

Length of newborns was assessed in ten studies (26%, 10/38) and four of them (11%, 4/38) reported the results.^{194,201,219,246} Head circumference of newborns was assessed in 11 studies (29%, 11/38) and five of them (13%, 5/38) reported the results.^{194,201,219,221,308} Length and head circumference were reported only in term babies in one study,²⁰¹ and EGA was not considered in other studies.

The timing of anthropometric assessment was specified in 13 studies (34%, 13/38): within 24 hours (n=5),^{194,198,221,303,308} 48 hours (n=1);²⁴⁶ 72 hours (n=4);^{211,219,237,268} five days (n=1);²⁷⁵ and seven days (n=1).²⁵⁷ One registered trial specified the timing as ‘as soon as possible after delivery’.²³⁶ Five studies (16%, 5/31) specified the minimal precision of the body weight scales used,^{188,190,211,214,252} and one study specified the minimum digit of the measurement.³⁰³ Only two of 19 RCTs that followed women until delivery (10%, 2/19) stated that the physical assessment of babies was done by blinded investigators.^{192,219}

Congenital abnormality

Thirty-two studies (84%, 32/38) reported the presence of any congenital abnormalities. Six studies (16%, 6/38) did not report whether there were any newborns with congenital abnormality.^{193,242,302,307,310,312} No studies reported that cardiac auscultation was

specifically performed except one study in which newborn heart sounds were systematically documented.²¹⁹ Only one infant with congenital heart diseases was reported, and the EGA at drug exposure was 19 weeks.¹⁹² Twenty-five studies (66%, 25/38) followed the newborns for a period (from six weeks to three years) after delivery to assess mortality, congenital abnormality and development (Appendix 3-12). At least six different methods of developmental assessment³²⁹⁻³³⁴ were used in eight studies,^{192,194,198,201,211,219,236,255} and three other studies reported using developmental milestones.^{188,190,266}

Neonatal mortality and condition of newborns at birth

APGAR score was assessed in four studies (11%, 4/38),^{192,198,201,237} and only one of them reported the results.¹⁹⁸ Neonatal jaundice was assessed in eight studies (21%, 8/38),^{198,201,237,243,247,255,257,303} and five of them reported the results.^{198,247,255,257,303} Neonatal death was defined in six studies (16%, 6/38) (Appendix 3-11). Two of them reported early neonatal death defined by death within the first week of life.^{192,201} Four studies used a month: a month,^{214,268} 27 days³⁰³ and 32 days.²¹⁹ Perinatal death was defined in two studies (5%, 2/38). One study included miscarriage, stillbirth and neonatal death (within 27 days after birth).³⁰³ The other study defined it as death from 28 weeks gestational age until one week after delivery.²⁰⁵ Sixteen studies (42%, 16/38) did not define but reported the deaths of newborns after delivery.^{188,190,193,194,205,211,221,235,243,246,247,280,302,308,309,312}

Associated obstetric risk factors for adverse pregnancy outcomes

Risk factors for adverse pregnancy outcomes were not well-reported (Appendix 3-13). The previous history of pregnancy loss was considered in two studies (4%, 2/49), which excluded women with a history of multiple miscarriage or stillbirths.^{237,247} All other studies (96%, 47/49) did not report the previous history of pregnancy loss. Except one study reporting the history of previous PTB to explain a neonatal death,¹⁹⁴ the histories of previous PTB or LBW were not reported in any other studies. In 22 studies (45%, 22/49), women with known chronic diseases (including renal/hepatic/cardiac/mental diseases) were excluded from the study. One study reported the proportion of women with diabetes,²⁰¹ and another study reported there were no women with chronic diseases included.²³² No studies reported the history of non-malarial febrile illness during the pregnancy but two studies excluded women with other diseases associated with fever²³⁸ or severe underlying diseases presenting with fever.²⁴⁸ Eleven studies mentioned HIV status of the participants. Four studies included women with HIV.^{192,242,247,303} Two studies included women with HIV only if cotrimoxazole prophylaxis (and antiretroviral treatment) was not administered.^{237,312} Five studies excluded women with HIV.^{213,233,235,243,305}

Smoking status was assessed in six studies (12%, 6/49).^{192,211,214,219,232,236} One published study assessed the smoking status but did not report it.¹⁹² Women with a history of alcohol (or narcotic abuse) were excluded in four studies (8%, 4/49),^{194,219,236,275} and alcohol consumption was not reported in any other studies. One study reported the use of traditional

medicine in the description of a stillbirth,³⁰³ and two studies excluded women who used herbal medicine in the past four weeks at enrolment.^{238,248} Marital status was reported in three studies (6%, 3/49).^{252,303,312} Consanguinity was reported as a possible reason for a stillbirth in a study.²¹⁹ Education level was reported in eight studies (16%, 8/49).^{213,236,239,243,247,252,303,312} Maternal height was assessed in eleven studies (22%, 11/49),^{198,213,214,232,236,238,243,248,252,253,266} and five of them also reported body mass index (BMI).^{213,232,238,248,252} Three studies reported BMI without the maternal height.^{211,219,233} No studies reported any other nutritional status assessment. The number of ANC visits was not reported except one study reporting the proportion of women who attended the ANC at least two times.²⁵²

3-III.4 Discussion

This literature search revealed the variability of antimalarial safety reporting in treatment efficacy studies in pregnancy. Understandably, pregnancy outcomes remain secondary to the primary objective of antimalarial efficacy studies and reporting of safety outcomes may have been omitted or truncated to meet journal length restrictions. Safety assessment in the context of malaria is further stymied by the paucity of reliable background data on pregnancy outcomes and early childhood norms in the target populations in LMICs.¹⁵⁰ Methodological difficulties, variability of definitions and measurement of key indicators, such as gestational age and birthweight,³³⁵ and lack of integrated malaria, antenatal and delivery activities prevent confident pooling of published data, which could potentially fill the gaps of current knowledge about the safety of antimalarials in pregnancy.

Pregnancy outcomes can be affected by many factors. Malaria can affect pregnancy outcomes by acute disease effects (e.g. fever), acute and chronic effects (e.g. placental sequestration) and drug effects. Fever by non-malaria causes, common in tropical areas, can cause pregnancy loss or preterm labour.³³⁶ Placental sequestration can cause inflammation of the placenta and reduction in the blood flow to the placenta, which lead to impaired placental function and IUGR.^{26,337,338}

For meaningful interpretation of pregnancy outcomes, gestational age needs specific attention¹⁶⁵ as it is necessary for interpretation of laboratory results, infant birthweight (as appropriate, large, or small for gestational age), as well as to evaluate the likelihood that a certain drug exposure could have a causative relationship with an adverse outcome. Preterm delivery and miscarriage are important outcomes associated both with infection and certain medications and can only be evaluated with accurate estimation of gestational age. However, gestational age was assessed differently across the included studies and with each method there is invariably a degree of increasing error as pregnancy progresses: after 24 weeks ultrasound cannot reliably determine gestational age and last menstrual period becomes more difficult to recall.³³⁹ Gestational age assessment relies on quality control, which was often not reported. Modifying superficial and neurological items in clinical newborn gestational age assessment reduces the accuracy of these tests.³⁴⁰

Laboratory results differ from non-pregnant participants owing to the physiological changes of gestation.³⁴¹ Although haematologic markers were commonly assessed, the

physiological change of haemoglobin over pregnancy³⁴² was not considered. A simple comparison between one measurement at the time of malaria and one at delivery may not estimate the effect of malaria or antimalarials correctly. The impact of repeated malaria infection also needs to be assessed.

As exposures to artemisinin derivatives in early pregnancy were shown to be related to cardiovascular abnormalities in animal studies,¹⁸⁵ echocardiography should be ideally used but is rarely feasible in low resource settings. In the absence of echocardiography, serial cardiac auscultation and clinical exams could be used to identify murmurs, signs of congestive heart failure, or cyanosis. Unfortunately, affected children are often difficult to identify against the high background incidence of respiratory infections.^{66,343} Shortening of long bones is also reported in animal studies, and length of the newborn should be measured and compared with a standard that accounts for sex and an adequately estimated gestational age. Ongoing follow-up and clinical exams to assess the development of infants can be useful to detect musculoskeletal abnormalities. Mode of feeding and other morbidities including malaria, need to be considered in the developmental assessment. In addition to the technical difficulties of developmental assessment, efficacy studies are unlikely to provide sufficient numbers of participants to assess for congenital abnormalities, which are rare,³⁴⁴ and it will be necessary to combine data from these studies with information from other sources.

It is difficult to compare birthweight between studies and even between different study arms in antimalarial efficacy trials. Assessment and comparison require a precise scale

as the reported magnitude of reduction in birthweight is 35–310 g.¹⁰ However, comparison with this precision is difficult because of the variability of methodology, inaccuracy of measurements and lack of information on confounding factors.³³⁵ Firstly, birthweight for comparison should be measured in undressed live singleton newborns without any apparent congenital abnormalities within 24 hours. Body weight on day 3–5 can physiologically be $\geq 10\%$ lower than the birthweight.^{345,346} Accuracy and precision of the body weight scale also need to be specified before concluding the difference in different treatment arms. Secondly, birthweight is affected by the gestational age and sex. Preterm babies are more likely to be categorized as being LBW, but LBW does not necessarily equate to SGA.³⁴⁷ LBW infants include a mixture of preterm and intrauterine growth restricted babies, and these categories should be distinguished as they reflect different aetiologies and outcomes. The impact of malaria on birthweight is more accurately assessed using SGA.³⁴⁷ International standards to assess birthweight adjusted for gestational age and sex are available such as INTERGROWTH-21st, which also covers populations in malaria endemic areas.¹⁶⁵

It is necessary to determine whether adverse outcomes are related to treatment rather than malaria. This can be only done when the risk is compared between different treatments (or with a well-established population prevalence). Several risk factors for adverse pregnancy outcomes, such as smoking, hypertension, low BMI and primigravida were also reported in malaria endemic settings,³⁴⁷ and there are some other potential local risk factors such as traditional medicine and consanguinity. These should be recorded and assessed, especially in

non-comparative studies.³² Parity and gravidity were not consistently reported and if some manuscripts report parity and others gravidity, pooling of data becomes difficult. A distinction between antepartum stillbirth and intrapartum stillbirth is important, as intrapartum stillbirth is mainly affected by the availability and the level of delivery support.¹⁷⁸ Finally, the impact of the timing and frequency of malaria episodes and drug administration in each pregnancy need to be assessed with parasitological confirmation,¹⁸⁹ and this is most appropriately done in prospective studies. A simple checklist of for reporting pregnancy-related information in antimalarial efficacy studies is provided in Chapter 9.

3-III.5 Conclusions

Much is still not known about the impact of antimalarial treatment on the consequences of malaria infection in pregnancy. Every effort should be made to make the most of each treatment episode in an efficacy study by gathering a minimal set of important safety data to fill this knowledge gap. This valuable data can then be pooled with data from other sources of clinical safety information such as pharmacovigilance studies or exposure registry to strengthen safety conclusions. Standardization of assessment and reporting will be a foundation for research with more comparable and reliable outputs and will generate the needed evidence to guide policy.

Chapter 4. Efficacy and tolerability of artemisinin-based and quinine-based treatments for uncomplicated falciparum malaria in pregnancy: a WorldWide Antimalarial Resistance Network individual patient data meta-analysis

* Part of this chapter has been published in a peer-reviewed journal and is freely available as follows under the Creative Commons Attribution licence. Saito M, Mansoor R, Kennon K, McGready R, Nosten F, Guérin PJ, Stepniewska K. The efficacy of artemisinin-based and quinine-based treatments for uncomplicated falciparum malaria in pregnancy: a protocol for systematic review and individual patient data (IPD) meta-analysis. *BMJ Open*. 2019;9:e027503.

4.1 Introduction

Pregnant women are vulnerable to malaria, which affects both mothers and fetuses disproportionately. Both effective prevention and effective treatment are required to limit the cumulative adverse impact of malaria during pregnancy. However, this vulnerable but important population, nurturing future generations, are understudied.⁵ Pregnant women are often excluded from clinical trials in general, and antimalarial studies are no exception.³

A previous systematic literature review in Chapter 3-II revealed that pregnant women had been systematically excluded from standard antimalarial treatment efficacy studies over the past 30 years.¹⁸⁹ There were a number of reasons for this, the first of which was concern over the teratogenicity and embryotoxicity of artemisinin derivatives shown in animal studies.¹⁴⁵ Over the last 20 years, however, data on the human safety of artemisinin derivatives in the first trimester have been accumulated, leading to a collaborative effort that concluded that artemisinin derivatives are at least as safe as quinine.^{32,33,207} The second reason was that there are no agreed guidelines to assess antimalarial drug efficacy in pregnant women. The current WHO guidelines on the assessment of antimalarial efficacy exclude pregnant women,¹⁷⁴ which is in a sense reasonable considering that pregnant women are different from the non-pregnant population in a number of ways. These include: altered immunity; gestational physiological changes affecting pharmacokinetics /pharmacodynamics; and the presence of the placenta, which may provide a haven for malaria parasites.^{26,112,249} The study design needs to be tailored for pregnant women. Nonetheless, the lack of standard study

design for pregnant women makes clinical trials in pregnancy more challenging, which in turn means there is insufficient evidence to establish a study design tailored for pregnant women.

The diversity of study designs extrapolated from the WHO guidelines limit aggregated data meta-analysis.¹⁸⁹ Additionally, most antimalarial studies in pregnancy were single-arm studies, which cannot be included in conventional aggregated data meta-analysis. Consequently, there was limited evidence supporting the current WHO recommendation¹⁴⁰ of quinine (with clindamycin if available) for first trimester women and ACT for second- or third-trimester women. Now that evidence on the safety of artemisinin-derivatives in pregnancy has been accumulated, the evidence on the efficacy of antimalarials for uncomplicated falciparum malaria in pregnancy needs to be summarized.

The study in this chapter aims to contribute to the body of evidence by pooling the individual patient data (IPD) of the studies on the efficacy of currently recommended antimalarials (namely, quinine-based treatment and artemisinin-based treatment) for uncomplicated falciparum malaria in pregnancy.

4.2 Methods

Search strategy and inclusion criteria

A systematic review and IPD meta-analysis on the efficacy of artemisinin-based and quinine-based treatment on uncomplicated falciparum malaria in pregnancy was conducted using the systematic literature review described in Chapter 3-II.¹⁸⁹ Details of the data acquisition and standardization are described in Chapter 2. The protocol for the IPD meta-

analysis is registered on PROSPERO (CRD42018104013).

For this IPD meta-analysis, studies were included if *P. falciparum* parasitaemia was confirmed by microscopy before the treatment, the length of active follow-up was ≥ 28 days and PCR was used for recurrence of falciparum malaria.

Outcomes and statistical analysis

The outcomes assessed included PCR-corrected efficacy, PCR-uncorrected efficacy, parasite clearance, fever clearance, gametocyte development, and acute adverse symptoms. Pregnancy outcomes are analysed in Chapter 6. Only those who were treated with (and completed) normal dosing range according to the WHO guideline¹⁴⁰ were included in the analyses, except PCR-uncorrected efficacy, which included all recorded episodes. Patients with indeterminate PCR was excluded from PCR-corrected efficacy analyses. Dose-response of AL was analysed by including women who completed the standard dose. Gametocyte development in day 1–6 and day 7–31 was assessed by including only those who did not have gametocytaemia on day 0 and did not have recurrence within 28 ± 3 days. Adverse symptoms were regarded as present if the symptom was recorded on any day between day 1 and day 7.

For descriptive analysis, PCR-corrected and PCR-uncorrected efficacy at fixed time points (i.e. day 28, 42 and 63) was estimated by the Kaplan-Meier method for each study at each site, and then pooled by the aggregated data meta-analysis methods.

To assess the risk factors and compare different treatments, one-stage IPD meta-

analyses with mixed-effects model for study site were used. Hazard ratios were used for PCR-corrected efficacy, and odds ratios were used for the other outcomes. PCR-uncorrected efficacy was only summarized for each transmission. A complete case analysis was conducted, and variables with $\geq 50\%$ of missing were excluded from the analyses. Models were built by backward elimination using $p < 0.05$ by likelihood ratio test as the cut-off. Two variables, namely treatment and baseline parasitaemia, were included in the multivariable models on PCR-corrected efficacy as *a priori* forced variables regardless of statistical significance. Few women had multiple episodes recorded, thus previous history of malaria in 28 days was included to take account of this. Interaction between gravidity (parity) and malaria transmission, or age and transmission was assessed if age or gravidity was included in the multivariable model, as the impact of age and gravidity (i.e. pregnancy-specific immunity) can be different depending on transmission. *P. vivax* intercalated infection (i.e. *P. vivax* mono-infection before the recurrence of *P. falciparum* parasitaemia) was regarded as censored if the original study did not test for falciparum recurrences after intercalated vivax infection by PCR, following the WHO guidelines.¹⁷⁴ If the original study tested PCR for falciparum recurrences regardless of intercalated vivax infection, vivax infection was regarded as a time-dependent covariate. Global test was used for checking the proportional hazard assumption. The piecewise-Cox model by introducing an interaction term between time was attempted for the violation of this assumption.

For other outcomes, treatment group was always included in the models.

Additionally, baseline parasitaemia (for parasite clearance) and the presence of the symptom on day 0 (for adverse symptoms) were always included in the model.

As a sensitivity analysis, the model was refitted excluding one study at a time to identify any influential studies.

4.3 Results

Study inclusion

A systematic literature review identified 30 studies with PCR-corrected efficacy. Excluding two unpublished studies, IPD of 4,968 episodes (92%: 4968/5360) in 19 studies^{188,190,192,194,211,214,219,232,233,237,246,252,257,268,275,280,303,306,313} were shared and pooled for the analyses of PCR-corrected efficacy (Figure 4-1).

The included studies were conducted between 1995–2014 in ten different countries: nine studies (3,813 episodes) were in sub-Saharan Africa and ten (1,155 episodes) were in Asia (Figure 4-2). The designs and qualities of the shared and unshared studies are summarized in Appendix Table 4-1.

Baseline characteristics

Ten antimalarial treatments were included in this analysis of PCR-corrected treatment efficacy: AL (n= 1278, 89.7% of episodes in the literature), ASAQ (n= 840, 91.8%), ASMQ (n= 1028, 96.0%), DP (n= 872, 98.6%), ASSP (n= 173, 84.4%), AS (n= 230, 100%), AC (n= 142, 100%), AAP (n= 91, 100%), quinine (n= 244, 81.6%) and QC (n= 67, 100%).

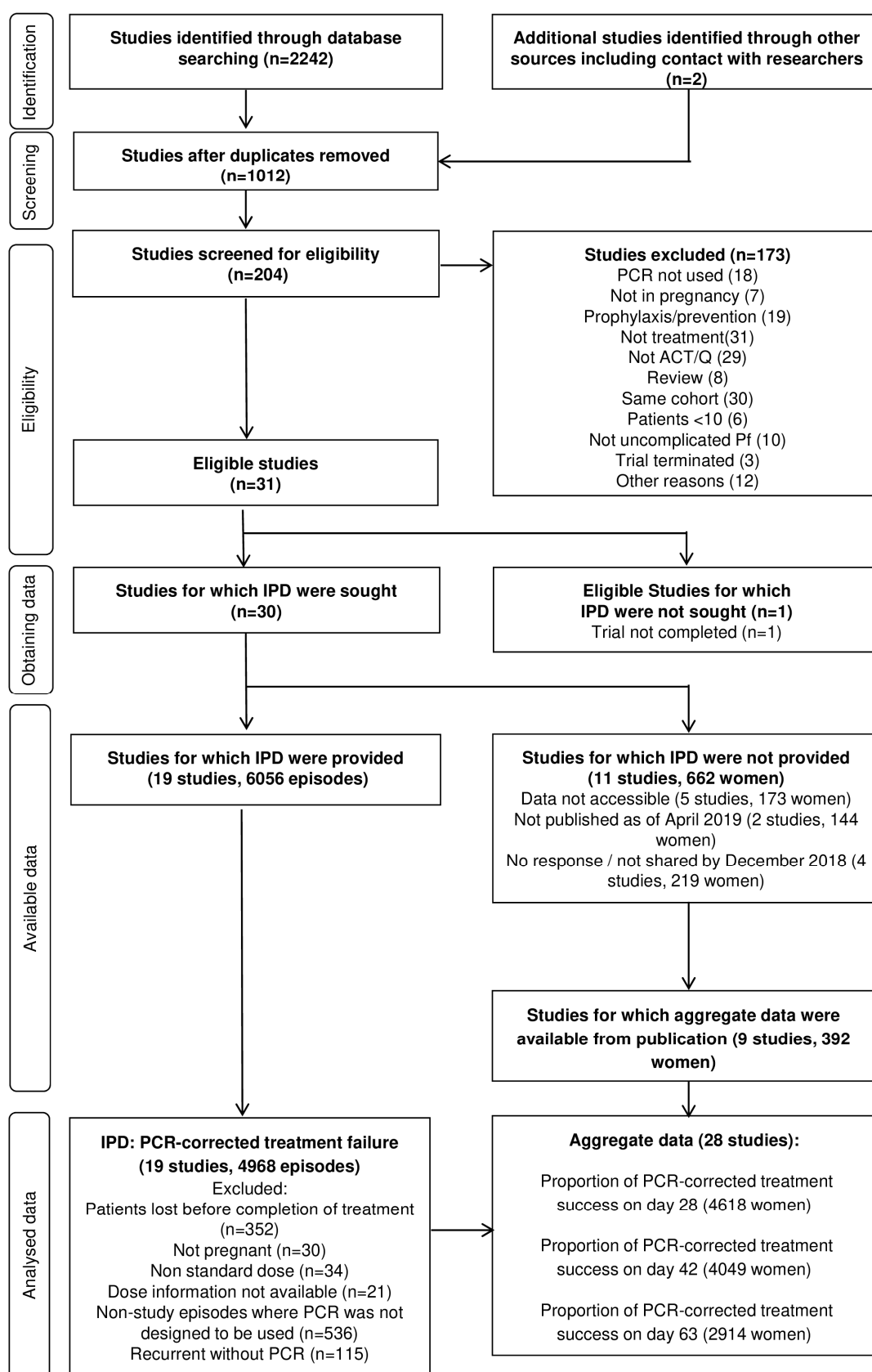


Figure 4-1. Flowchart of inclusion of the eligible studies

Fixed dose was used in all ASAQ and 93.6% (962/1028) of ASMQ.

Of the 4968 women, the mean age was 23.5 (standard deviation [SD] 6.0), and the



Figure 4-2. Geographical locations of the included studies

median parity was 1 (range 0–10) (Table 4-1). The majority of episodes were either in the second (67.0%, 3327/4965) or third trimester (32.4%, 1610/4965). Of these, 98.9% (4914/4968) were *P. falciparum* mono-infections and the remainder were mixed infections (1.1%, 54/4968). The geometric mean parasite density was 1,189 (range 1–447,638) asexual parasites / μL , with 1.6% (79/4968) hyperparasitaemic ($>100,000$ / μL). Only 10.4% (504/4854) of the women were febrile at presentation (>37.5 °C).

PCR-corrected efficacy at day 28, 42, 63

With the exception of quinine monotherapy, the PCR-corrected treatment efficacy by the Kaplan-Meier method was more than 90% at day 28, 42 or 63 pooled by random effects (Table 4-2). The efficacy of quinine monotherapy was the lowest: 87.7 % (95% confidence interval [CI] 58.2 to 99.3%) on day 28 and 84.9% (95% CI 51.6 to 99.3%) on day 63. The efficacy of AL on day 63 was 93.6% (95% CI 89.1 to 96.7%) while the efficacy was $>95\%$ for all the other ACTs. The PCR-corrected treatment efficacy was almost unchanged when studies for which IPD were not shared were included in the pooled estimates (Appendix Table 4-2).

Table 4-1. Baseline characteristic of pregnant women assessed for PCR-corrected efficacy

Characteristic	All		AL		ASAQ		ASMQ		DP	
	N	% (N)	N	% (N)	N	% (N)	N	% (N)	N	% (N)
		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)
EGA	4965	24.7 (5.9)	1278	24.4 (5.8)	840	24.1 (5.2)	1028	25.1 (5.4)	872	24.3 (5.7)
Age (years)	4968	23.5 (6.0)	1278	22.9 (5.8)	841	23.6 (5.9)	1028	23.6 (5.7)	874	22.8 (5.8)
Parity 0	4903	43.4 (2130)	1240	44.8 (555)	814	41.6 (339)	1028	42.9 (441)	874	47.4 (414)
1		21.2 (1040)		20.3 (252)		22.5 (183)		20.9 (215)		22.0 (192)
≥2		35.3 (1733)		34.9 (433)		35.9 (292)		36.2 (372)		30.7 (268)
Height (cm)	4256	155.8 (7.0)	1081	156.4 (6.9)	815	157.8 (6.5)	986	156.1 (7.5)	874	155.3 (6.3)
Weight (kg)	4943	54.0 (8.5)	1266	54.2 (7.7)	841	56.7 (9.3)	1028	54.1 (8.7)	874	54.5 (8.3)
BMI	4243	22.2 (3.0)	1081	21.9 (2.5)	815	22.6 (3.4)	986	22.3 (3.1)	874	22.6 (2.9)
Fever (temperature >37.5)	4854	10.4 (504)	1232	9.7 (119)	841	6.2 (52)	1027	9.4 (97)	871	4.8 (42)
Haemoglobin on day 0 (g/dL)	4917	9.9 (1.4)	1252	10.2 (1.4)	841	10.0 (1.3)	1026	9.8 (1.4)	866	10.0 (1.4)
Anaemia										
no anaemia	4917	29.0 (1428)	1252	31.0 (388)	841	25.6 (215)	1026	24.2 (248)	866	26.3 (228)
moderate		70.6 (3470)		68.8 (861)		74.3 (625)		75.6 (776)		73.3 (635)
severe		0.4 (19)		0.2 (3)		0.1 (1)		0.2 (2)		0.3 (3)
HIV infection	3233	1.4 (45)	934	1.4 (13)	605	0.5 (3)	746	0.1 (1)	679	1.2 (8)
Parasitaemia (log10/μL)	4968	3.1 (0.9)	1278	3.2 (0.9)	841	2.9 (0.8)	1028	3.0 (0.8)	874	2.9 (0.8)
Hyperparasitaemia	4968	1.6 (79)	1278	1.9 (24)	841	0.1 (1)	1028	1.1 (11)	874	1.1 (10)
Presence of gametocytes	4826	4.1 (197)	1242	4.3 (54)	815	2.9 (24)	1007	1.7 (17)	874	3.7 (32)
Mixed infection	4968	1.1 (54)	1278	1.0 (13)	841	0.0 (0)	1028	0.0 (0)	874	1.1 (10)
Previous history of malaria										
No treatment	4967	87.1 (4328)	1278	83.9 (1072)	841	100.0 (841)	1028	98.3 (1011)	874	95.3 (833)
Confirmed recrudescence in 28 days		2.1 (105)		2.2 (28)		0.0 (0)		0.0 (0)		1.6 (14)
All other antimalarial use in 28 days		4.7 (232)		4.6 (59)		0.0 (0)		0.2 (2)		2.7 (24)
First ANC visit		6.1 (302)		9.3 (119)		0.0 (0)		1.5 (15)		0.3 (3)
Malaria transmission intensity										
Low	4968	26.5 (1319)	1278	15.6 (200)	841	3.1 (26)	1028	18.3 (188)	874	9.4 (82)
Moderate		52.2 (2594)		60.7 (776)		57.3 (482)		49.3 (507)		84.4 (738)
High		21.2 (1055)		23.6 (302)		39.6 (333)		32.4 (333)		6.2 (54)

AAP: Artesunate with atovaquone-proguanil, AC: artesunate with clindamycin, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, DP: Dihydroartemisinin-piperaquine, EGA: Estimated gestational age, Q: Quinine monotherapy, QC: Quinine with clindamycin, SD: standard deviation.

Table 4-1 continued.

Characteristic	ASSP		AS		AC		AAP		Q		QC	
	N	% (N)	N	% (N)	N	% (N)	N	% (N)	N	% (N)	N	% (N)
		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)
EGA	173	24.5 (5.7)	230	26.1 (7.5)	142	29.9 (5.8)	91	26.2 (7.0)	244	23.2 (6.6)	67	26.2 (6.6)
Age (years)	173	22.5 (4.3)	230	26.4 (7.4)	142	26.3 (7.5)	91	24.1 (5.9)	244	23.7 (5.5)	67	25.2 (6.1)
Parity 0	173	57.2 (99)	230	32.2 (74)	142	32.4 (46)	91	37.4 (34)	244	43.9 (107)	67	31.3 (21)
1		22.5 (39)		17.4 (40)		19.7 (28)		23.1 (21)		22.5 (55)		22.4 (15)
≥2		20.2 (35)		50.4 (116)		47.9 (68)		39.6 (36)		33.6 (82)		46.3 (31)
Height (cm)	122	150.1 (6.9)	148	151.6 (5.6)	135	152.2 (5.3)	40	151.2 (7.9)	54	152.6 (5.0)	1	154.0
Weight (kg)	173	48.2 (7.0)	230	49.3 (6.4)	129	51.2 (6.3)	91	49.8 (6.3)	244	54.1 (9.2)	67	49.3 (5.5)
BMI	122	20.6 (2.9)	148	21.4 (2.6)	122	22.1 (2.2)	40	21.8 (3.8)	54	21.1 (2.3)	1	19.4
Fever (temperature >37.5)	121	25.6 (31)	228	24.1 (55)	133	19.5 (26)	91	25.3 (23)	243	18.9 (46)	67	19.4 (13)
Haemoglobin on day 0 (g/dL)	171	9.3 (1.4)	230	9.5 (1.5)	139	9.3 (1.6)	90	9.3 (1.5)	237	10.1 (1.7)	65	9.2 (1.6)
Anaemia												
no anaemia	171	11.7 (20)	230	30.4 (70)	139	48.2 (67)	90	50.0 (45)	237	48.5 (115)	65	49.2 (32)
moderate		88.3 (151)		66.5 (153)		51.8 (72)		50.0 (45)		51.1 (121)		47.7 (31)
severe		0.0 (0)		3.0 (7)		0.0 (0)		0.0 (0)		0.4 (1)		3.1 (2)
HIV infection	159	6.3 (10)	0		3	0.0 (0)	0		107	9.3 (10)	0	
Parasitaemia (log10/μL)	173	3.4 (0.8)	230	3.3 (1.0)	142	3.3 (1.0)	91	3.5 (1.1)	244	3.4 (0.9)	67	3.2 (1.0)
Hyperparasitaemia	173	2.9 (5)	230	5.2 (12)	142	4.9 (7)	91	6.6 (6)	244	1.2 (3)	67	0.0 (0)
Presence of gametocytes	122	3.3 (4)	225	9.3 (21)	141	12.8 (18)	91	4.4 (4)	243	7.4 (18)	66	7.6 (5)
Mixed infection	173	0.0 (0)	230	7.0 (16)	142	6.3 (9)	91	1.1 (1)	244	0.4 (1)	67	6.0 (4)
Previous history of malaria												
No treatment	173	98.3 (170)	230	58.3 (134)	142	47.9 (68)	91	52.7 (48)	243	43.2 (105)	67	68.7 (46)
Confirmed recrudescence in 28 days		0.6 (1)		10.4 (24)		21.8 (31)		7.7 (7)		0.0 (0)		0.0 (0)
All other antimalarial use in 28 days		1.2 (2)		17.8 (41)		30.3 (43)		26.4 (24)		11.9 (29)		11.9 (8)
First ANC visit		0.0 (0)		13.5 (31)		0.0 (0)		13.2 (12)		44.9 (109)		19.4 (13)
Malaria transmission intensity												
Low	173	70.5 (122)	230	100.0 (230)	142	99.3 (141)	91	100.0 (91)	244	70.5 (172)	67	100.0 (67)
Moderate		10.4 (18)		0.0 (0)		0.7 (1)		0.0 (0)		29.5 (72)		0.0 (0)
High		19.1 (33)		0.0 (0)		0.0 (0)		0.0 (0)		0.0 (0)		0.0 (0)

AAP: Artesunate with atovaquone-proguanil, AC: artesunate with clindamycin, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, DP: Dihydroartemisinin-piperaquine, EGA: Estimated gestational age, Q: Quinine monotherapy, QC: Quinine with clindamycin, SD: standard deviation.

Table 4-2. PCR-corrected efficacy on day 28, 42 and 63 by Kaplan-Meier estimates pooled by random effects model

Treatment	PCR-corrected treatment success*					
	Day 28		Day 42		Day 63	
	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)
AL	1168	96.9 (94.5-98.5)	929	95.5 (92.6-97.5)	598	93.6 (89.1-96.7)
ASAQ	811	99.6 (99.2-99.9)	782	99.2 (98.5-99.7)	611	98.8 (97.2-99.6)
ASMQ	982	99.9 (99.6-100)	948	99.5 (98.3-99.9)	717	99.2 (97.0-99.9)
DP	815	99.5 (98.0-99.9)	799	99.4 (97.0-99.9)	705	98.4 (95.6-99.6)
ASSP	159	99.0 (95.4-99.9)	120	99.2 (95.7-99.9)	113	99.2 (95.7-99.9)
AS	169	95.9 (88.4-99.1)	140	95.6 (87.3-99.1)	91	95.3 (85.7-99.2)
AC	106	99.5 (97.4-99.9)	73	98.6 (85.4-100)	39	97.5 (71.4-100)
AAP	58	99.9 (98.4-100)	49	98.9 (91.5-100)	40	98.5 (91.4-99.9)
Q	181	87.7 (58.2-99.3)	164	86.8 (56.6-99.3)	128	84.9 (51.6-99.3)
QC	43	99.9 (97.6-100)	37	99.9 (97.0-100)	28	99.9 (96.3-100)

* Estimated by pooling the Kaplan-Meier survival estimates in each study by random effects method.

AAP: Artesunate with atovaquone-proguanil, AC: artesunate with clindamycin, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, PCR: polymerase chain reaction, Q: Quinine monotherapy, QC: Quinine with clindamycin.

Risk factors for PCR-corrected treatment failure

The risk factors for PCR-corrected treatment failure were analysed. Treatment effect was assessed by the piece-wise Cox model, which split the observation period into 0–28 days and >28 days, in order to satisfy the proportional hazards assumption. In the univariable analysis, the risk of PCR-corrected treatment failure was higher in quinine monotherapy both in day 0–28 (hazard ratio [HR] 7.63, 95% CI 3.09 to 18.83, $p < 0.001$) and after day 28 (HR 3.14, 95% CI 1.00 to 9.82, $p = 0.05$) than AL (Table 4-3). On the other hand, the risk of PCR-corrected treatment failure was lower in ASAQ (HR 0.14, 95% CI 0.04 to 0.47, $p = 0.001$), ASMQ (HR 0.05, 95% CI 0.01 to 0.35, $p = 0.003$) and DP (HR 0.19, 95% CI 0.06 to 0.57, $p = 0.003$) in day 0–28. The risk of failure after day 28 was also lower in ASAQ (HR 0.40, 95% CI 0.18 to 0.88, $p = 0.02$). The risks were not different between AS and AL in both

periods. The risks of ASSP were higher in both periods but not significantly different from those of AL. The risk of treatment failure was lower in AC in day 0–28, but this was based on a small number of observations. There was no failure in QC and AAP in day 0–28. Among the baseline characteristics (Table 4-4), lower age (HR 0.97, 95% CI 0.95 to 1.00, $p=0.03$) and higher baseline parasitaemia (HR 2.00 for a 10-fold increase, 95% CI 1.67 to 2.38, $p<0.001$) were associated with a higher risk of PCR-corrected treatment failure. The risks of treatment failure were lower in primiparous women (HR 0.59, 95% CI 0.39 to 0.90, $p=0.01$) and multiparous (i.e. parity ≥ 2) women (HR 0.63, 95% CI 0.44 to 0.90, $p=0.01$) than nulliparous women.

Table 4-3. Risk of PCR-corrected treatment failure for different antimalarial treatments in two periods after treatment

Treatment	Proportion	Univariable		Multivariable*	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Day0-28					
AL	40/1278	Reference		Reference	
ASAQ	3/841	0.14 (0.04-0.47)	0.001	0.15 (0.04-0.49)	0.002
ASMQ	1/1028	0.05 (0.01-0.35)	0.003	0.05 (0.01-0.36)	0.003
DP	4/874	0.19 (0.06-0.57)	0.003	0.19 (0.06-0.59)	0.004
ASSP	2/173	1.46 (0.21-9.96)	0.70	1.26 (0.18-8.92)	0.82
AS	12/230	0.76 (0.37-1.58)	0.47	0.78 (0.38-1.63)	0.52
AC	1/142	0.08 (0.01-0.59)	0.01	0.08 (0.01-0.58)	0.01
AAP	0/91	No failure			
Q	24/244	7.63 (3.09-18.83)	<0.001	7.96 (3.17-19.99)	<0.001
QC	0/67	No failure			
Day 29-					
AL	28/1036	Reference		Reference	
ASAQ	9/804	0.40 (0.18-0.88)	0.02	0.40 (0.18-0.86)	0.02
ASMQ	24/971	1.13 (0.63-2.04)	0.68	1.08 (0.60-1.94)	0.80
DP	10/811	0.55 (0.25-1.19)	0.13	0.53 (0.24-1.15)	0.11
ASSP	2/135	8.86 (0.74-106.21)	0.09	7.62 (0.65-89.68)	0.11
AS	3/160	0.35 (0.10-1.24)	0.10	0.34 (0.10-1.20)	0.10
AC	5/100	1.23 (0.43-3.48)	0.70	1.14 (0.40-3.21)	0.81
AAP	2/57	0.81 (0.15-4.36)	0.81	0.78 (0.14-4.22)	0.77
Q	7/174	3.14 (1.00-9.82)	0.05	3.29 (1.04-10.36)	0.04
QC	1/43	1.01 (0.09-11.28)	1.00	0.97 (0.09-11.13)	0.98

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, HR: hazard ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for previous antimalarial exposure, parity and baseline parasitaemia (in log10 scale).

Table 4-4. Baseline characteristics associated with the risk of PCR-corrected treatment failure

Characteristic	Proportion	Univariable*		Multivariable*	
		HR (95% CI)	p-value	HR (95% CI)	p-value
EGA	178/4965	0.99 (0.96-1.01)	0.36		
Age (years)	178/4968	0.97 (0.95-1.00)	0.03		
Gravidity 1	78/1763	Reference			
2	36/1118	0.65 (0.43-0.97)	0.03		
≥3	60/1989	0.59 (0.42-0.84)	0.004		
Parity 0	89/2130	Reference		Reference	
1	31/1040	0.59 (0.39-0.90)	0.01	0.59 (0.39-0.90)	0.01
≥2	56/1733	0.63 (0.44-0.90)	0.01	0.63 (0.44-0.90)	0.01
Height (cm)	153/4256	1.01 (0.98-1.04)	0.44		
Weight (kg)	177/4943	1.00 (0.98-1.02)	0.76		
BMI	153/4243	0.99 (0.93-1.06)	0.78		
Fever (temperature >37.5)	32/504	1.00 (0.66-1.51)	0.99		
no fever	142/4350	Reference			
Haemoglobin on day 0 (g/dL)	175/4917	0.93 (0.84-1.04)	0.21		
HIV infection	1/45	1.08 (0.13-8.64)	0.94		
negative/not confirmed	177/4923	Reference			
Parasitaemia (log ₁₀ /μL)	178/4968	2.00 (1.67-2.38)	<0.001	1.93 (1.61-2.32)	<0.001
Hyperparasitaemia	6/79	0.75 (0.31-1.82)	0.53		
No	172/4889	Reference			
Presence of gametocytes	12/197	1.34 (0.72-2.47)	0.35		
no gametocytes	157/4629	Reference			
Mixed infection	5/54	1.65 (0.65-4.21)	0.30		
mono-infection	173/4914	Reference			
Intercalated vivax infection	10/233	0.82 (0.39-1.72)	0.59		
No vivax infection	168/4735	Reference			
Malaria transmission intensity					
Low	81/1319	Reference			
Moderate	49/2594	0.86 (0.33-2.28)	0.77		
High	48/1055	1.22 (0.36-4.13)	0.75		

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, HR: hazard ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for treatment, previous antimalarial exposure, their interactions with time after treatment (0-28 days and after 28 days) and baseline parasitaemia (on log₁₀ scale).

In the multivariable analyses adjusted for parity and baseline parasitaemia, quinine monotherapy remained associated with a higher risk of PCR-corrected treatment failure in both in day 0–28 (adjusted HR 7.96, 95% CI 3.17 to 19.99, p<0.001) and after day 28 (aHR 3.29, 95% CI 1.04 to 10.36, p=0.04). Similarly, the risk of PCR-corrected treatment failure was lower in ASAQ (aHR 0.15, 95% CI 0.04 to 0.497, p=0.002), ASMQ (aHR 0.05, 95% CI

0.01 to 0.36, $p=0.003$) and DP (aHR 0.19, 95% CI 0.06 to 0.59, $p=0.004$) in day 0–28. The risk of failure after day 28 was also lower in ASAQ (aHR 0.40, 95% CI 0.18 to 0.86, $p=0.02$). The risk of treatment failure was lower in AC in day 0–28 (aHR 0.08, 95% CI 0.01 to 0.58, $p=0.01$) and higher in ASSP both in day 0–28 (aHR 1.26, 95% CI 0.18 to 8.92, $p=0.82$) and after day 28 (aHR 7.62, 95% CI 0.65 to 89.68, $p=0.11$), but these were based on relatively few observations. Higher baseline parasitaemia (aHR 1.93 for 10-fold increase, 95% CI 1.61 to 2.32, $p<0.001$) and nulliparous women were associated with a higher risk of PCR-corrected treatment failure.

The effect of parity was different depending on the malaria transmission intensity (p -value for interaction 0.02). Compared with nulliparous women, the risks of treatment failure were lower in primiparous women in moderate (aHR 0.29, 95% CI 0.10 to 0.81, $p=0.02$) or high endemic areas (aHR 0.72, 95% CI 0.34 to 1.56, $p=0.41$); and in multiparous (i.e. parity ≥ 2) women in moderate (aHR 0.22, 95% CI 0.08 to 0.63, $p=0.005$) or high endemic areas (aHR 0.47, 95% CI 0.24 to 0.91, $p=0.03$). In low endemic areas, the risk was not different between nulliparous women and primiparous (aHR 0.84, 95% CI 0.45 to 1.55, $p=0.57$) or multiparous women (aHR 1.10, 95% CI 0.65 to 1.83, $p=0.73$).

As a sensitivity analysis, a model was developed without splitting the data (thus ignoring the violation of proportional hazards assumption). Compared with AL, the risk of PCR-corrected treatment failure was lower in ASAQ (aHR 0.27, 95% CI 0.14 to 0.52, $p<0.001$), ASMQ (aHR 0.56, 95% CI 0.34 to 0.94, $p=0.03$), DP (aHR 0.35, 95% CI 0.18 to

0.68, $p=0.002$) and AC (aHR 0.37, 95% CI 0.15 to 0.91, $p=0.03$), but higher in quinine monotherapy (aHR 6.11, 95% CI 2.57 to 14.54, $p<0.001$). The risk was not different in ASSP (aHR 2.05, 95% CI 0.38 to 11.03, $p=0.40$), artesunate monotherapy (aHR 0.64, 95% CI 0.34 to 1.23, $p=0.18$), AAP (aHR 0.31, 95% CI 0.06 to 1.61, $p=0.17$) or QC (aHR 0.48, 95% CI 0.04 to 5.24, $p=0.55$) (Appendix Table 4-3).

Another sensitivity analysis was conducted with *P. vivax* intercalated infection being censored on the day of *P. vivax* appearance (following the current WHO guidelines for non-pregnant populations). The estimates for this model were similar to the main analysis above (Appendix Table 4-4, 4-5).

Dose-response

The dose-response was analysed only for AL, which was at a higher risk of treatment failure than other ACTs. The dose-response of quinine was not investigated as quinine was administered based on body weight (i.e. patients received a very similar dose in mg/kg of body weight).

The same standard dose (total 2,880 mg of lumefantrine) was given to women ($n=1262$, from 11 study sites in eight studies) regardless of body weight, following the study protocols in each study. Twelve women did not have information on body weight and were thus excluded from the analyses. Overall, there was no apparent dose-response (aHR 0.99 per 1 mg/kg of lumefantrine, 95% CI 0.96 to 1.02, $p=0.59$) and only baseline parasitaemia (aHR

Table 4-5. The effect of lumefantrine dose on the risk of PCR-corrected treatment failure for each malaria transmission intensity area

Characteristic	Proportion	Univariable*		Multivariable*	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Lumefantrine dose (mg/kg)					
Low transmission	19/190	0.94 (0.88-1.00)	0.048	0.94 (0.88-1.00)	0.048
Moderate transmission	30/758	1.00 (0.96-1.05)	0.84	1.00 (0.96-1.05)	0.84
High transmission	18/302	1.04 (0.97-1.11)	0.29	1.04 (0.97-1.11)	0.29

CI: confidence interval, HR: hazard ratio. *Adjusted for dose, parasitaemia and previous antimalarial exposure. The p-value for interaction between lumefantrine dose and malaria transmission intensity was 0.088.

2.03 per 10-fold increase, 95% CI 1.54 to 2.69, $p < 0.001$) was associated with a higher risk of PCR-corrected treatment failure (Appendix Table 4-6). However, when the dose effect was assessed for each malaria transmission intensity area (p-value for interaction 0.09), a linear dose-response was indicated in the low transmission, but not in moderate or high endemic areas (Table 4-5). In low transmission intensity areas, women given a higher dose (i.e. women with lower body weight) were associated with a lower risk of PCR-corrected treatment failure (aHR 0.94, 95% CI 0.88 to 1.00, $p = 0.048$).

Parasite clearance

Parasite clearance was analysed by mixed-effects logistic regression, including those with recurrence without PCR. The risk of positive parasitaemia on day 1 (adjusted by baseline parasitaemia) was higher in quinine monotherapy (odds ratio [OR] 5.49, 95% CI 3.28 to 9.19, $p < 0.001$) and QC (OR 2.05, 95% CI 0.61 to 9.19, $p = 0.25$) than AL (Table 4-6). The risk was lower in ASAQ (OR 0.30, 95% CI 0.21 to 0.43, $p < 0.001$) and ASMQ (OR 0.43, 95% CI 0.32 to 0.58, $p < 0.001$), DP (OR 0.35, 95% CI 0.25 to 0.50, $p < 0.001$) and ASSP (OR 0.31, 95% CI 0.14 to 0.65, $p = 0.002$). Other baseline characteristics associated with a higher risk of positive

Table 4-6. Univariable and multivariable logistic regression of the risk of positive parasitaemia on day 1

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
AL	375/1174	Reference		Reference	
ASAQ	70/837	0.30 (0.21-0.43)	<0.001	0.30 (0.21-0.43)	<0.001
ASMQ	164/1014	0.43 (0.32-0.58)	<0.001	0.43 (0.32-0.57)	<0.001
DP	120/866	0.35 (0.25-0.50)	<0.001	0.35 (0.25-0.50)	<0.001
ASSP	32/174	0.31 (0.14-0.65)	0.002	0.29 (0.13-0.61)	0.001
AS	181/227	1.02 (0.50-2.11)	0.95	0.95 (0.46-1.96)	0.88
AC	99/120	0.95 (0.43-2.07)	0.89	0.74 (0.33-1.66)	0.47
AAP	72/92	1.03 (0.37-2.89)	0.96	0.95 (0.33-2.71)	0.92
Q	172/263	5.49 (3.28-9.19)	<0.001	6.02 (3.51-10.31)	<0.001
QC	53/63	2.05 (0.61-6.87)	0.25	1.92 (0.57-6.46)	0.29
EGA	1338/4827	1.02 (1.00-1.03)	0.04	1.02 (1.00-1.03)	0.04
Age (years)	1338/4830	0.98 (0.96-1.00)	0.02		
Gravidity 1	519/1737	Reference			
2	285/1112	0.73 (0.58-0.93)	0.01		
≥3	521/1954	0.78 (0.63-0.97)	0.03		
Parity 0	611/2079	Reference		Reference	
1	251/1018	0.69 (0.54-0.89)	0.005	0.69 (0.54-0.90)	0.01
≥2	463/1704	0.83 (0.67-1.04)	0.10	0.85 (0.67-1.08)	0.18
Height (cm)	997/4162	1.01 (0.99-1.02)	0.33		
Weight (kg)	1326/4815	1.00 (0.99-1.01)	0.94		
BMI	987/4149	0.97 (0.93-1.01)	0.11		
Fever (temperature >37.5)	279/476	1.37 (1.00-1.87)	0.05		
no fever	1036/4279	Reference			
Haemoglobin on day 0 (g/dL)	1327/4800	0.91 (0.85-0.98)	0.01	0.92 (0.86-0.99)	0.02
HIV infection	9/45	0.53 (0.20-1.39)	0.20		
negative/not confirmed	1329/4785	Reference			
Parasitaemia (log ₁₀ /μL)	1338/4830	5.28 (4.60-6.05)	<0.001	5.02 (4.36-5.78)	<0.001
Hyperparasitaemia	59/70	0.70 (0.30-1.63)	0.41		
No	1279/4760	Reference			
Presence of gametocytes	88/186	1.07 (0.67-1.70)	0.78		
no gametocytes	1219/4557	Reference			
Mixed infection	36/50	1.00 (0.40-2.52)	1.00		
mono-infection	1302/4780	Reference			
Previous history of malaria					
no treatment	957/4228	Reference			
recrudescence	90/99	2.47 (1.06-5.76)	0.04		
other antimalarial use	154/209	0.94 (0.57-1.55)	0.80		
first ANC visit	136/293	0.94 (0.59-1.50)	0.80		
Malaria transmission intensity					
Low	804/1276	Reference			
Moderate	354/2507	1.04 (0.56-1.91)	0.91		
High	180/1047	0.19 (0.04-1.02)	0.05		

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for treatment and parasitaemia.

parasitaemia on day 1 included later pregnancy (OR 1.02 per gestational week, 95% CI 1.00 to 1.03, $p=0.04$), younger age (OR 0.98 per year, 95% CI 0.96 to 1.00, $p=0.02$), primigravida/nullipara, presence of fever (OR 1.37, 95% CI 1.00 to 1.87, $p=0.05$), lower haemoglobin on day 0 (OR 0.91 per g/dL, 95% CI 0.85 to 0.98, $p=0.01$) and higher parasitaemia (OR 5.28 per 10-fold increase, 95% CI 4.60 to 6.05, $p<0.001$).

When adjusted for EGA, parity, baseline haemoglobin and baseline parasitaemia, the risk of positive parasitaemia on day 1 was higher in quinine monotherapy (adjusted OR [aOR] 6.02, 95% CI 3.51 to 10.31, $p<0.001$), and lower in ASAQ (aOR 0.30, 95% CI 0.21 to 0.43, $p<0.001$), ASMQ (aOR 0.43, 95% CI 0.32 to 0.57, $p<0.001$), DP (aOR 0.35, 95% CI 0.25 to 0.50, $p<0.001$) and ASSP (aOR 0.29, 95% CI 0.13 to 0.61, $p=0.001$) than AL. After adjustment, later pregnancy (aOR 1.02 per gestational week, 95% CI 1.00 to 1.03, $p=0.04$), lower haemoglobin on day 0 (aOR 0.92 per g/dL, 95% CI 0.86 to 0.99, $p=0.02$) and higher parasitaemia (aOR 5.02 per 10-fold increase, 95% CI 4.36 to 5.78, $p<0.001$) were associated with a higher risk of positive parasitaemia on day 1. The risk was lower in primiparous (aOR 0.69, 95% CI 0.54 to 0.90, $p=0.01$) or multiparous women (aOR 0.85, 95% CI 0.67 to 1.08, $p=0.18$) than nulliparous women.

The risk of positive parasitaemia on day 2 (adjusted by baseline parasitaemia) was higher in quinine monotherapy (OR 20.04, 95% CI 8.92 to 45.01, $p<0.001$), QC (OR 19.32, 95% CI 6.79 to 54.94, $p<0.001$) and AC (OR 2.22, 95% CI 1.20 to 4.09, $p=0.01$) than AL (Table 4-7). The risks were not different in other ACTs. Other baseline characteristics

associated with a higher risk of positive parasitaemia on day 2 included later pregnancy (OR 1.03 per gestational week, 95% CI 1.00 to 1.05, $p=0.03$) and higher parasitaemia (OR 3.49 per 10-fold increase, 95% CI 2.90 to 4.20, $p<0.001$).

When adjusted for EGA, weight and baseline parasitaemia, the risk of positive parasitaemia on day 2 was higher in quinine monotherapy (aOR 33.63, 95% CI 13.63 to 82.96, $p<0.001$) and QC (aOR 22.92, 95% CI 7.90 to 66.48, $p<0.001$) than AL. The risk of positive parasitaemia on day 2 was higher in DP but not significant (aOR 2.72, 95% CI 0.57 to 12.91, $p=0.21$). Higher body weight was associated with a lower risk of positive parasitaemia on day 2 in the adjusted analysis (aOR 0.97 per kg, 95% CI 0.94 to 0.99, $p=0.01$). After adjustment, later pregnancy (aOR 1.04 per gestational week, 95% CI 1.01 to 1.06, $p=0.005$) and higher parasitaemia (aOR 3.60 per 10-fold increase, 95% CI 2.97 to 4.35, $p<0.001$) remained associated with a higher risk of positive parasitaemia on day 2.

On day 3, parasitaemia was negative in all patients treated with ASAQ ($n=851$), ASMQ ($n=1021$) and ASSP ($n=174$) and also in women in high transmission intensity areas ($n=1089$). Positive parasitaemia on day 3 was observed in AL (0.9%, 11/1214), DP (0.7%, 6/874), artesunate monotherapy (6.7%, 15/225), AC (9.0%, 11/122), AAP (2.2%, 2/90), quinine monotherapy (16.4%, 43/262) and QC (32.8%, 20/61). Further analyses were not conducted.

Table 4-7. Univariable and multivariable logistic regression of the risk of positive parasitaemia on day 2

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
AL	45/1212	Reference		Reference	
ASAQ	3/843	0.85 (0.16-4.67)	0.85	1.02 (0.19-5.61)	0.98
ASMQ	11/1019	1.04 (0.36-3.03)	0.95	1.35 (0.44-4.11)	0.60
DP	25/871	2.32 (0.49-11.06)	0.29	2.72 (0.57-12.91)	0.21
ASSP	4/175	0.97 (0.16-5.77)	0.98	1.23 (0.21-7.41)	0.82
AS	68/225	1.46 (0.83-2.56)	0.19	1.47 (0.83-2.60)	0.19
AC	47/120	2.22 (1.20-4.09)	0.01	1.79 (0.93-3.41)	0.08
AAP	20/91	1.27 (0.47-3.42)	0.63	1.76 (0.60-5.13)	0.30
Q	93/259	20.04 (8.92-45.01)	<0.001	33.63 (13.63-82.96)	<0.001
QC	39/61	19.32 (6.79-54.94)	<0.001	22.92 (7.90-66.48)	<0.001
EGA	355/4873	1.03 (1.00-1.05)	0.03	1.04 (1.01-1.06)	0.005
Age (years)	355/4876	0.99 (0.97-1.01)	0.45		
Gravidity 1	108/1762	Reference			
2	86/1119	1.44 (0.94-2.19)	0.09		
≥3	160/1968	1.08 (0.75-1.54)	0.68		
Parity 0	124/2108	Reference			
1	86/1023	1.26 (0.84-1.89)	0.27		
≥2	144/1714	1.04 (0.73-1.48)	0.85		
Height (cm)	220/4205	0.99 (0.96-1.03)	0.75		
Weight (kg)	349/4860	0.98 (0.95-1.00)	0.06	0.97 (0.94-0.99)	0.01
BMI	215/4192	1.00 (0.92-1.09)	0.95		
Fever (temperature >37.5)	117/476	1.38 (0.94-2.02)	0.10		
no fever	235/4295	Reference			
Haemoglobin on day 0 (g/dL)	352/4843	1.04 (0.94-1.15)	0.49		
HIV infection	4/45	1.86 (0.48-7.13)	0.37		
negative/not confirmed	351/4831	Reference			
Parasitaemia (log ₁₀ /μL)	355/4876	3.49 (2.90-4.20)	<0.001	3.60 (2.97-4.35)	<0.001
Hyperparasitaemia	30/72	1.10 (0.51-2.35)	0.82		
No	325/4804	Reference			
Presence of gametocytes	31/190	1.05 (0.63-1.77)	0.84		
no gametocytes	314/4599	Reference			
Mixed infection	16/48	0.88 (0.40-1.96)	0.76		
mono-infection	339/4828	Reference			
Previous history of malaria					
no treatment	189/4279	Reference			
recrudescence	36/95	1.57 (0.90-2.74)	0.11		
other antimalarial use	71/209	1.31 (0.86-2.02)	0.21		
first ANC visit	59/292	1.33 (0.81-2.19)	0.26		
Malaria transmission intensity					
Low	333/1272	Reference			
Moderate	17/2521	1.03 (0.35-3.03)	0.96		
High	5/1083	0.18 (0.02-2.02)	0.16		

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for treatment and parasitaemia.

Fever clearance

Body temperature was assessed on day 0 (17 studies), 1 (14 studies), 2 (14 studies) and 3 (15 studies). Only 15.1% (95% CI 10.5% to 20.3%, 514/4964) of the women were febrile ($>37.5^{\circ}\text{C}$) on day 0, but the proportion of febrile women pooled by random effects was higher in low malaria transmission areas (24.5%, 95% CI 18.9% to 30.6 %) compared with moderate (7.2%, 95% CI 3.7% to 11.7%) or high (8.3%, 95% CI 3.7% to 14.5%) transmission areas. After treatment, 1.8% (95% CI 0.9% to 3.0%, 103/4509) of the women were febrile on day 1, but the majority were afebrile on day 2 (0.0%, 17/4420) and day 3 (0.0%, 13/4207). The proportions of febrile patients for each drug on day 0, 1, 2 and 3 are summarized in Table 4-8. Further analyses were not conducted because of the small number of febrile women and different policies on antipyretic drug use in different studies.

Table 4-8. Proportions of febrile women on day 0 to day 3 after different treatment, pooled by random effects

Treatment	Pooled proportion (95% CI) of febrile women ($>37.5^{\circ}\text{C}$)			
	Day 0	Day 1	Day 2	Day 3
AL	13.7 (7.0–22.1)	2.1 (0.1–5.7)	0.0 (0.0–0.2)	0.0 (0.0–0.3)
ASAQ	7.3 (2.3–14.6)	0.1 (0.0–1.3)	0.0 (0.0–0.4)	0.0 (0.0–0.2)
ASMQ	8.2 (4.1–13.3)	1.5 (0.2–3.7)	0.3 (0.0–0.9)	0.0 (0.0–0.5)
DP	4.5 (1.2–9.5)	0.6 (0.0–2.2)	0.0 (0.0–0.3)	0.0 (0.0–0.0)
ASSP	25.6 (18.7–34.1)	4.2 (1.8–9.5)	2.5 (0.8–7.0)	1.7 (0.5–6.0)
AS	26.2 (13.7–40.7)	6.2 (3.0–10.1)	0.0 (0.0–0.6)	0.1 (0.0–2.7)
AC	14.1 (0.5–37.0)	No data	No data	No data
AAP	24.7 (7.6–46.9)	10.9 (1.4–25.9)	0.0 (0.0–2.5)	0.0 (0.0–7.9)
Q	17.8 (13.1–22.9)	2.9 (0.0–8.4)	0.1 (0.0–1.7)	0.0 (0.0–0.9)
QC	19.7 (11.9–30.8)	6.5 (2.5–15.4)	1.9 (0.3–9.9)	2.8 (0.5–14.2)

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, Q: Quinine monotherapy, QC: Quinine with clindamycin.

Gametocyte development after treatment

The proportion of patients with gametocytaemia on different days among those who were without gametocytaemia on day 0 is summarized in Table 4-9. Further analyses of the risk of gametocyte development among women without gametocytaemia on day 0 were conducted by categorizing treatments into ABT and QBT because of the small number of outcomes in each treatment. Overall, the Kaplan-Meier estimates of women who were without gametocytaemia at baseline but in whom it developed within 28 days pooled by random effects were 3.7% (95% CI 2.2% to 5.8%, 143/4256) after ABT and 20.6% (95% CI 6.9% to 39.3%, 37/260) after QBT.

Table 4-9. Proportions of women with gametocytaemia after different treatment on day 1-6 and day 7-31 in women without gametocytaemia on day 0, pooled by random effects

Treatment	Pooled proportion (95% CI) of women with positive gametocytaemia	
	Day 1-6	Day 7-31
Women without gametocytaemia on day 0		
AL	2.4 (0.7-5.0)	0.0 (0.0-0.5)
ASAQ	2.3 (0.4-5.5)	0.9 (0.2-2.1)
ASMQ	1.1 (0.0-3.2)	0.0 (0.0-0.2)
DP	1.8 (0.0-5.3)	0.2 (0.0-1.3)
ASSP	0.9 (0.2-4.7)	0.0 (0.0-3.2)
AS	12.4 (5.7-20.6)	2.0 (0.0-5.8)
AC	3.6 (0.5-8.5)	1.3 (0.0-5.5)
AAP	7.8 (2.6-14.8)	0.0 (0.0-2.4)
Q	16.9 (3.4-35.9)	9.8 (0.0-35.5)
QC	18.6 (10.7-30.4)	11.6 (5.1-24.5)

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, Q: Quinine monotherapy, QC: Quinine with clindamycin.

In the univariable analysis, the risk of developing gametocytaemia was higher after QBT than ABT on day 1–6 (OR 2.34, 95% CI 1.31 to 4.20, $p=0.004$) and on day 7–31 (OR 12.17, 95% CI 4.23 to 35.04, $p<0.001$) (Table 4-10, 4-11). In the multivariable analyses adjusted for other risk factors, the risk of developing gametocytaemia remained higher after QBT than ABT on day 1–6 (aOR 2.06, 95% CI 1.14 to 3.73, $p=0.02$) and on day 7–31 (aOR 13.22, 95% CI 4.60 to 37.97, $p<0.001$). On day 1–6, lower haemoglobin on day 0 (aOR 0.86, 95% CI 0.77 to 0.97, $p=0.01$) and higher baseline parasitaemia (aOR 1.87 per 10-fold

Table 4-10. Univariable and multivariable logistic regression of the risk of positive gametocytaemia on day 1–6 among women without gametocytaemia on day 0

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
Artemisinin-based treatment	125/4182	Reference		Reference	
Quinine-based treatment	31/259	2.34 (1.31-4.20)	0.004	2.06 (1.14-3.73)	0.02
EGA	156/4438	0.97 (0.94-1.00)	0.03	0.96 (0.93-0.99)	<0.001
Age group <20	51/1405	Reference			
20-24	47/1406	0.79 (0.52-1.20)	0.26		
25-29	28/879	0.62 (0.38-1.01)	0.05		
≥30	30/751	0.63 (0.39-1.03)	0.07		
Gravidity 1	60/1569	Reference			
2	37/1029	0.89 (0.58-1.38)	0.61		
≥3	59/1838	0.61 (0.42-0.90)	0.01		
Parity 0	65/1884	Reference			
1	40/949	1.00 (0.66-1.53)	0.98		
≥2	51/1603	0.62 (0.42-0.91)	0.02		
Height (cm)	108/3908	1.00 (0.96-1.03)	0.83		
Weight (kg)	155/4427	0.98 (0.96-1.01)	0.16		
BMI	107/3896	1.00 (0.92-1.08)	0.92		
Fever (temperature >37.5)	34/405	1.85 (1.21-2.83)	0.005		
no fever	120/3996	Reference			
Haemoglobin on day 0 (g/dL)	156/4410	0.86 (0.77-0.97)	0.01	0.86 (0.77-0.97)	0.01
HIV infection	1/30	1.02 (0.13-7.99)	0.99		
negative/not confirmed	155/4411	Reference			
Parasitaemia (log ₁₀ /μL)	156/4441	1.81 (1.50-2.19)	<0.001	1.87 (1.54-2.27)	<0.001
Hyperparasitaemia	8/60	3.31 (1.43-7.64)	0.01		
No	148/4381	Reference			
Mixed infection	6/38	2.16 (0.84-5.55)	0.11		
mono-infection	150/4403	Reference			
Malaria transmission intensity					
Low	89/1080	Reference		Reference	
Moderate	25/2396	0.15 (0.06-0.38)	<0.001	0.20 (0.08-0.52)	0.001
High	42/965	0.36 (0.12-1.13)	0.08	0.44 (0.13-1.55)	0.203

BMI: body mass index, EGA: estimated gestational age, CI: confidence interval, OR: odds ratio. *Adjusted for treatment.

increase, 95% CI 1.54 to 2.27, $p<0.001$) were associated with a higher risk of developing gametocytaemia. Women in areas of moderate malaria transmission intensity were associated with lower risk (aOR 0.20, 95% CI 0.08 to 0.52, $p=0.001$) than women in areas of low transmission intensity. On day 7–31, higher baseline parasitaemia (aOR 2.07 per 10-fold increase, 95% CI 1.49 to 2.88, $p<0.001$) and lower body weight (aOR 0.95, 95% CI 0.90 to 0.99, $p=0.02$) were associated with a higher risk of developing gametocytaemia.

Table 4-11. Univariable and multivariable logistic regression of the risk of positive gametocytaemia on day 7–31 among women without gametocytaemia on day 0

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
Artemisinin-based treatment	34/4153	Reference		Reference	
Quinine-based treatment	17/233	12.17 (4.23-35.04)	<0.001	13.22 (4.60-37.97)	<0.001
EGA	51/4383	0.96 (0.92-1.01)	0.11		
Age group <20	19/1374	Reference			
20-24	16/1404	0.72 (0.36-1.46)	0.37		
25-29	6/867	0.35 (0.14-0.92)	0.03		
≥30	10/741	0.47 (0.20-1.09)	0.08		
Gravidity 1	25/1538	Reference			
2	10/1003	0.58 (0.27-1.25)	0.16		
≥3	16/1817	0.39 (0.20-0.76)	0.01		
Parity 0	26/1853	Reference			
1	10/945	0.61 (0.28-1.31)	0.20		
≥2	15/1582	0.45 (0.23-0.88)	0.02		
Height (cm)	40/3870	0.99 (0.93-1.04)	0.66		
Weight (kg)	50/4363	0.95 (0.90-1.00)	0.03	0.95 (0.90-0.99)	0.02
BMI	39/3859	0.86 (0.74-1.00)	0.05		
Fever (temperature >37.5)	8/400	1.15 (0.51-2.60)	0.73		
no fever	42/3936	Reference			
Haemoglobin on day 0 (g/dL)	51/4342	0.88 (0.72-1.06)	0.18		
HIV infection	0/31	omitted			
negative/not confirmed	51/4355				
Parasitaemia ($\log_{10}/\mu\text{L}$)	51/4386	2.07 (1.50-2.88)	<0.001	2.07 (1.49-2.88)	<0.001
Hyperparasitaemia	1/61	0.87 (0.11-7.03)	0.90		
No	50/4325	Reference			
Mixed infection	1/36	0.90 (0.11-7.20)	0.92		
mono-infection	50/4350	Reference			
Malaria transmission intensity					
Low	35/1051	Reference			
Moderate	9/2380	0.10 (0.02-0.52)	0.01		
High	7/955	0.51 (0.09-2.75)	0.43		

BMI: body mass index, EGA: estimated gestational age, CI: confidence interval, OR: odds ratio.

*Adjusted for treatment.

PCR-uncorrected treatment failure

The PCR-uncorrected treatment efficacy on day 28, 42 or 63 by the Kaplan-Meier method was pooled by random effects, for each malaria transmission intensity (Appendix Table 4-7). On day 28 in high transmission areas, the PCR-uncorrected treatment efficacy of quinine and AL was 42.0% (95% CI 27.7 to 59.8) and 89.6% (95% CI 84.9 to 93.3), respectively. The PCR-uncorrected efficacy was higher in lower endemic areas, but even in the low transmission areas, the day 28 PCR-uncorrected efficacy of quinine and AL was 79.4% (95% CI 68.6 to 88.3) and 93.8% (95% CI 81.6 to 99.0). The day 28 efficacy was >95% for all other ACTs regardless of transmission intensity.

Adverse events

The symptoms that developed within a week after starting treatment were analysed by mixed-effects logistic regression on nine commonly assessed symptoms (Figure 4-3).

The risk of abdominal pain was higher in ASAQ (aOR 2.03, 95% CI 1.40 to 2.96, $p<0.001$), quinine (aOR 1.81, 95% CI 1.07 to 3.04, $p=0.03$), and ASMQ (aOR 1.44, 95% CI 1.03 to 2.02, $p=0.03$) than AL (Appendix Table 4-8). No other baseline characteristics (except the presence of headache on day 0) were associated with the risk of headache.

The risk of anorexia was higher in quinine (aOR 4.29, 95% CI 1.99 to 9.23, $p<0.001$), ASAQ (aOR 3.88, 95% CI 2.36 to 6.38, $p<0.001$), QC (aOR 3.41, 95% CI 1.19 to 9.79, $p=0.02$), and ASMQ (aOR 2.41, 95% CI 1.53 to 3.79, $p<0.001$) than AL (Appendix

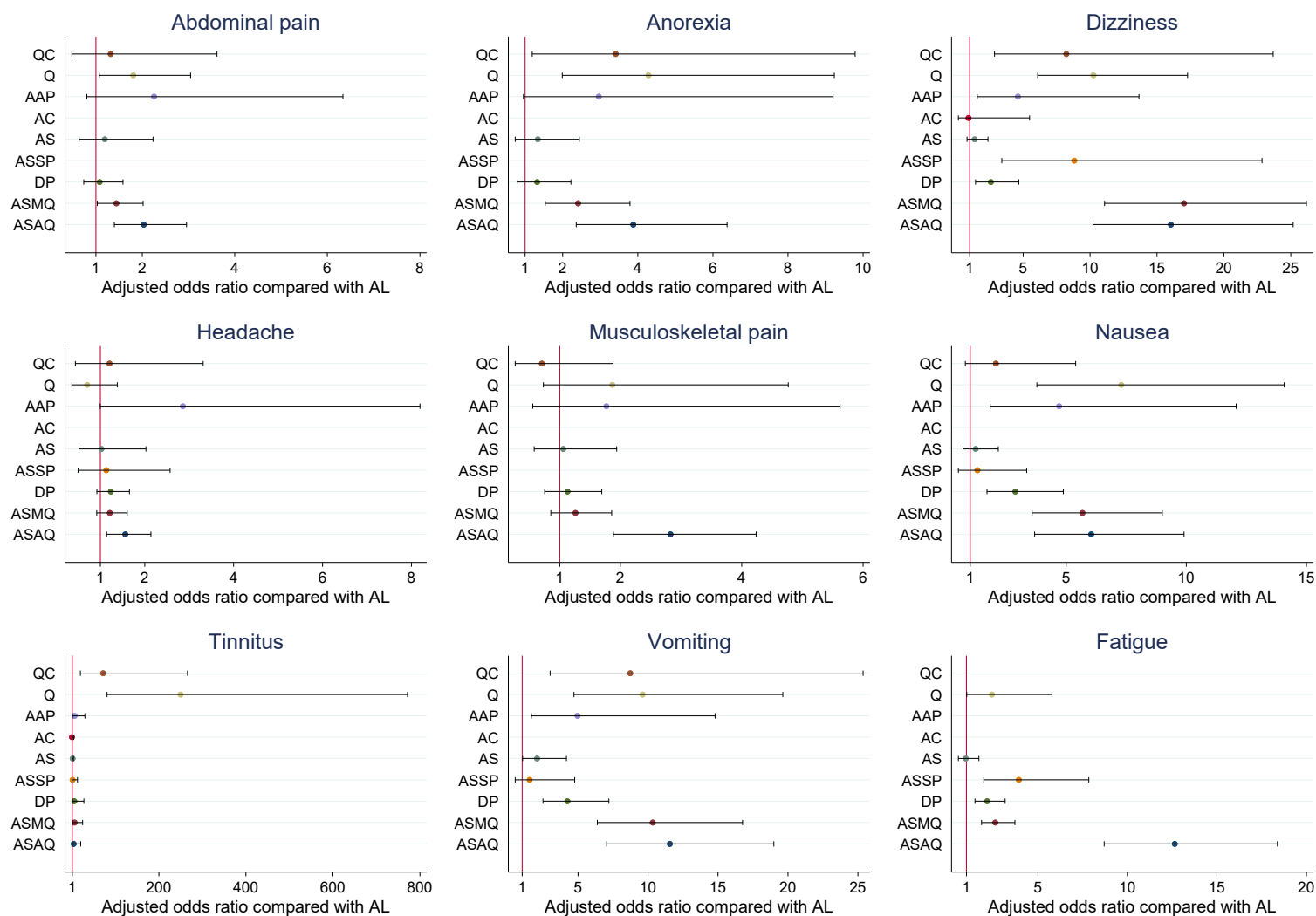


Figure 4-3. Adjusted odds ratio of developing adverse symptoms after treatment compared with artemether-lumefantrine

Table 4-9). Higher parasitaemia was associated with an increased risk of anorexia (aOR 1.23 per 10-fold increase, 95% CI 1.07 to 1.40, $p=0.003$). Younger age (< 20 years old) was associated with a higher risk of anorexia.

The risk of dizziness was higher in ASMQ (aOR 17.03, 95% CI 11.08 to 26.16, $p<0.001$), ASAQ (aOR 16.04, 95% CI 10.21 to 25.18, $p<0.001$), quinine (aOR 10.25, 95% CI 6.08 to 17.28, $p<0.001$), ASSP (aOR 8.82, 95% CI 3.40 to 22.85, $p<0.001$), QC (aOR 8.22, 95% CI 2.86 to 23.68, $p<0.001$), AAP (aOR 4.60, 95% CI 1.55 to 13.65, $p=0.01$), and DP (aOR 2.58, 95% CI 1.43 to 4.67, $p=0.02$) than AL (Appendix Table 4-10). Women with hyperparasitaemia (aOR 2.26, 95% CI 1.08 to 4.74, $p=0.03$) were associated with a higher risk of dizziness. EGA was associated with decreased risk of dizziness (aOR 0.98 per week, 95% CI 0.97 to 1.00, $p=0.04$). Compared with nulliparous women, multiparous women (aOR 1.33, 95% CI 1.09 to 1.63, $p=0.01$) were more likely to have dizziness but similar in primiparous women (aOR 1.14, 95% CI 0.90 to 1.45, $p=0.27$).

The risk of headache was higher in ASAQ (aOR 1.56, 95% CI 1.14 to 2.14, $p=0.01$) than AL (Appendix Table 4-11). No other baseline characteristics (except the presence of headache on day 0) were associated with the risk of headache.

The risk of musculoskeletal pain was uniquely higher in ASAQ (aOR 2.83, 95% CI 1.88 to 4.24, $p<0.001$) than AL (Appendix Table 4-12). Women with fever on day 0 (aOR 1.58, 95% CI 1.11 to 2.24, $p=0.01$) and women in later pregnancy (aOR 1.02 per week, 95% CI 1.00 to 1.04, $p=0.03$) were more likely to have musculoskeletal pain. Compared with

nulliparous women, multiparous women (aOR 1.45, 95% CI 1.14 to 1.85, $p=0.003$) were more likely to have musculoskeletal pain but similar in primiparous women (aOR 1.08, 95% CI 0.80 to 1.45, $p=0.61$).

The risk of nausea was higher in quinine (aOR 7.29, 95% CI 3.78 to 14.08, $p<0.001$), ASAQ (aOR 6.04, 95% CI 3.68 to 9.91, $p<0.001$), ASMQ (aOR 5.68, 95% CI 3.58 to 9.00, $p<0.001$), AAP (4.70, 95% CI 1.83 to 12.07, $p=0.001$), and DP (aOR 2.88, 95% CI 1.70 to 4.88, $p<0.001$) than AL (Appendix Table 4-13). The risk of nausea was higher in QC (aOR 2.07, 95% CI 0.80 to 5.39, $p=0.14$) but not significant. Women of higher age (aOR 1.02 per year, 95% CI 1.01 to 1.04, $p=0.01$) and women in earlier pregnancy (aOR 0.98, 95% CI 0.97 to 1.00, $p=0.05$) were more likely to develop nausea.

The risk of tinnitus was higher in quinine (aOR 249.84, 95% CI 80.90 to 771.56, $p<0.001$) and QC (aOR 71.91, 95% CI 19.45 to 265.86, $p<0.001$) than AL (Appendix Table 4-14). Among ACTs, the risk was higher in AAP (aOR 6.29, 95% CI 1.33 to 29.86, $p=0.02$), ASMQ (aOR 6.22, 95% CI 1.56 to 24.82, $p=0.01$), DP (aOR 5.75, 95% CI 1.19 to 27.77, $p=0.03$), and ASAQ (aOR 4.24, 95% CI 0.89 to 20.29, $p=0.07$) than AL. No other baseline characteristics (except the presence of tinnitus on day 0) were associated with the risk of tinnitus.

The risk of vomiting was higher in ASAQ (aOR 11.56, 95% CI 7.04 to 19.00), ASMQ (aOR 10.34, 95% CI 6.38 to 16.76, $p<0.001$), quinine (aOR 9.61, 95% CI 4.70 to 19.63, $p<0.001$) and QC (aOR 8.73, 95% CI 3.00 to 25.37, $p<0.001$) than AL (Appendix

Table 4-15). To a lesser extent, the risk was also higher in AAP (aOR 4.96, 95% CI 1.667 to 14.80, $p=0.004$), DP (aOR 4.24, 95% CI 2.49 to 7.21, $p<0.001$), and AS (aOR 2.07, 95% CI 1.03 to 4.17, $p=0.04$). Women in later pregnancy (aOR 0.98, 95% CI 0.96 to 1.00, $p=0.03$) were associated with a lower risk of vomiting.

The risk of fatigue was particularly higher in ASAQ (aOR 12.65, 95% CI 8.70 to 18.38, $p<0.001$) than AL (Appendix Table 4-16). To a lesser extent, the risk was also higher in ASSP (aOR 3.93, 95% CI 1.97 to 7.84, $p<0.001$), ASMQ (aOR 2.62, 95% CI 1.84 to 3.71, $p<0.001$), quinine (aOR 2.43, 95% CI 1.02 to 5.78, $p=0.05$), and DP (aOR 2.16, 95% CI 1.48 to 3.16, $p<0.001$). Women of higher age (aOR 1.03 per year, 95% CI 1.01 to 1.04, $p<0.001$) and lower baseline haemoglobin (aOR 0.93 per g/dL, 95% CI 0.87 to 1.00, $p=0.04$) were more likely to develop fatigue.

4.4 Discussion

This IPD meta-analysis, which included 92% of the published data from 10 different countries, provides compelling evidence that quinine monotherapy should be withdrawn from the recommendation for pregnant women. The efficacy of evaluated antimalarials was $>95\%$ except for quinine monotherapy ($<90\%$ after day 28) and AL on day 63 (93.6%); the former is recommended for the first trimester and the latter is the most widely used ACT in pregnancy. For both drugs, the difference remained even when adjusted for baseline parasitaemia loads and parity. The slightly lower efficacy of AL might be due to its under-dosing in pregnant women, which was confirmed in pharmacokinetic studies (as the lower blood level of

lumefantrine on day 7) in some areas²³⁴ but was not consistent across different populations.³¹⁴

Although the pooled point estimates of the efficacy of ASSP was >95%, the adjusted risk of treatment failure was even higher than AL. This apparent inconsistency was because most treatment failure after ASSP took place between 28 and 42 days in a study, which did not follow up for 42 days. Therefore, these failures were not captured by pooling efficacy at fixed time points but properly included in the survival analyses. ASSP virtually worked as artesunate monotherapy in areas of high prevalence of SP resistance. Considering the extent of SP resistance,²⁸⁷ ASSP, like artesunate monotherapy, should be withdrawn from the recommendation for pregnant women. As was shown in non-pregnant populations,³⁴⁸ adding clindamycin improved the efficacy of quinine. QC can be a second-line option for pregnant women, although supervision would be required.

Higher baseline parasitaemia loads were associated with a higher risk of PCR-corrected treatment failure as is known in non-pregnant populations. Nulliparous women were more likely to experience treatment failure in moderate and high endemic areas, but this effect, which was largely due to pregnancy-specific (parity-dependent) immunity, was not observed in low endemic areas as expected. These findings imply that in an era of declining malaria endemicity, pregnant women may lose pregnancy-specific immunity and the risk of treatment failure may increase in areas of currently higher transmission.⁵⁷ Active screening, together with early and effective treatment will potentially lower the risk of treatment failure, and thus protect mothers and fetuses, particularly in this situation.

As with PCR-corrected treatment efficacy, the risk of recurrent falciparum malaria (i.e. PCR-uncorrected failure) was high after AL and quinine. As was expected from the drug half-life (i.e. post-treatment prophylactic effect), the PCR-uncorrected efficacy of AL decreased rapidly over time, while recurrences of other ACTs were predominantly observed after day 42. As the recurrence of malaria can affect the fetus cumulatively,^{31,349} PCR-uncorrected treatment efficacy can be important when choosing the most appropriate drug for pregnant women, particularly in high transmission settings.

Parasite clearance was fast after treatment with ACTs. By day 3, more than 99% of patients had cleared peripheral parasitaemia after ACTs, while more than 10% of the women treated with quinine or QC were still positive. Although it has been shown in many studies that parasite clearance is faster after treatment with artemisinin derivatives than quinine,³⁵⁰ this difference is important bearing in mind the adherence of quinine (with clindamycin) over seven days of treatment and the higher risks of adverse symptoms discussed later. Parasite clearance was slower in women with higher baseline parasitaemia and women treated with AL (than in women treated with other ACTs), as was shown in a large IPD meta-analysis for non-pregnant populations,³⁵¹ although in the vast majority of women treated with AL, parasitaemia had cleared by day 3 when the treatment was completed. Later pregnancy (higher EGA) was associated with slower clearance, which might be because elimination of drugs generally increases as the gestation progresses.³⁵²

The use of QBT leads to a higher risk of gametocyte development than ABT, since

artemisinin derivatives have a gametocidal effect but quinine does not.^{143,353} Lower baseline haemoglobin and lower body weight were associated with a higher risk of immediate and later development of gametocytes, respectively. Together with higher baseline parasitaemia, these three factors were shown to be associated with a higher risk of gametocyte carriage after treatment with ACT in non-pregnant populations.^{354,355} Lower baseline haemoglobin may reflect a more extended period of infection before treatment²² and can also stimulate gametocyte development by itself.³⁴ The proportion of women who developed gametocytaemia after ABT was 3.7% (95% CI 2.2 to 5.8%) even excluding those who had recurrent parasitaemia within 28 days. This was higher than reported in non-pregnant populations (1.9%, 95% CI 1.7 to 2.1),³⁵⁴ which supports a previous notion that pregnant women can be important reservoirs of malaria parasites.³⁵⁶ In pregnant women, this is further complicated by earlier findings that SP (given as IPTp) does not clear gametocytes³⁵⁷ but can increase infectivity³⁵⁸ by altering the sex ratio of the gametocytes.^{356,359,360}

QBT was associated with higher risks of abdominal pain, anorexia, dizziness, nausea, tinnitus and vomiting, than AL, limiting the practical use of QBT in the field without supervision. Tolerability is important since pregnant women infected with malaria were generally less symptomatic. This poor adherence is particularly problematic considering that QBT is currently recommended for women in the first trimester when suffering from morning sickness: higher risks of nausea and vomiting were also confirmed to be associated with earlier pregnancy in this study.

Among ACTs, AL followed by DP were the drugs with the lowest frequency of adverse symptoms with risks similar to those of artesunate monotherapy (i.e. no partner drugs). Musculoskeletal pain and fatigue/weakness were uniquely associated with ASAQ. Similarly, the risk of anorexia, nausea, vomiting and dizziness was higher in ASAQ and ASMQ than in AL or DP. All studies included in this pooled analysis were open-label; therefore, the complaints of adverse symptoms reported by women (and also the way investigators asked the question) could have been affected by knowledge or previous experiences of the given drug. Pregnancy outcomes are described in detail in Chapter 6, but there was no difference among the four major ACTs (AL, ASAQ, ASMQ and DP).

This study has some limitations. Firstly, although the efficacy of ACTs shown in this study was satisfactory, this summary is not directly applicable to specific areas with different prevalence of drug resistance. While the data from Southeast Asia included in this study were conducted before the widespread artemisinin resistance in the area, the current spread of resistance in the greater sub-Mekong region will affect the general recommendation of antimalarials, requiring some other forms of treatment (such as triple combination ACT, ACT combined with quinine¹³⁷ or extended administration of ACT³⁶¹). Sensitivity to the partner drugs differs depending on the location and study year, although a sensitivity analysis in which one study site was removed at a time did not change the conclusion of the IPD meta-analysis regardless of study design or study quality (data not shown). Nine studies^{193,198,221,231,239,247,256,266,308} (8% of IPD) were not included. However, the pooled

efficacy at the fixed time points was almost the same when aggregated data of those unshared studies were included. These confirm the robustness of our IPD meta-analysis as the current global summary.

Secondly, the women were mostly enrolled at ANCs by screening peripheral parasitaemia; thus the women included in this pooled analysis were more likely to be asymptomatic and with lower baseline parasitaemia than most of the antimalarial efficacy studies in non-pregnant populations.³⁵⁴ Thus, the efficacy at fixed time points may not be directly comparable to the results in non-pregnant populations. Indeed, as baseline parasitaemia is a known risk factor for PCR-corrected treatment failure, the pooled efficacy at fixed time points in this study could have been overestimated. Continuous monitoring of the efficacy of antimalarials, particularly AL, in pregnant women is needed.

4.5 Conclusions

This IPD meta-analysis provides compelling evidence that quinine monotherapy should be withdrawn from the recommendation for pregnant women. Quinine monotherapy is associated with higher risks of both PCR-corrected and PCR-uncorrected treatment failure. Although the efficacy of QC was as high as that of ACTs, the practical use of QBT is less favourable considering the slow parasite clearance and the longer treatment period, complicated by higher risk of adverse symptoms. The higher risk of gametocyte development after QBT makes them even less favourable, particularly bearing in mind concerns about the higher risk of gametocyte development in pregnant women. Although AL has fewer adverse

symptoms, its efficacy can be lower than other ACTs with the current dosing. In addition, the post-treatment prophylactic period of AL is shorter, leading to a higher risk of recurrences, which can cumulatively affect the fetus. Early detection and treatment with highly effective drugs, particularly in nulliparous women and all women in low endemic areas, can reduce the risk of recurrent malaria, leading to better outcomes for both mothers and fetuses.

Chapter 5. Optimal duration of follow-up for assessing antimalarial efficacy in pregnancy: a retrospective analysis of clinical trials on the Thailand-Myanmar border

Part of this chapter has been published in a peer-reviewed journal and is freely available as follows under the Creative Commons Attribution licence. Saito M, Mansoor R, Wiladphaingern J, Paw MK, Pimanpanarak M, Proux S, Guérin PJ, White NJ, Nosten F, McGready R. Optimal duration of follow-up for assessing antimalarial efficacy in pregnancy: a retrospective analysis of a cohort followed up until delivery on the Thailand-Myanmar border. *Open Forum Infect Dis.* 2019;6(7):ofz264.

5.1 Introduction

Effective antimalarial medicines are required to limit the adverse effects of malaria in pregnancy, which affects both the mother and fetus. Global implementation of highly effective ACTs has changed the treatment of falciparum malaria dramatically.¹⁹ The efficacy of antimalarial drugs needs to be monitored because of the continued emergence and/or spread of resistance. WHO recommends that antimalarial policy be changed if the efficacy of treatments is $\leq 90\%$.¹⁴⁰ A declining trend in efficacy over time requires closer monitoring,¹⁴⁰ particularly in vulnerable populations such as pregnant women.

According to the current WHO guidelines, in evaluating the efficacy of antimalarial treatment, patients should be followed up actively with clinical and parasitological assessments by microscopy to detect recurrence of *P. falciparum* malaria.¹⁷⁴ Recurrence of parasitemia can be either recrudescence of the same parasite “strain” that caused the initial infection (i.e. treatment failure) or reinfection with a new strain. Provided there is sufficient genetic diversity in the prevailing parasite populations, this is distinguished by PCR genotyping of polymorphic loci.¹⁴⁴ The currently recommended duration of follow-up for non-pregnant populations is a minimum of 28 days (for regimens containing amodiaquine, artemisinin derivatives, chloroquine, lumefantrine, quinine or SP) to 42 days (for piperazine and mefloquine).¹⁷⁴

Currently, there is no standardized guideline for assessing antimalarial efficacy in pregnancy and the guideline for non-pregnant patients is frequently adopted as was shown in Chapter 3-II.^{112,189} Both the efficacy and the optimal duration of follow-up could be different

in pregnant women because of altered immunity, physiological changes affecting antimalarial pharmacokinetics and a unique parasite haven in the placenta.^{43,112,190,310,322} It seems that pregnant women can harbour blood stage infections for protracted periods: previous studies have reported recrudescences more than 100 days after treatment in pregnant women both in high transmission areas in sub-Saharan Africa^{192,303,323} and also in lower transmission areas in Southeast Asia.^{194,310}

The objective of the study in this chapter was to characterize the factors that affect the time to recrudescence of *P. falciparum* infections in pregnancy and to predict what proportion of recrudescence infections would be detected by days 28, 42, and 63 for each treatment, after adjusting for other risk factors.

5.2 Methods

Study settings

This study includes secondary data collected prospectively in treatment efficacy studies on uncomplicated falciparum malaria in pregnancy.^{188,190,194,211,214,219,268,275,280,310} The studies included were all conducted on the Thailand-Myanmar border, where the transmission of falciparum malaria is low and unstable.^{170,171} In this setting, pregnant women presenting to the ANCs were screened for malaria by checking peripheral blood smear by microscopy every 1–2 weeks regardless of their symptoms. The pregnant women actively detected in this way were enrolled into prospective treatment studies, provided they gave fully informed consent, and were then followed-up with malaria screening until delivery (or 42–63 days, whichever

came later). The treatment was given under direct observation and doses used in these studies were within the WHO-recommended ranges.¹⁴⁰ PCR genotyping (based on polymorphic segments of *msp1*, *msp2*, and *glurp*) was done for *P. falciparum* recurrences regardless of the intervening interval or any intercalated *P. vivax* infections. If all three-locus genotypes were the same before and after the treatment, the recurrence was considered a recrudescence.¹⁴⁴ The reliability of the use of these three molecular markers in this area has been validated previously.³⁶² This analysis uses data collected between 1994 and 2010, before the artemisinin resistance started to be observed widespread in this area.^{134,170}

Inclusion criteria

Recurrent malaria episodes (defined here as the reappearance of peripheral falciparum malaria parasitaemia detected by microscopy regardless of the symptoms) with PCR-confirmed recrudescence in pregnant women in any trimester who completed an ABT or QBT were included in the analysis. Only late treatment failure (i.e. recurrence of parasitemia on and after day seven),¹⁷⁴ which requires PCR genotyping, was included. Treatment groups with less than five recrudescence infections were excluded from the analyses.

Modelling the distribution of time to recrudescence

For each treatment group, baseline characteristics were described as median and range for continuous variables, and as proportions for categorical variables.

To model the time to treatment failure, episodes of PCR-confirmed recrudescence

were analysed by accelerated failure time (AFT) models (namely, exponential, Weibull, log-normal and log-logistic distributions). These were compared without any covariates using the Akaike information criterion (AIC).³⁶³ To assess the fit of the model visually, fitted survival curves by each model were shown together with observed Kaplan-Meier survival estimate.³⁶⁴

The covariates with $p < 0.05$ by the Wald test in univariable analysis were all included in the multivariable model. Two variables, namely the treatment group and baseline asexual parasite densities, were always included in the multivariable model as they were known *a priori* to affect recrudescence risk. With selected covariates, different parametric models were again compared using AIC to choose the final model.^{363,365} Adequacy of the assumption of final parametric model was assessed graphically.^{363,366} Goodness of fit of the final model is assessed by Cox-Snell residuals.^{364,366}

P. vivax intercalated infections preceding PCR-confirmed *P. falciparum* recrudescence were included in the analysis but regarded as being censored on the day of *P. vivax* infection. A sensitivity analysis without censoring of *P. vivax* infections was also conducted. Each episode was regarded as unique in the analysis. A complete case analysis was conducted.

As a sensitivity analysis, flexible parametric models with 0–2 knots were constructed,³⁶⁷ but the model without knots was selected as the final model because of the simplicity and interpretability; only the model without knots can be interpreted as AFT model. Another sensitivity analysis was conducted including mefloquine monotherapy, which is neither an ABT nor QBT.

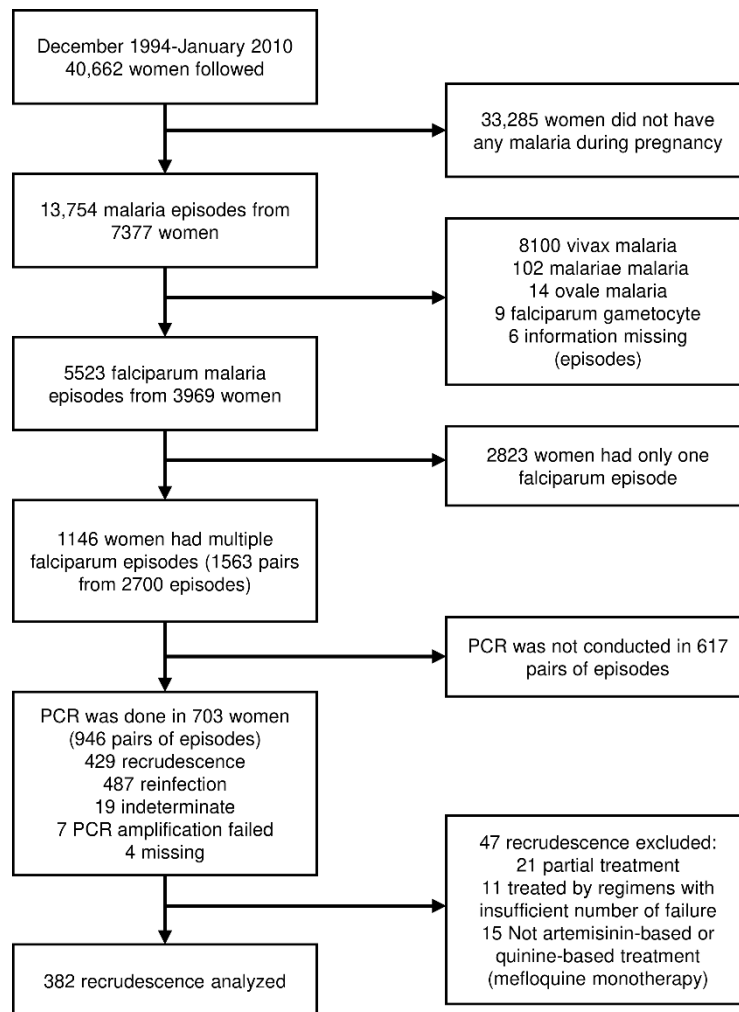


Figure 5-1. Number of recurrent malaria episodes in pregnant women attending antenatal care on the Thailand-Myanmar border, 1994–2010
PCR, polymerase chain reaction.

The cumulative probability densities with the commonly used follow-up durations (i.e. days 28, 42 and 63) were predicted using the final model. Probability density curves based on the final model were drawn for each drug assess the distribution of recrudescence over time.

5.3 Results

Baseline characteristics

Of 40,662 pregnant women followed in this prospective cohort between December

1994 and January 2010, 3969 (10%) had at least one episode of falciparum malaria (Figure 5-1). During a median of 97 days (interquartile range 37–160) follow-up, 1,146 women had more than one episode of falciparum malaria. Among these recurrences, 946 paired isolates from 703 women were PCR genotyped. The median duration of follow-up for each genotyped recurrence was 129 days (interquartile range 83–174). There were 429 PCR-confirmed recrudescence infections, and 382 eligible recrudescence episodes in 341 women were included in the analysis. Five antimalarial treatments were included in this analysis: quinine monotherapy (n=295), AS (n=43), AL (n=19), AC (n=19) and DP (n=6).

Table 5-1. Baseline characteristics of pregnant women with PCR-confirmed recrudescence of falciparum malaria

Baseline characteristic	Quinine (n = 295)		AS (n = 43)		AL (n = 19)		AC (n = 19)		DP (n = 6)	
	N (%)	Median (Range)	N (%)	Median (Range)	N (%)	Median (Range)	N (%)	Median (Range)	N (%)	Median (Range)
Age (years)										
<20	65 (22)		9 (21)		2 (11)		7 (37)		1 (17)	
20-24	75 (25)		8 (19)		4 (21)		7 (37)		0	
25-29	87 (29)		11 (26)		5 (26)		1 (5)		1 (17)	
≥30	68 (23)		15 (35)		8 (42)		4 (21)		4 (67)	
Gravidity										
1	85 (29)		9 (21)		3 (16)		9 (47)		1 (17)	
2	67 (23)		8 (19)		5 (26)		1 (5)		0	
≥ 3	143 (48)		26 (60)		11 (58)		9 (47)		5 (83)	
Trimester										
1st trimester	99 (34)		1 (2)		0		2 (11)		0	
2nd trimester	157 (53)		28 (65)		10 (53)		10 (53)		5 (83)	
3rd trimester	39 (13)		14 (33)		9 (47)		7 (37)		1 (17)	
Weight (kg)	295	46 (32–77)	43	46 (36–62)	19	50 (40–65)	19	49 (31–58)	6	52.5 (47–57)
Fever (>37.5 °C)										
Yes	81 (27)		13 (30)		6 (32)		4 (21)		1 (17)	
No	179 (61)		27 (63)		13 (68)		15 (79)		5 (83)	
Missing	35 (12)		3 (7)		0		0		0	
Parasitemia (log ₁₀ (/ μ L))	295	3.6 (1.5–5.3)	43	4.0 (1.5–5.6)	19	4.2 (2.1–5.2)	19	3.1 (1.5–5.3)	6	4.2 (3.3–5.1)
Species										
Falciparum only	276 (94)		38 (88)		19 (100)		18 (95)		6 (100)	
Mixed infection	19 (6)		5 (12)		0		1 (5)		0	
Previous antimalarial treatment in 28 days										
Yes	45 (15)		19 (44)		11 (58)		7 (37)		4 (67)	
No	250 (85)		24 (56)		8 (42)		12 (63)		2 (33)	

AC, artesunate with clindamycin; AL, artemether-lumefantrine; AS, artesunate monotherapy; CI, confidence interval; DP, dihydroartemisinin-piperaquine

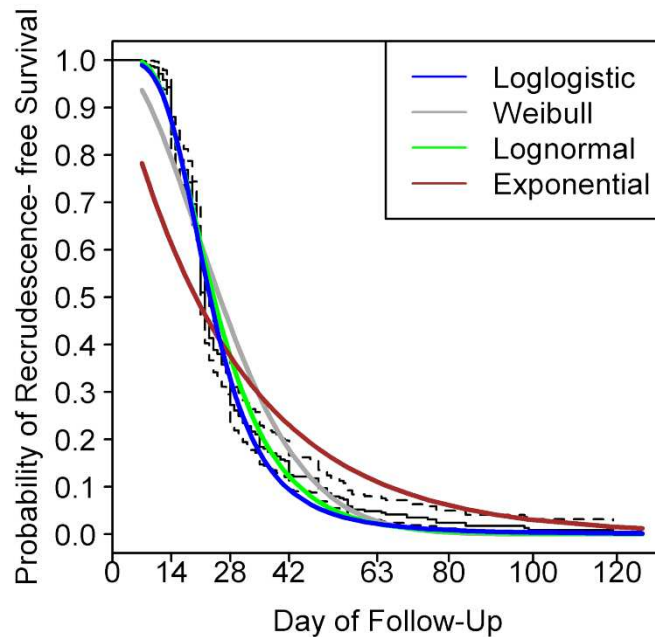


Figure 5-2. Recrudescence-free survival curves in pregnant women treated for uncomplicated falciparum malaria

95% confidence intervals using Kaplan-Meier estimates are shown in dotted lines.

Of the 382 episodes, 357 (93%) were *P. falciparum* mono-infections and the remainder were mixed infections with *P. vivax* (Table 5-1). The geometric mean parasite density was 4287 (range 32–422,016) asexual parasites / μL . Because of the active screening, only 31% (105/344) of women were febrile at presentation ($>37.5\text{ }^{\circ}\text{C}$), although 73% (274/373) reported a history of fever in the last 48 hours before admission.

There was no apparent difference in baseline characteristics among the treatment groups except for EGA and previous history of antimalarial use. Despite its poor efficacy and tolerability profile, quinine is still the drug of choice recommended by WHO in the first trimester of pregnancy, so EGA was lower and fewer patients had antimalarial treatment in the previous 28 days in the quinine group. ABT (namely, AC, AL, AS, and DP) were used as either the first-line (in the second or third trimester) or rescue treatment for quinine-failures.

Factors affecting the time to recrudescence

Among the different AFT models, a log-logistic model was chosen for the final analysis (Figure 5-2). In the univariable analysis, treatment was associated with time to recrudescence (Table 5-2). With quinine as the reference, the interval to recrudescence (95% CI) was 1.04-times (0.90 to 1.20) longer after AS, 1.08-times (0.87 to 1.35) after AL, 1.30-times (1.03 to 1.63) after AC, and 1.46-times (1.02 to 2.08) after DP. Although presence of fever and *P. vivax* co-infection showed shorter times to recrudescence, none fulfilled the

Table 5-2. Univariable and multivariable analyses for the time to recrudescence in pregnant women by log-logistic accelerated failure time models

Baseline characteristic	Univariable analysis		Multivariable analysis	
	Acceleration factor (95% CI)	p-value	Acceleration factor (95% CI)	p-value
Age				
< 20 years	Reference			
20–24 years	0.90 (0.78–1.03)	0.12		
25–29 years	0.92 (0.80–1.05)	0.20		
≥30 years	0.96 (0.84–1.10)	0.56		
Gravidity				
1	1.05 (0.94–1.17)	0.38		
2	0.99 (0.88–1.10)	0.80		
≥ 3	Reference			
Estimated gestational week	1.00 (0.99–1.01)	0.98		
Weight (kg)	1.00 (1.00–1.01)	0.28		
Parasitaemia (log ₁₀ (/μL))	0.98 (0.93–1.03)	0.35	0.97 (0.93–1.02)	0.29
Fever (>37.5 °C)				
Yes	0.96 (0.86–1.06)	0.40		
No	Reference			
Mixed Infection				
Yes	0.87 (0.73–1.04)	0.12		
No	Reference			
Previous antimalarial treatment in 28 days				
Yes	1.00 (0.90–1.12)	0.96		
No	Reference			
Treatment				
Quinine	Reference		Reference	
AS	1.04 (0.90–1.20)	0.61	1.05 (0.91–1.21)	0.54
AL	1.08 (0.87–1.35)	0.50	1.09 (0.87–1.36)	0.45
AC	1.30 (1.03–1.63)	0.03	1.29 (1.03–1.62)	0.03
DP	1.46 (1.02–2.08)	0.04	1.48 (1.03–2.11)	0.03

AC, artesunate with clindamycin; AL, artemether-lumefantrine; AS, artesunate monotherapy; CI, confidence interval; DP, dihydroartemisinin-piperaquine

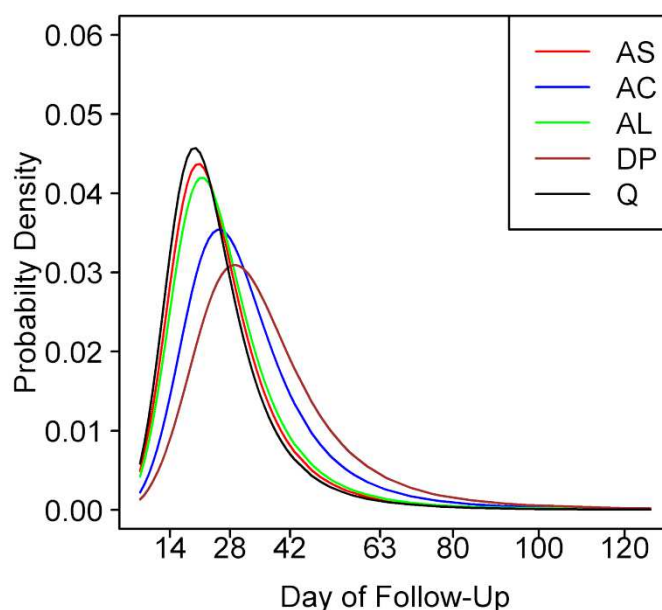


Figure 5-3. Adjusted probability of recrudescence over time by treatment in pregnant women

The mean value in $\log_{10}$ scale (4186 / μL) is used for baseline parasitemia. AC, artesunate with clindamycin; AL, artemether-lumefantrine; AS, artesunate monotherapy; DP, dihydroartemisinin-piperaquine; Q, quinine monotherapy.

inclusion criteria for multivariable models ($p < 0.05$).

In the multivariable analysis, the type of treatment was associated with time to recrudescence adjusted by baseline parasitemia. The adjusted interval to recrudescence compared with that after quinine was 1.05-times (95% CI 0.91 to 1.21) longer after AS, 1.09-times (0.87 to 1.36) after AL, 1.29-times (1.03 to 1.62) after AC, and 1.48-times (1.03 to 2.11) after DP.

Distribution of intervals to recrudescence

Based on the final multivariable model, the predicted probability density functions (Figure 5-3) and the predicted cumulative probabilities of recrudescence (Appendix Figure 5-1) were calculated assuming the mean value of the baseline parasitemia on the $\log_{10}$ scale

Table 5-3. Predicted cumulative proportions of treatment failure detected by days 28, 42 and 63 after antimalarial treatment in pregnant women

Treatment	Predicted cumulative proportion of treatment failure detected by different times (95% CI)		
	Day 28	Day 42	Day 63
Quinine	0.70 (0.65–0.74)	0.92 (0.89–0.94)	0.98 (0.97–0.99)
AS	0.66 (0.53–0.76)	0.90 (0.84–0.94)	0.98 (0.96–0.99)
AL	0.62 (0.42–0.79)	0.89 (0.77–0.95)	0.97 (0.94–0.99)
AC	0.46 (0.26–0.67)	0.80 (0.63–0.91)	0.95 (0.89–0.98)
DP	0.34 (0.11–0.67)	0.71 (0.38–0.91)	0.92 (0.74–0.98)

AC: artesunate with clindamycin, AL: artemether-lumefantrine, AS: artesunate monotherapy, CI: confidence interval, DP: dihydroartemisinin-piperaquine.

(4186/ μ L). The modal interval to recrudescence was 20 days for quinine, 21 for AS and AL, 25 for AC and 29 days for DP.

The proportion (cumulative probability) of total treatment failures detected by the end of currently recommended follow-up durations was derived from the final model (Table 5-3). The predicted cumulative proportion of treatment failures detected was low if the follow-up was only for 28 days: 70% (95% CI 65 to 74) for quinine, 66% (95% CI 53 to 76) for AS, 62% (95% CI 42 to 79) for AL, 46% (95% CI 26 to 67) for AC and 34% (95% CI 11 to 67) for DP. Similarly, 10% or more treatment failures would not be detected if the follow-up duration was 42 days. Follow-up for 63 days was predicted to detect $\geq 95\%$ of the treatment failures except for DP, for which it was predicted that 92% (95% CI 74 to 98) of treatment failures would be detected. In order to detect 95% of all treatment failures in pregnancy, it was estimated that 53 days (95% CI 41 to 65) follow up was required for AL and 72 days (95%CI 46 to 97) for DP.

The predicted proportions of *P. falciparum* recrudescences detected by a model without *P. vivax* infection censoring, and by a flexible parametric model were similar to the

predictions by the final AFT model (Appendices 5-2, 5-3). Another sensitivity analysis was conducted including mefloquine monotherapy. The adjusted interval to recrudescence was longer by 1.08-times (0.86 to 1.37) after mefloquine than that after quinine. The predictions for other treatments were similar to those by the final AFT model (Appendix 5-4).

The model fit was good and model assumptions were adequately satisfied (Appendix 5-5).

5.4 Discussion

Highly effective treatment can limit the cumulative deleterious impact of recurrent malaria during pregnancy on the mother and fetus.³¹ This demands correct assessment of treatment efficacy, which in turn requires an adequate length of follow-up. This large study from an area of low and seasonal transmission suggests that recommended durations of follow-up to assess antimalarial drug efficacy for non-pregnant populations (i.e. 28–42 days) are too short for pregnant populations.

The duration of follow-up in a survival analysis needs to be long enough to capture the majority of events (i.e. recrudescence in antimalarial efficacy studies) so that appropriate statistical analysis can be performed.^{324,363} This is particularly important when the events are rare, as is the case for ACTs where treatment failure should be less than 5%, and the study size is small, as is the case for studies in pregnancy.

Drug elimination rate (assessed conventionally by the terminal half-life) is the main factor determining the interval to recrudescence (i.e. as it reflects the time during which a drug suppresses parasite growth). However, the duration of post-treatment prophylaxis is

longer than the drug half-life,³⁶⁸ which indicates that there are other factors that need to be considered. Before being detected by microscopy, parasites need to increase in number above the detectable threshold ($\sim 50/\mu\text{L}$).⁴⁰ The treatment with higher efficacy results in lower residual parasitaemia (if not cleared completely), and the lower the parasitaemia, the longer becomes the time to become detectable.⁴⁰ Immunity against malaria affects the survival and growth of parasites independently of the antimalarial drug.⁴² The pregnant status can affect both drug elimination and immunity,^{26,249} presumably enhancing the survival and growth of parasites.

Previous studies on non-pregnant populations, which assumed 63 days of follow-up captured all recrudescences, concluded that patients should be followed up for at least 28 days for antimalarial drugs with a short half-life (e.g. quinine, AS, and AC), for 42 days with an intermediate half-life (e.g. AL) and for 63 days with long half-life drugs (e.g. DP, mefloquine).^{322,324} Based on these studies, WHO recommends to follow up non-pregnant populations for 28 days to 42 days (for piperazine and mefloquine).¹⁷⁴ Following this recommendation, a systematic literature review on AL reported that only 36%(58/161) of studies on AL followed for 42 days or longer, and only 4% (7/161) followed 63 days or longer in non-pregnant populations.³⁶⁹

These recommendations appear insufficient for pregnant women, at least in a low transmission setting. This study shows that in pregnant women, a 28-day follow-up can miss $\geq 30\%$ of the recrudescences, even for quinine or AS. For AL, 11 (95% CI: 5 to 23) % of recrudescences are predicted to be missed even with 42-day follow-up. For DP, around 10%

of recrudescences are predicted to occur even after 63 days, although this prediction is based on very small number of observations. Under the currently recommended dosing regimens, this study suggests longer follow-up periods, such as 53 days (95% CI 41 to 65) for AL and 72 days (95%CI 46 to 97) for DP are required to capture 95% of all recrudescences in pregnancy. In the context of antenatal care, this length of time is shorter than one trimester and should not be too burdensome. This study did not extend to the post-partum period and rapidity of reversion to the non-pregnant adult therapeutic response is unknown.

One previous study simulated the time to recrudescence based solely on the drug half-life.³⁷⁰ In that study, after ACT with a partner drug with a half-life of ≤ 1 week (e.g. AL), the peak of recrudescence was predicted to be day 28. In this current analysis, the peak of recrudescence was around day 21 after AL (and 29 after DP). This difference can be explained partly by the shorter drug terminal elimination half-lives of lumefantrine and piperaquine in pregnant compared with non-pregnant women.²⁴⁹ Compared with the simulated distribution curves based on drug half-lives,³⁷⁰ the distribution curves modelled in this study had a slightly longer distal tail. Immunity, placental sequestration and relatively low baseline parasitemia due to active detection may contribute to this longer “tail” of recrudescences.

The time to recrudescence was longer in AC than expected from the drug half-lives of artesunate (<1 hour) and clindamycin (2–4 hours). However, AC, AS, and quinine were given over seven days whereas other treatments were given over three days. Another possibility is that higher efficacy achieved by adding clindamycin reduced the residual parasite burden. It is demonstrated that 7-day treatment by artemisinin derivatives is more

effective to decrease the parasitaemia, particularly when the baseline parasitaemia is high,^{371,372} treating recrudescence²¹⁹ or even strains less sensitive to artemisinin.³⁶¹ The initial lower number of residual parasites will result in the longer time to become detectable by microscopy.³⁷³ Similarly, an additional analysis including mefloquine monotherapy, which was used when high grade resistance to mefloquine was already prevalent and well described in the non-pregnant population in this area,³⁷⁴ shows that higher grade drug resistance shortens the time to recrudescence, which is consistent with a previous mathematical modelling.³⁷⁵ To detect lower grade resistance, a longer duration of follow-up may be needed.

This is the first study to use AFT models to analyse the time to treatment failure in malaria. There are several reasons why the AFT model is better suited to this purpose than the Cox proportional hazards model. Biologically, the assumption of the AFT models is more plausible; the distribution of time to recrudescence is shorter (or longer) depending on factors such as the half-life of the drug. Clinically, the AFT models analyse the times to events directly (rather than hazard ratios), thus the interpretation of the output is clinically relevant and meaningful.³⁷⁶⁻³⁷⁹ The AFT model prediction is also better than that of the Cox model for the “right-hand” or distal tail of the distribution, which is the main research interest in this study. Because of its flexibility, predicted baseline hazard function in Cox model can be shaggy and also more vulnerable to the outliers particularly in small sample size.³⁶⁷ The disadvantage of the conventional parametric model is the model fit, but in our data, the model fit was adequate and the predicted capturing proportion by a flexible parametric model (with a

better fit) was similar to or even slightly lower than the figures by the final AFT model.

Despite these advantages, there are also limitations. Firstly, the number of observations included in this analysis was low for all drugs except quinine. However, modelling enables prediction of the temporal distributions for drugs with fewer observations based on the distributions for other drugs with more observations but with a proportional change in the time to events, as discussed above. The effects of covariates (e.g. gestational age) might be different for each drug, but this was not assessed fully.

Secondly, this study is based on the data collected on the Thailand-Myanmar border where the falciparum malaria transmission was low and unstable. This limits generalizability. Nonetheless, available evidence from other studies is scarce.¹⁸⁹ There are three other published studies that report follow-up of patients beyond the current framework of follow-up (i.e. for more than 63 days) with PCR genotyping for recurrences, but two of them did not have enough recrudescences (i.e. <5) for analysis^{192,303} and the third followed up patients less frequently (i.e. monthly or less).³¹³ Recently, more studies in pregnancy followed up patients for 63 days, which is reassuring for assessing the efficacy of drug with a shorter drug half-life (such as AL or quinine) as was shown in this study. However, as the prediction model implied, 63 days may not be long enough for ACTs with a partner drug of a longer half-life, such as DP. In a recent multi-centre trial conducted in four sub-Saharan African countries reported that the most of the recrudescences after DP occurred during 56–63 days,²³⁷ implying the possibility of recrudescence after the follow-up of 63 days. As was shown in Chapter 3-II, the systematic review highlighted the necessity of more antimalarial treatment studies in

pregnancy with an adequate length of follow-up.¹⁸⁹

These predictions need to be tested in high transmission areas, although this is made more difficult because of a higher risk of misclassification with genotyping and the confounding effects of malaria immunity. In high transmission areas, concomitant infection with multiple discrete genotypes is common^{356,380,381} and the rates of reinfection are high.³⁷⁰ Both lead to a higher probability of misclassification of reinfection as recrudescence, particularly in the late follow-up period during which the post-treatment prophylactic effect has waned and the risk of reinfection substantially outweighs that of recrudescence. In all areas, this type of study is difficult because of the long follow-up and large sample size needed when studying effective treatments. This particular cohort is unique with a consistent method of data collection over many years, including quality control of microscopy diagnosis and with a long follow-up length of median ≥ 100 days, ensuring that nearly all recrudescences were captured.

The possibility of predicting the ‘true’ efficacy of antimalarial treatments by extrapolating the observed efficacy with a shorter follow-up needs cautious consideration. As was implied in this analysis, the shorter the duration of follow-up, the more uncertain the prediction of ‘true’ efficacy becomes. This uncertainty becomes greater if the number of recrudescences is small. Efforts should be made to capture most of the recrudescences so that accurate assessments of the failure rate can be made.

Further studies with >63 days of follow-up will be needed if the dosing is tailored for pregnant women. Dose optimization based on pharmacokinetics is most likely increase of the

dose, which will lead to less recrudescence and probably longer time to recrudescence.

5.5 Conclusions

Insufficient length of follow-up in antimalarial therapeutic assessments underestimates recrudescence rates and thus overestimates treatment efficacy. This can lead to continuation of a less effective drug, or insufficient dosage resulting in more treatment failures at the individual level and a higher risk of selecting resistance. This study suggests that the currently recommended length of follow-up of 28–42 days in therapeutic assessments may be insufficient for pregnant women, at least in low transmission areas.

Chapter 6. Pregnancy outcomes and risk of placental malaria following artemisinin-based and quinine-based treatment for uncomplicated falciparum malaria in pregnancy: individual patient data meta-analysis

6.1 Introduction

Malaria during pregnancy has adverse impacts on the fetus: increased risks of pregnancy loss,¹¹ PTB,³¹ IUGR and SGA^{31,337,349} have been reported. Pathologically, a recent study showed that malaria infection in early pregnancy was associated with impaired vascular development of the placenta, which was associated with both IUGR and shorter duration of pregnancy.⁹⁸ PTB is the major reason for neonatal and post-neonatal infant mortality globally, while the burden of mortality occurs in LMICs.⁶⁹ SGA leads to a higher risk of stillbirth,²⁸⁹ and is associated with increased short-term mortality.⁶⁹ SGA is a proxy for IUGR, which is associated with a higher risk of metabolic disorders in later adult life.^{71,72}

The most widely reported adverse impact by malaria in pregnancy is LBW. Historically, LBW has been used as a proxy for fetal growth. However, LBW, commonly defined as birthweight less than 2,500 g, does not distinguish between PTB and IUGR, which are associated with different neonatal mortality rates. Despite this limitation, LBW is still widely used to assess the impact of malaria in pregnancy.^{70,288} For the purpose of summarizing the evidence, LBW is not comparable between studies (or study arms) as a proxy for fetal growth if the gestational age at delivery is different between studies. A better proxy for IUGR is SGA, defined as <10th percentile of the standard growth chart, which takes account of sex and gestational age. A recent advancement is that an international standard fetal growth chart for defining SGA has become available¹⁶⁵ and SGA can thus be used to summarize the evidence of the impact of malaria on fetal growth across different studies.

Placental malaria (i.e. sequestration of malaria parasites to the placenta) is one of the primary pathogenic mechanisms by which malaria during pregnancy can cause these adverse effects on the fetus.^{26,27} Many studies have been conducted to assess the relationship between placental malaria and adverse pregnancy outcomes, both of which were assessed at delivery, and have confirmed the association.³⁸²⁻³⁸⁴ Placental malaria is a direct consequence of malaria infection, which should be assessed as one of the efficacy outcomes after treatment or IPTp.^{112,115}

ACT is highly effective with generally $\geq 95\%$ treatment success (clearance of peripheral parasitaemia) as was shown in an IPD meta-analysis (see Chapter 4). However, few studies have been powered to specifically explore the impact of different treatments on adverse pregnancy outcomes and placental malaria.

In this chapter, IPD from treatment efficacy studies have been pooled to describe the risks of placental malaria (i.e. a direct consequence) and adverse pregnancy outcomes (i.e. indirect consequences) after treatment of uncomplicated falciparum malaria in pregnancy.

6.2 Methods

Search strategy and inclusion criteria

A systematic review and IPD meta-analysis on the efficacy of ABT and QBT on uncomplicated falciparum malaria in pregnancy was conducted as described in Chapter 2. Details of the systematic review are described in Chapter 3-2 and 3-3. The same IPD used in Chapter 4 to assess the treatment efficacy were used in this chapter to assess pregnancy

outcomes after treatment of uncomplicated falciparum malaria in pregnancy.

Definition of outcomes

The following definitions were used in this analysis: miscarriage was defined as fetal loss before 28 weeks of gestation and stillbirth was defined as fetal loss at 28 weeks or more of gestation.^{177,178} PTB was defined as babies born alive before 37 weeks of gestation:¹⁷⁹ extremely PTB (less than 28 weeks), very PTB (28 to less than 32 weeks) and moderate to late PTB (32 to less than 37 weeks).¹⁸⁰ SGA was defined as birthweight lower than the 10th percentile^{181,182} of the standard growth curve derived from the INTERGROWTH-21st international standard.¹⁶⁵

Placental histopathology was categorized according to the combination of malaria parasites and malaria pigment as follows:³¹⁵ no infection (both parasites and pigment are negative); acute infection (only parasites are present); past infection (only pigment present); chronic infection (both parasites and pigment are present). The presence of malaria pigment was analysed in order to assess the effect of malaria infection during pregnancy on placental malaria as the presence of parasites in the placenta is reportedly more closely associated with concurrent peripheral parasitaemia at delivery.²⁷

Inclusion criteria at the individual level

Only the first recorded study episode of falciparum malaria was used for the analysis, which did not necessarily mean the first malaria episode in the pregnancy had been captured.

The risk of congenital abnormality after artemisinin derivatives use during the first trimester (organogenesis period) is no greater than quinine (or the background population level),³³ and almost all the women enrolled in clinical trials on antimalarial efficacy were in the second or third trimester.¹⁸⁹

Only singleton births without congenital abnormality were analysed, except for calculating the proportion of congenital abnormality. The proportion of miscarriage was calculated excluding episodes after 28 weeks of gestation. PTB included only live singleton births and excluded pregnant women who had the first recorded malaria after 28 (for extremely PTB), 32 (for very PTB), or 37 (for moderate to late PTB) weeks of gestation. Only live singleton births delivered before 42 weeks 6 days were included for assessing SGA. Newborns whose birthweight was assessed within three days were included in the analyses.

Statistical analysis

Stillbirth was analysed by the stratified Cox model taking account of delayed entry (in terms of gestational age). PTB and SGA were analysed by mixed-effects logistic regression. The impact of treated falciparum malaria episodes on the development of placental malaria was analysed by mixed-effects logistic regression using the deposition of malaria pigment in the placenta as the outcome.

Multiple imputation was used for missing variables (see Appendix 6 for details). Variables that were missing more than 5% included HIV status (~18%) and height (~14%),

thus dataset was imputed for 20 times under fully conditional specification using *mi impute mvn* command of Stata 15.1 MP. As recommended when multiple imputation is used, the Wald test was used for model building.^{385,386} Firstly, variables with $p < 0.05$ by the Wald test were included in the multivariable analyses. Secondly, variables with $p > 0.05$ were dropped one at a time from the multivariable model until all variables had $p < 0.05$. Other variables were again put in the multivariable model to see if any then had $p < 0.05$. Treatment was always included in the model regardless of its significance. Separately, the effect of any recurrence within 63 days was assessed for placental malaria.

For SGA and PTB, an interaction term between gestational age and treatment was added to the final models to see the difference among different antimalarials used in different gestational periods. Because the majority of the studies included second- or third-trimester women, the gestational period between 14 and 37 weeks was divided into four periods that have a roughly similar number of women. Gravidity (G) and previous history of pregnancy loss were jointly categorized (G1, G2 with no loss, $G \geq 3$ with no loss, G2 with 1 loss, $G \geq 3$ with 1 loss and $G \geq 3$ with ≥ 2 losses) and always adjusted in the multivariable analyses as a confounder for assessing stillbirth, PTB and SGA.^{13,289} Previous history of pregnancy loss was estimated by (gravidity – parity – 1), with a lower limit of 0. HIV status was always adjusted as a confounder except models assessing placental malaria.¹³ For placental malaria, malaria transmission was included in the multivariable model as an *a priori* confounder, and interaction between parity and malaria transmission was assessed because pregnancy-specific

(parity-dependent) immunity can be different depending on malaria transmission.

The non-linear relationship of continuous variables was assessed by categorizing them into four or five groups. Within-study-site clustering was accounted for by mixed-effects logistic or stratified Cox regressions. If the number of overall observations (or outcomes) was small, only the effect of treatment was assessed by univariable mixed-effects logistic regression. The proportion (and 95% CI) was pooled by random effects.

Sensitivity analysis

In some studies, EGA in weeks (without days) was available at malaria episodes but not at delivery. When the EGA at delivery was calculated from the EGA at malaria episode, the difference of a maximum of seven days (i.e. day 0 or day 6 of the same week) can affect the binary category of PTB and SGA. For those studies, results assuming day 0 of the week were presented as the primary analyses, and those assuming day 6 of the week were presented as sensitivity analyses (shown in Appendices).

6.3 Results

Study inclusion

Of the 28 identified studies with PCR-corrected efficacy, 22 studies assessed pregnancy outcomes, resulting in a total of 5,015 women in the literature. IPD of 4,765 women (95.0%) in 16 studies were pooled for the analyses. Delivery information was available for 4,503 women with 5.5% (262/4765) lost-to-follow-up. After excluding twin

pregnancies (1.4%, 63/4503), 4,440 women with singleton deliveries were included.

Ultrasound was used to estimate EGA in eight studies, comprising 91.5 % (4120/4503) of the women. One study did not have information on gestational age. Another study, which EGA by ultrasound at the time of the malaria episode, reported EGA only in weeks.

Histopathology of the placenta was assessed in up to 3,128 women in six studies with PCR-corrected efficacy in the literature. IPD of 3,033 women (97.0%) in four studies were analysed.

Congenital abnormality

The overall pooled proportion of congenital abnormality was 0.4% (58/4440, 95% CI 0.1 to 0.9, I^2 39%). However, congenital abnormalities were only reported in live born infants. As they are a known contributor to stillbirths and were not recorded, congenital abnormalities were likely to be underestimated. The highest crude proportion of congenital abnormality was observed in the AAP group (2/83, 2.4%, 95% CI 0.7% to 8.4%) and there was no difference among different treatments ($p=0.68$). No further analyses were conducted because of the small number of cases. These 58 pregnancies with congenital abnormality were excluded from the following analyses.

Miscarriage

The overall pooled proportion of miscarriage was 0.0% (21/2932, 95% CI 0.0 to 0.1,

I² 0%) among pregnant women who had malaria before 28 weeks of gestation and were followed up until delivery. The highest crude proportion of miscarriage was observed in the quinine monotherapy group (3/140, 2.1%, 95% CI 0.7% to 6.1%) and there was no difference among different treatments (p=0.60). No further analyses were conducted because of the small number of cases.

Stillbirth

Overall summary and baseline characteristics

The overall pooled proportion of stillbirth was 1.1% (84/4361, 95% CI 0.6 to 1.7, I² 29%), among pregnant women who were followed up until delivery (n=4382), excluding those who had a miscarriage. Pregnant women for whom there was no information on EGA (n=27) were excluded from the subsequent analysis on stillbirth.

Nine antimalarial treatments of the 4334 women were included in the analyses on stillbirth: AL (n=1087), ASAQ (n=775), ASMQ (n=965), DP (n=837), ASSP (n=154), AS (n=193), AAP (n=81), quinine monotherapy (n=184) and QC (n=58) (Table 6-1).

The median age was 22 (interquartile range 19–27) and gravidity was 2 (interquartile range 1–4). The included malaria episodes occurred mostly in the second (68.0%, 2949/4334) or third trimester (31.6%, 1371/4334). The majority of the episodes (99.3%, 4302/4334) were *P. falciparum* mono-infections and the remainder were mixed infections. The geometric mean parasite density was 1059 (range 1–447,638) asexual parasites / μ L. Only 9.2% (395/4292) of

women were febrile at presentation ($>37.5^{\circ}\text{C}$). HIV status was assessed in 3019 women (69.7%) in six studies, with a crude proportion of 1.2% (35/3019) positive. Of the rest, 643 (14.8%) women were included in nine studies conducted on the Thailand-Myanmar border where the seroprevalence of HIV is $<0.5\%$,³⁸⁷ thus regarded as HIV-negative in the following analyses. None of the included women received antiretroviral treatment during pregnancy.

Risk factors for stillbirth

In the univariable analysis, higher baseline parasitaemia (hazard ratio [HR] 1.41 per 10-fold increase of parasitaemia, 95% CI 1.06 to 1.88, $p=0.02$) and BMI $<18.5\text{ kg/m}^2$ (HR 2.23, 95% CI 1.03 to 4.84, $p=0.04$) compared with BMI 18.5–24.9 kg/m^2 were associated with a higher risk of stillbirth. Different antimalarial treatments were not associated with stillbirth using AL as the reference (Table 6-2).

In the multivariable analysis adjusted for HIV status and gravidity combined with a previous history of pregnancy loss, BMI $<18.5\text{ kg/m}^2$ (adjusted hazard ratio [aHR] 2.22, 95% CI 1.02 to 4.84, $p=0.05$) remained associated with a higher risk of stillbirth, while baseline parasitaemia became not significant after adjusting for gravidity (aHR 1.32 per 10-fold increase of parasitaemia, 95% CI 0.99 to 1.77, $p=0.06$). Although statistically not significant, the risk of stillbirth was higher in women with HIV infection (aHR 3.17, 95% CI 0.72 to 13.89, $p=0.12$) and lower in pregnant women who had no previous pregnancy loss compared with primigravid. Different antimalarial treatments were not associated with stillbirth.

Table 6-1. Baseline characteristics of pregnant women assessed for stillbirth

Characteristic	All		AL		ASAQ		ASMQ		DP	
	N	% (N)	N	% (N)	N	% (N)	N	% (N)	N	% (N)
		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)
EGA (week)	4334	24.6 (5.7)	1087	24.3 (5.7)	775	24.1 (5.1)	965	25.3 (5.3)	837	24.4 (5.7)
Age group <20	4334	32.0 (1388)	1087	36.5 (397)	775	31.7 (246)	965	28.1 (271)	837	39.8 (333)
20-24		32.3 (1399)		31.1 (338)		31.6 (245)		33.6 (324)		30.0 (251)
25-29		19.3 (836)		17.8 (194)		20.1 (156)		21.2 (205)		16.6 (139)
30-34		9.7 (419)		8.1 (88)		10.7 (83)		10.6 (102)		8.4 (70)
>35		6.7 (292)		6.4 (70)		5.8 (45)		6.5 (63)		5.3 (44)
Gravidity 1	4297	35.4 (1519)	1053	35.1 (370)	774	36.4 (282)	965	33.2 (320)	837	38.4 (321)
2		23.4 (1005)		23.2 (244)		22.5 (174)		24.1 (233)		24.9 (208)
≥3		41.3 (1773)		41.7 (439)		41.1 (318)		42.7 (412)		36.8 (308)
Parity 0	4329	42.7 (1847)	1083	42.6 (461)	774	41.1 (318)	965	42.6 (411)	837	47.6 (398)
1		21.8 (945)		20.9 (226)		22.4 (173)		20.9 (202)		22.6 (189)
≥2		35.5 (1537)		36.6 (396)		36.6 (283)		36.5 (352)		29.9 (250)
Height (cm)	3809	155.8 (6.9)	934	156.1 (6.6)	775	157.9 (6.5)	927	156.1 (7.3)	837	155.3 (6.3)
Weight (kg)	4333	53.9 (8.3)	1086	53.7 (7.4)	775	56.3 (8.8)	965	54.0 (8.5)	837	54.4 (8.2)
BMI <18.5	3809	6.3 (239)	934	5.8 (54)	775	4.9 (38)	927	6.8 (63)	837	5.5 (46)
18.5-24.9		79.9 (3044)		84.7 (791)		78.8 (611)		77.9 (722)		77.8 (651)
25.0-29.9		12.0 (458)		8.9 (83)		12.4 (96)		13.4 (124)		15.4 (129)
≥30		1.8 (68)		0.6 (6)		3.9 (30)		1.9 (18)		1.3 (11)
Fever (temperature >37.5)	4292	9.2 (395)	1086	8.2 (89)	775	4.8 (37)	964	9.8 (94)	834	4.2 (35)
Haemoglobin on day 0 (g/dL)	4308	10.0 (1.4)	1075	10.2 (1.4)	775	10.0 (1.3)	963	9.9 (1.4)	834	10.0 (1.4)
Parasitaemia (log ₁₀ /μL)	4334	3.0 (0.9)	1087	3.1 (0.8)	775	2.8 (0.8)	965	3.0 (0.8)	837	2.9 (0.8)
Presence of gametocyte	4271	3.8 (161)	1083	3.9 (42)	775	3.0 (23)	946	1.7 (16)	837	3.8 (32)
Mixed infection	4334	0.7 (32)	1087	0.9 (10)	775	0.0 (0)	965	0.0 (0)	837	0.7 (6)
HIV infection	2730	1.3 (35)	808	1.4 (11)	573	0.5 (3)	570	0.2 (1)	668	0.9 (6)

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, DP: Dihydroartemisinin-piperaquine, EGA: Estimated gestational age, Q: Quinine monotherapy, QC: Quinine with clindamycin, SD: standard deviation.

Table 6-1 continued.

Characteristic	ASSP		AS		AAP		Q		QC	
	N	% (N)	N	% (N)	N	% (N)	N	% (N)	N	% (N)
		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)
EGA (week)	154	24.4 (5.8)	193	26.6 (7.4)	81	26.2 (7.1)	184	23.8 (6.3)	58	26.8 (6.5)
Age group <20	154	20.1 (31)	193	19.2 (37)	81	28.4 (23)	184	20.7 (38)	58	20.7 (12)
20-24		55.2 (85)		24.9 (48)		27.2 (22)		39.7 (73)		22.4 (13)
25-29		17.5 (27)		21.2 (41)		22.2 (18)		20.1 (37)		32.8 (19)
30-34		3.9 (6)		14.5 (28)		13.6 (11)		13.0 (24)		12.1 (7)
>35		3.2 (5)		20.2 (39)		8.6 (7)		6.5 (12)		12.1 (7)
Gravidity 1	154	48.7 (75)	193	27.5 (53)	81	29.6 (24)	182	32.4 (59)	58	25.9 (15)
2		24.7 (38)		15.0 (29)		21.0 (17)		25.8 (47)		25.9 (15)
≥3		26.6 (41)		57.5 (111)		49.4 (40)		41.8 (76)		48.3 (28)
Parity 0	154	56.5 (87)	193	30.1 (58)	81	33.3 (27)	184	37.0 (68)	58	32.8 (19)
1		23.4 (36)		19.7 (38)		24.7 (20)		25.0 (46)		25.9 (15)
≥2		20.1 (31)		50.3 (97)		42.0 (34)		38.0 (70)		41.4 (24)
Height (cm)	118	150.0 (7.0)	136	151.7 (5.6)	33	150.2 (8.3)	49	153.3 (5.1)	0	
Weight (kg)	154	47.8 (6.9)	193	49.5 (6.4)	81	49.8 (6.6)	184	55.2 (9.4)	58	49.8 (5.5)
BMI <18.5	118	18.6 (22)	136	8.1 (11)	33	6.1 (2)	49	6.1 (3)	0	
18.5-24.9		72.0 (85)		83.8 (114)		78.8 (26)		89.8 (44)		
25.0-29.9		9.3 (11)		7.4 (10)		9.1 (3)		4.1 (2)		
≥30		0.0 (0)		0.7 (1)		6.1 (2)		0.0 (0)		
Fever (temperature >37.5)	117	24.8 (29)	193	25.4 (49)	81	24.7 (20)	184	16.8 (31)	58	19.0 (11)
Haemoglobin on day 0 (g/dL)	152	9.3 (1.4)	193	9.5 (1.5)	80	9.4 (1.5)	179	10.2 (1.8)	57	9.1 (1.6)
Parasitaemia (log ₁₀ /μL)	154	3.4 (0.8)	193	3.3 (1.0)	81	3.6 (1.1)	184	3.4 (0.9)	58	3.3 (1.1)
Presence of gametocyte	118	3.4 (4)	189	10.1 (19)	81	3.7 (3)	184	9.2 (17)	58	8.6 (5)
Mixed infection	154	0.0 (0)	193	6.2 (12)	81	1.2 (1)	184	0.0 (0)	58	5.2 (3)
HIV infection	27	22.2 (6)	0		0		84	9.5 (8)	0	

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, DP: Dihydroartemisinin-piperaquine, EGA: Estimated gestational age, Q: Quinine monotherapy, QC: Quinine with clindamycin, SD: standard deviation.

Table 6-2. Univariable and multivariable analyses on the risk of stillbirth

Characteristic	Proportion	Univariable*		Multivariable	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Treatment					
AL	19/1086	Reference		Reference	
ASAQ	13/769	0.74 (0.32-1.71)	0.48	0.68 (0.29-1.59)	0.37
ASMQ	23/961	0.82 (0.38-1.76)	0.61	0.78 (0.36-1.69)	0.53
DP	19/834	0.87 (0.40-1.87)	0.71	0.85 (0.39-1.84)	0.68
ASSP	4/154	0.28 (0.03-3.07)	0.30	0.26 (0.02-2.85)	0.27
AS	2/193	0.92 (0.06-14.69)	0.95	0.79 (0.05-12.69)	0.87
AAP	1/81	omitted			
Q	2/184	0.67 (0.11-3.99)	0.66	0.72 (0.12-4.27)	0.71
QC	1/58	0.78 (0.02-39.47)	0.90	0.62 (0.01-31.40)	0.81
Age (year)					
<20	27/1384	Reference			
20-24	27/1393	0.93 (0.53-1.61)	0.79		
25-29	18/835	1.00 (0.54-1.87)	1.00		
30-34	6/417	0.72 (0.29-1.79)	0.48		
≥35	6/291	1.05 (0.42-2.66)	0.91		
Gravidity and previous history of pregnancy loss					
G1	33/1516	Reference		Reference	
G2 with no loss	11/736	0.63 (0.31-1.26)	0.19	0.65 (0.32-1.32)	0.24
G≥3 with no loss	15/1163	0.55 (0.29-1.04)	0.07	0.60 (0.31-1.16)	0.13
G2 with 1 loss	8/264	1.18 (0.53-2.67)	0.68	1.18 (0.52-2.66)	0.70
G≥3 with 1 loss	11/458	1.04 (0.51-2.09)	0.92	1.09 (0.53-2.24)	0.81
G≥3 with ≥2 losses	5/145	0.99 (0.37-2.66)	0.99	1.00 (0.37-2.72)	1.00
Height (cm)	73/3795	0.98 (0.95-1.02)	0.37		
BMI					
<18.5	8/239	2.23 (1.03-4.84)	0.04	2.22 (1.02-4.84)	0.05
18.5-24.9	49/3034	Reference		Reference	
25.0-29.9	12/454	0.96 (0.51-1.83)	0.91	1.10 (0.58-2.09)	0.77
≥30	4/68	1.67 (0.57-4.90)	0.35	1.84 (0.60-5.60)	0.28
Fever (>37.5°C)	8/395	1.28 (0.57-2.83)	0.55		
No fever	73/3883	Reference			
Haemoglobin on day 0 (g/dL)					
	83/4294	1.06 (0.90-1.24)	0.49		
Parasitaemia (log ₁₀ /μL)	84/4320	1.41 (1.06-1.88)	0.02	1.32 (0.99-1.77)	0.06
Presence of gametocyte	2/161	0.80 (0.19-3.36)	0.76		
Without gametocyte	79/4096	Reference			
Mixed infection	0/32	omitted			
Monoinfection	84/4288	Reference			
HIV infection	3/35	3.24 (0.76-13.87)	0.11	3.17 (0.72-13.89)	0.12
not confirmed	61/3619	Reference		Reference	
Malaria transmission					
Low	12/993	Reference			
Moderate	57/2336	1.41 (0.23-8.41)	0.71		
High	15/991	1.14 (0.13-10.10)	0.91		

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, G: gravidity, HR: hazard ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. * Adjusted for treatment.

Preterm birth

Overall summary and baseline characteristics

The overall pooled proportion of extremely PTB (less than 28 weeks), very PTB (28 to less than 32 weeks) and moderate to late PTB (32 to less than 37 weeks) was 0·0% (8/2488, 95% CI 0·0 to 0·0, I^2 0%), 0·4% (47/3131, 95% CI 0·1 to 0·9, I^2 11%) and 10·0% (619/4131, 95% CI 7·0 to 13·4, I^2 88%), respectively. Since the number of outcomes of extremely PTB and very PTB was small even when pooled, they were not analysed further. The risks of moderate to late PTB among different treatments used in four different pregnancy periods were investigated.

Nine antimalarial treatments of 4,131 women were included in this analysis: AL (n=1035), ASAQ (n=747), ASMQ (n=926), DP (n=804), ASSP (n=147), AS (n=174), AAP (n=76), quinine (n=171) and QC (n=51). Baseline characteristics (Table 6-3) were similar to pregnant women who were included in the previous analysis of stillbirth. The gestational period of pregnancy was divided into <14 weeks (n=38), 14–19.9 weeks (n=863), 20–23.9 weeks (n=1023), 24–27.9 weeks (n=917), 28–36.9 weeks (n=1290).

Table 6-3. Baseline characteristics of pregnant women who were assessed for moderate to late preterm birth

Characteristic	All		AL		ASAQ		ASMQ		DP	
	N	% (N)	N	% (N)	N	% (N)	N	% (N)	N	% (N)
		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)
Age group <20	4131	32.0 (1323)	1035	36.2 (375)	747	31.7 (237)	926	28.0 (259)	804	39.8 (320)
20-24		32.2 (1330)		30.7 (318)		31.6 (236)		33.4 (309)		30.1 (242)
25-29		19.4 (803)		18.3 (189)		20.1 (150)		21.5 (199)		16.7 (134)
30-34		9.7 (401)		8.3 (86)		10.8 (81)		10.7 (99)		8.3 (67)
>35		6.6 (274)		6.5 (67)		5.8 (43)		6.5 (60)		5.1 (41)
Gravidity 1	4095	35.1 (1439)	1002	34.6 (347)	746	36.2 (270)	926	32.8 (304)	804	38.4 (309)
2		23.3 (955)		23.0 (230)		22.5 (168)		24.4 (226)		24.4 (196)
≥3		41.5 (1701)		42.4 (425)		41.3 (308)		42.8 (396)		37.2 (299)
Parity 0	4126	42.4 (1750)	1031	41.9 (432)	746	40.8 (304)	926	42.2 (391)	804	47.6 (383)
1		21.7 (896)		20.9 (215)		22.3 (166)		21.0 (194)		22.1 (178)
≥2		35.9 (1480)		37.2 (384)		37.0 (276)		36.8 (341)		30.2 (243)
Height (cm)	3655	155.9 (6.9)	894	156.1 (6.6)	747	158.0 (6.5)	890	156.1 (7.4)	804	155.4 (6.3)
Weight (kg)	4130	53.9 (8.3)	1034	53.7 (7.4)	747	56.3 (8.8)	926	54.0 (8.4)	804	54.5 (8.2)
BMI <18.5	3655	6.1 (224)	894	5.7 (51)	747	5.0 (37)	890	6.4 (57)	804	5.5 (44)
18.5-24.9		80.3 (2936)		85.1 (761)		79.1 (591)		78.3 (697)		78.2 (629)
25.0-29.9		11.8 (431)		8.5 (76)		12.2 (91)		13.5 (120)		14.9 (120)
≥30		1.8 (64)		0.7 (6)		3.7 (28)		1.8 (16)		1.4 (11)
Fever (temperature >37.5)	4094	9.2 (375)	1034	8.2 (85)	747	4.6 (34)	925	9.7 (90)	801	4.2 (34)
Haemoglobin on day 0 (g/dL)	4107	10.0 (1.4)	1025	10.2 (1.4)	747	10.0 (1.3)	924	9.9 (1.4)	801	10.0 (1.4)
Parasitaemia (log ₁₀ /μL)	4131	3.0 (0.9)	1035	3.0 (0.8)	747	2.8 (0.8)	926	3.0 (0.8)	804	2.9 (0.8)
Presence of gametocyte	4075	3.8 (153)	1031	4.1 (42)	747	2.9 (22)	908	1.7 (15)	804	3.7 (30)
Mixed infection	4131	0.7 (29)	1035	0.9 (9)	747	0 (0)	926	0 (0)	804	0.6 (5)
HIV infection	2618	1.2 (31)	771	1.3 (10)	555	0.5 (3)	550	0.2 (1)	642	0.9 (6)
EGA										
<14 week	4131	0.9 (38)	1035	0.7 (7)	747	0.1 (1)	926	0.4 (4)	804	1.1 (9)
14-19.9 week		20.9 (863)		24.2 (250)		22.9 (171)		14.4 (133)		21.4 (172)
20-23.9 week		24.8 (1023)		25.9 (268)		23.3 (174)		24.7 (229)		25.6 (206)
24-27.9 week		22.2 (917)		20.8 (215)		27.6 (206)		23.9 (221)		19.7 (158)
28-36.9 week		31.2 (1290)		28.5 (295)		26.1 (195)		36.6 (339)		32.2 (259)

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, DP: Dihydroartemisinin-piperaquine, EGA: Estimated gestational age, Q: Quinine monotherapy, QC: Quinine with clindamycin, SD: standard deviation.

Table 6-3 continued.

Characteristic	ASSP		AS		AAP		Q		QC	
	N	% (N)	N	% (N)	N	% (N)	N	% (N)	N	% (N)
		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)
Age group <20	147	19.0 (28)	174	20.1 (35)	76	28.9 (22)	171	21.6 (37)	51	19.6 (10)
20-24		56.5 (83)		23.6 (41)		28.9 (22)		39.2 (67)		23.5 (12)
25-29		17.0 (25)		21.3 (37)		21.1 (16)		20.5 (35)		35.3 (18)
30-34		4.1 (6)		14.4 (25)		11.8 (9)		12.9 (22)		11.8 (6)
>35		3.4 (5)		20.7 (36)		9.2 (7)		5.8 (10)		9.8 (5)
Gravidity 1	147	47.6 (70)	174	27.0 (47)	76	31.6 (24)	169	32.5 (55)	51	25.5 (13)
2		25.2 (37)		15.5 (27)		19.7 (15)		25.4 (43)		25.5 (13)
≥3		27.2 (40)		57.5 (100)		48.7 (37)		42.0 (71)		49.0 (25)
Parity 0	147	55.8 (82)	174	29.9 (52)	76	35.5 (27)	171	36.3 (62)	51	33.3 (17)
1		23.1 (34)		19.5 (34)		23.7 (18)		25.7 (44)		25.5 (13)
≥2		21.1 (31)		50.6 (88)		40.8 (31)		38.0 (65)		41.2 (21)
Height (cm)	116	150.0 (7)	128	151.6 (5.7)	32	149.9 (8.2)	44	153.3 (5.1)	0	
Weight (kg)	147	47.7 (6.9)	174	48.9 (5.9)	76	49.7 (6.6)	171	55.1 (9.4)	51	49.4 (5.4)
BMI <18.5	116	18.1 (21)	128	7.8 (10)	32	3.1 (1)	44	6.8 (3)	0	
18.5-24.9		72.4 (84)		84.4 (108)		81.3 (26)		90.9 (40)		
25.0-29.9		9.5 (11)		7.0 (9)		9.4 (3)		2.3 (1)		
≥30		0 (0)		0.8 (1)		6.3 (2)		0 (0)		
Fever (temperature >37.5)	115	24.3 (28)	174	27.0 (47)	76	25.0 (19)	171	15.8 (27)	51	21.6 (11)
Haemoglobin on day 0 (g/dL)	145	9.2 (1.4)	174	9.5 (1.5)	75	9.3 (1.5)	166	10.2 (1.8)	50	9.2 (1.7)
Parasitaemia (log ₁₀ /μL)	147	3.4 (0.8)	174	3.4 (1)	76	3.6 (1)	171	3.4 (0.9)	51	3.3 (1.1)
Presence of gametocyte	116	3.4 (4)	171	10.5 (18)	76	3.9 (3)	171	8.2 (14)	51	9.8 (5)
Mixed infection	147	0 (0)	174	6.9 (12)	76	0 (0)	171	0 (0)	51	5.9 (3)
HIV infection	23	17.4 (4)	0		0		77	9.1 (7)	0	
EGA										
<14 week	147	2.0 (3)	174	4.6 (8)	76	2.6 (2)	171	2.3 (4)	51	0 (0)
14-19.9 week		18.4 (27)		20.1 (35)		22.4 (17)		28.1 (48)		19.6 (10)
20-23.9 week		25.9 (38)		18.4 (32)		19.7 (15)		30.4 (52)		17.6 (9)
24-27.9 week		23.1 (34)		14.9 (26)		18.4 (14)		17.0 (29)		27.5 (14)
28-36.9 week		30.6 (45)		42.0 (73)		36.8 (28)		22.2 (38)		35.3 (18)

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, DP: Dihydroartemisinin-piperaquine, EGA: Estimated gestational age, Q: Quinine monotherapy, QC: Quinine with clindamycin, SD: standard deviation.

Risk factors for moderate to late preterm birth

In the univariable analysis, different antimalarial treatments were not associated with moderate to late PTB (Table 6-4).

In the multivariable analysis adjusted for gestational period, maternal age, height, BMI, HIV status, malaria transmission and gravidity combined with the previous history of pregnancy loss, different antimalarial treatments remained not associated with moderate to late PTB compared with AL. Between 14 and 37 weeks of gestation, the risk of PTB increased over the gestational period. Taller height (adjusted odds ratio [aOR] 0.98 per cm, 95% CI 0.97 to 1.00, $p=0.01$) and higher BMI (aOR 0.94 per kg/m^2 , 95% CI 0.90 to 0.97, $p=0.001$) were associated with a lower risk of PTB. Teenagers and primigravid women were independently associated with a higher risk of PTB. Compared with primigravid women, the risk of PTB was lower in G2 women with no previous pregnancy loss (aOR 0.65, 95% CI 0.48 to 0.89, $p=0.007$) and $G\geq 3$ women with no loss (aOR 0.67, 95% CI 0.47 to 0.95, $p=0.03$) or one loss (aOR 0.66, 95% CI 0.44 to 0.98, $p=0.04$), but was not different in G2 women with one loss (aOR 0.95, 95% CI 0.65 to 1.40, $p=0.80$), or $G\geq 3$ women with equal to or more than two losses (aOR 1.01, 95% CI 0.56 to 1.80, $p=0.98$). Compared with women <20 years old, the risk of PTB was lower in 20–24 year olds (aOR 0.67, 95% CI 0.51 to 0.87, $p=0.003$), but not different in 25–29 year olds (aOR 0.82, 95% CI 0.56 to 1.19, $p=0.29$), 30–34 (aOR 0.72, 95% CI 0.46 to 1.13, $p=0.15$) or ≥ 35 (aOR 1.00, 95% CI 0.61 to 1.65, $p=1.00$). The risk of PTB was higher in moderate or high malaria transmission area compared with low

transmission area.

When the risk of PTB after each treatment was assessed in each gestational period by introducing the interaction term between treatment and gestational period (Table 6-5), the adjusted risk of PTB was higher in AS in 24–27.9 weeks (aOR 5.45, 95% CI 1.65 to 17.96, $p=0.01$) but it was based on a small number of observations (6/26). For all other drugs, the risk was not different in any gestational periods. The first trimester (<14 weeks) was not assessed because of the small number of women.

A sensitivity analysis was conducted considering the imprecision of EGA at a maximum of plus one week in one study. The overall pooled proportion of extremely PTB (less than 28 weeks), very PTB (28 to less than 32 weeks) and moderate to late PTB (32 to less than 37 weeks) was 0.0% (5/2610, 95% CI 0.0 to 0.0, I^2 0%), 0.1% (34/3279, 95% CI 0.0 to 0.4, I^2 0%) and 7.8% (438/4147, 95% CI 5.6 to 10.3, I^2 81%), respectively. The results of the univariable and multivariable analyses were similar to the primary analysis results (Appendix Table 6-1, 6-2), except that malaria transmission intensity was not associated with the risk of PTB.

Table 6-4. Univariable and multivariable analyses of the risk of moderate to late preterm birth

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
AL	156/1035	Reference		Reference	
ASAQ	124/747	1.04 (0.79-1.38)	0.76	1.05 (0.79-1.39)	0.75
ASMQ	128/926	1.10 (0.84-1.46)	0.48	1.07 (0.81-1.42)	0.65
DP	151/804	1.28 (0.98-1.66)	0.07	1.22 (0.93-1.60)	0.15
ASSP	22/147	1.78 (0.87-3.64)	0.11	1.83 (0.88-3.79)	0.11
AS	15/174	1.42 (0.63-3.21)	0.40	1.49 (0.63-3.49)	0.36
AAP	4/76	0.51 (0.15-1.72)	0.28	0.69 (0.19-2.45)	0.56
Q	15/171	1.17 (0.56-2.41)	0.68	1.66 (0.78-3.51)	0.19
QC	4/51	1.04 (0.26-4.14)	0.96	1.39 (0.34-5.66)	0.65
Age (year)					
<20	284/1323	Reference		Reference	
20-24	154/1330	0.55 (0.44-0.68)	<0.001	0.67 (0.51-0.87)	0.003
25-29	100/803	0.61 (0.47-0.78)	<0.001	0.82 (0.56-1.19)	0.29
30-34	46/401	0.54 (0.38-0.77)	<0.001	0.72 (0.46-1.13)	0.15
≥35	35/274	0.75 (0.51-1.12)	0.16	1.00 (0.61-1.65)	1.00
Gravidity and previous history of pregnancy loss					
G1	284/1439	Reference		Reference	
G2 with no loss	81/704	0.57 (0.43-0.74)	<0.001	0.65 (0.48-0.89)	0.007
G≥3 with no loss	141/1128	0.61 (0.49-0.77)	<0.001	0.67 (0.47-0.95)	0.03
G2 with 1 loss	41/251	0.86 (0.59-1.25)	0.43	0.95 (0.65-1.40)	0.80
G≥3 with 1 loss	51/435	0.61 (0.44-0.84)	0.003	0.66 (0.44-0.98)	0.04
G≥3 with ≥2 losses	18/137	0.94 (0.55-1.60)	0.82	1.01 (0.56-1.80)	0.98
Height (cm)	579/3655	0.99 (0.97-1.00)	0.07	0.98 (0.97-1.00)	0.01
BMI	579/3655	0.96 (0.93-1.00)	0.04	0.94 (0.90-0.97)	0.001
Fever (>37.5°C)	45/375	1.07 (0.76-1.51)	0.71		
No fever	563/3719	Reference			
Haemoglobin on day 0 (g/dL)					
	616/4107	0.94 (0.88-1.00)	0.05		
Parasitaemia (log ₁₀ /μL)	619/4131	1.11 (0.99-1.24)	0.07		
Presence of gametocyte	24/153	1.47 (0.91-2.37)	0.11		
Without gametocyte	584/3922	Reference			
Mixed infection	2/29	0.99 (0.22-4.40)	0.99		
Monoinfection	617/4102	Reference			
HIV infection	4/31	1.33 (0.47-3.75)	0.59	1.17 (0.41-3.39)	0.77
not confirmed	527/3462	Reference		Reference	
Gestational period					
<14 weeks	5/38	3.81 (1.36-10.67)	0.01	3.97 (1.40-11.27)	0.01
14-19.9 weeks	78/863	Reference		Reference	
20-23.9 weeks	136/1023	1.67 (1.23-2.26)	0.001	1.70 (1.25-2.31)	0.001
24-27.9 weeks	142/917	2.17 (1.60-2.94)	<0.001	2.37 (1.74-3.23)	<0.001
28-36.9 weeks	258/1290	3.62 (2.70-4.84)	<0.001	4.33 (3.21-5.84)	<0.001
Malaria transmission					
Low	72/929	Reference		Reference	
Moderate	397/2236	1.99 (1.11-3.58)	0.02	2.33 (1.23-4.41)	0.01
High	150/966	1.85 (0.92-3.75)	0.09	2.00 (0.91-4.39)	0.08

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, G: gravidity, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. * Adjusted for treatment.

Table 6-5. Univariable and multivariable analyses of the risk of moderate to late preterm birth after different treatments in different gestational periods of pregnancy

Treatment	Proportion	Univariable		Multivariable*	
		OR (95% CI)	p-value	OR (95% CI)	p-value
14-19.9 weeks					
AL	22/250	Reference		Reference	
ASAQ	19/171	1.08 (0.56-2.10)	0.81	1.11 (0.57-2.17)	0.75
ASMQ	10/133	1.38 (0.62-3.09)	0.43	1.49 (0.66-3.35)	0.34
DP	19/172	1.11 (0.57-2.15)	0.75	1.14 (0.58-2.21)	0.71
ASSP	1/27	0.90 (0.11-7.63)	0.92	0.98 (0.12-8.31)	0.98
AS	1/35	1.10 (0.13-9.33)	0.93	1.42 (0.17-12.14)	0.75
AAP	2/17	2.76 (0.49-15.62)	0.25	4.12 (0.72-23.47)	0.11
Q	3/48	1.97 (0.50-7.82)	0.33	2.55 (0.65-10.04)	0.18
QC	1/10	3.26 (0.31-33.97)	0.32	4.12 (0.40-42.35)	0.23
20-23.9 weeks					
AL	37/268	Reference		Reference	
ASAQ	22/174	0.80 (0.45-1.43)	0.44	0.84 (0.47-1.52)	0.57
ASMQ	22/229	0.95 (0.53-1.70)	0.87	0.97 (0.54-1.74)	0.91
DP	39/206	1.32 (0.80-2.21)	0.28	1.29 (0.77-2.16)	0.33
ASSP	7/38	2.41 (0.79-7.29)	0.12	2.44 (0.82-7.25)	0.11
AS	4/32	2.72 (0.75-9.81)	0.13	3.32 (0.91-12.06)	0.07
AAP	0/15	0.21 (0.02-1.85)	0.16	0.28 (0.03-2.51)	0.26
Q	5/52	1.83 (0.59-5.71)	0.30	2.37 (0.77-7.34)	0.13
QC	0/9	0.95 (0.16-5.73)	0.95	1.16 (0.20-6.88)	0.87
24-27.9 weeks					
AL	33/215	Reference		Reference	
ASAQ	33/206	0.98 (0.57-1.69)	0.95	1.03 (0.59-1.78)	0.93
ASMQ	30/221	1.21 (0.69-2.10)	0.50	1.14 (0.65-1.99)	0.65
DP	30/158	1.27 (0.72-2.24)	0.40	1.29 (0.73-2.29)	0.38
ASSP	6/34	2.34 (0.76-7.20)	0.14	2.22 (0.72-6.83)	0.16
AS	6/26	4.77 (1.45-15.65)	0.01	5.45 (1.65-17.96)	0.01
AAP	0/14	0.21 (0.02-1.85)	0.16	0.28 (0.03-2.51)	0.26
Q	3/29	1.93 (0.49-7.65)	0.35	2.53 (0.63-10.18)	0.19
QC	1/14	1.05 (0.11-10.50)	0.97	1.53 (0.16-15.18)	0.71
28-36.9 weeks					
AL	63/295	Reference		Reference	
ASAQ	50/195	1.30 (0.82-2.05)	0.26	1.23 (0.77-1.96)	0.39
ASMQ	65/339	1.07 (0.71-1.62)	0.74	1.03 (0.68-1.56)	0.89
DP	63/259	1.25 (0.81-1.91)	0.31	1.20 (0.78-1.85)	0.42
ASSP	7/45	1.43 (0.51-4.02)	0.50	1.40 (0.50-3.95)	0.52
AS	3/73	0.38 (0.10-1.48)	0.17	0.41 (0.11-1.60)	0.20
AAP	1/28	0.21 (0.02-1.85)	0.16	0.28 (0.03-2.51)	0.26
Q	4/38	1.09 (0.33-3.61)	0.88	1.19 (0.36-3.97)	0.78
QC	2/18	0.95 (0.16-5.73)	0.95	1.16 (0.20-6.88)	0.87

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin.

* Adjusted for age, height, BMI, malaria transmission, HIV status and gravidity combined with previous history of pregnancy loss.

Small-for-gestational-age

Overall summary and baseline characteristics

Among 4,277 live singleton births without congenital abnormality, 3,707 births were included in the analyses after excluding 570 births for which SGA was not assessed (248 without birthweight, 253 birthweight measured >3 days after delivery, one without information on sex of the baby, 41 born after 43 weeks, 27 without EGA at delivery). The overall pooled proportion of SGA was 32.3% (1007/3707, 95% CI 26.7 to 38.2, I² 90%).

Nine antimalarial treatments were included in this analysis: AL (n=973), ASAQ (n=700), ASMQ (n=820), DP (n=716), ASSP (n=129), AS (n=154), AAP (n=55), quinine (n=119) and QC (n=41).

Baseline characteristics (Table 6-6) were similar to pregnant women who were included in the previous analysis of stillbirth and PTB.

Table 6-6. Baseline characteristics of pregnant women who were assessed for small-for-gestational-age

Characteristic	All		AL		ASAQ		ASMQ		DP	
	N	% (N)	N	% (N)	N	% (N)	N	% (N)	N	% (N)
		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)
Age group <20	3707	32.4 (1202)	973	37.5 (365)	700	31.9 (223)	820	27.7 (227)	716	40.6 (291)
20-24		32.0 (1185)		29.8 (290)		31.0 (217)		33.7 (276)		30.0 (215)
25-29		19.3 (717)		17.9 (174)		20.1 (141)		21.3 (175)		16.1 (115)
30-34		9.6 (357)		8.3 (81)		11.0 (77)		10.5 (86)		8.2 (59)
>35		6.6 (246)		6.5 (63)		6.0 (42)		6.8 (56)		5.0 (36)
Gravidity 1	3671	35.8 (1316)	940	35.5 (334)	699	36.2 (253)	820	33.5 (275)	716	40.1 (287)
2		22.9 (839)		23.2 (218)		22.0 (154)		23.7 (194)		23.5 (168)
≥3		41.3 (1516)		41.3 (388)		41.8 (292)		42.8 (351)		36.5 (261)
Parity 0	3702	43.1 (1596)	969	43.1 (418)	699	40.8 (285)	820	42.6 (349)	716	49.0 (351)
1		21.1 (780)		20.4 (198)		21.7 (152)		20.6 (169)		21.2 (152)
≥2		35.8 (1326)		36.4 (353)		37.5 (262)		36.8 (302)		29.7 (213)
Height (cm)	3347	156.0 (6.9)	855	156.1 (6.5)	700	158.0 (6.6)	813	156.2 (7.4)	716	155.4 (6.2)
Weight (kg)	3707	54.1 (8.2)	973	53.7 (7.2)	700	56.1 (8.7)	820	54.1 (8.4)	716	54.7 (8.2)
BMI <18.5	3347	6.0 (201)	855	4.9 (42)	700	5.1 (36)	813	7.0 (57)	716	5.2 (37)
18.5-24.9		80.3 (2687)		85.5 (731)		79.4 (556)		78.4 (637)		77.8 (557)
25.0-29.9		12.0 (400)		8.9 (76)		11.7 (82)		12.9 (105)		15.6 (112)
≥30		1.8 (59)		0.7 (6)		3.7 (26)		1.7 (14)		1.4 (10)
Fever (temperature >37.5)	3677	8.8 (325)	973	7.8 (76)	700	5.0 (35)	819	9.4 (77)	715	4.3 (31)
Haemoglobin on day 0 (g/dL)	3690	10.0 (1.4)	964	10.2 (1.3)	700	10.0 (1.3)	820	9.9 (1.3)	714	10.0 (1.4)
Parasitaemia (log ₁₀ /μL)	3707	3.0 (0.8)	973	3.0 (0.8)	700	2.8 (0.8)	820	3.0 (0.8)	716	2.9 (0.8)
Presence of gametocyte	3655	3.6 (133)	970	4.0 (39)	700	3.1 (22)	801	1.2 (10)	716	3.6 (26)
Mixed infection	3707	0.7 (25)	973	1.0 (10)	700	0 (0)	820	0 (0)	716	0.1 (1)
HIV infection	2437	1.0 (24)	726	1.1 (8)	521	0.6 (3)	514	0.2 (1)	595	1.0 (6)
EGA										
<14 week	3707	0.8 (31)	973	0.7 (7)	700	0 (0)	820	0.2 (2)	716	1.0 (7)
14-19.9 week		20.6 (765)		23.6 (230)		23.3 (163)		13.0 (107)		21.2 (152)
20-23.9 week		24.7 (914)		25.7 (250)		23.4 (164)		25.5 (209)		25.6 (183)
24-27.9 week		22.1 (820)		21.2 (206)		27.6 (193)		24.1 (198)		19.3 (138)
28-36.9 week		30.7 (1138)		27.4 (267)		25.7 (180)		36.7 (301)		33.0 (236)
≥37 week		1.1 (39)		1.3 (13)		0 (0)		0.4 (3)		0 (0)

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, DP: Dihydroartemisinin-piperaquine, EGA: Estimated gestational ageQ: Quinine monotherapy, QC: Quinine with clindamycin, SD: standard deviation.

Table 6-6 continued.

Characteristic	ASSP		AS		AAP		Q		QC	
	N	% (N)	N	% (N)	N	% (N)	N	% (N)	N	% (N)
		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)
Age group <20	129	20.2 (26)	154	16.2 (25)	55	27.3 (15)	119	20.2 (24)	41	14.6 (6)
20-24		55.8 (72)		27.9 (43)		25.5 (14)		40.3 (48)		24.4 (10)
25-29		17.8 (23)		23.4 (36)		25.5 (14)		21.0 (25)		34.1 (14)
30-34		3.9 (5)		13.6 (21)		12.7 (7)		11.8 (14)		17.1 (7)
>35		2.3 (3)		18.8 (29)		9.1 (5)		6.7 (8)		9.8 (4)
Gravidity 1	129	50.4 (65)	154	25.3 (39)	55	29.1 (16)	117	31.6 (37)	41	24.4 (10)
2		24.8 (32)		15.6 (24)		20.0 (11)		23.9 (28)		24.4 (10)
≥3		24.8 (32)		59.1 (91)		50.9 (28)		44.4 (52)		51.2 (21)
Parity 0	129	58.9 (76)	154	27.9 (43)	55	32.7 (18)	119	37.0 (44)	41	29.3 (12)
1		22.5 (29)		20.1 (31)		21.8 (12)		22.7 (27)		24.4 (10)
≥2		18.6 (24)		51.9 (80)		45.5 (25)		40.3 (48)		46.3 (19)
Height (cm)	102	150.0 (7.2)	107	151.7 (5.6)	23	148.0 (8.5)	31	153.4 (5.4)	0	
Weight (kg)	129	47.8 (7.1)	154	50.0 (6.6)	55	50.9 (7.3)	119	55.6 (9.3)	41	49.3 (5.5)
BMI <18.5	102	18.6 (19)	107	7.5 (8)	23	78.3 (0)	31	6.5 (2)	0	
18.5-24.9		70.6 (72)		83.2 (89)		13.0 (18)		87.1 (27)		
25.0-29.9		10.8 (11)		8.4 (9)		8.7 (3)		6.5 (2)		
≥30		0 (0)		0.9 (1)		0 (2)		0 (0)		
Fever (temperature >37.5)	101	25.7 (26)	154	25.3 (39)	55	25.5 (14)	119	15.1 (18)	41	22.0 (9)
Haemoglobin on day 0 (g/dL)	127	9.2 (1.3)	154	9.5 (1.5)	54	9.2 (1.4)	116	10.4 (1.7)	41	9.3 (1.7)
Parasitaemia (log ₁₀ /μL)	129	3.4 (0.8)	154	3.3 (1.1)	55	3.6 (1.1)	119	3.4 (0.8)	41	3.3 (1.1)
Presence of gametocyte	102	2.9 (3)	151	11.3 (17)	55	5.5 (3)	119	8.4 (10)	41	7.3 (3)
Mixed infection	129	0 (0)	154	7.1 (11)	55	1.8 (1)	119	0 (0)	41	4.9 (2)
HIV infection	21	14.3 (3)	0		0		60	5.0 (3)	0	
EGA										
<14 week	129	1.6 (2)	154	4.5 (7)	55	3.6 (2)	119	3.4 (4)	41	0 (0)
14-19.9 week		19.4 (25)		22.1 (34)		21.8 (12)		29.4 (35)		17.1 (7)
20-23.9 week		26.4 (34)		16.2 (25)		18.2 (10)		28.6 (34)		12.2 (5)
24-27.9 week		20.9 (27)		10.4 (16)		18.2 (10)		18.5 (22)		24.4 (10)
28-36.9 week		31.8 (41)		38.3 (59)		30.9 (17)		18.5 (22)		36.6 (15)
≥37 week		0 (0)		8.4 (13)		7.3 (4)		1.7 (2)		9.8 (4)

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, DP: Dihydroartemisinin-piperaquine, EGA: Estimated gestational age, Q: Quinine monotherapy, QC: Quinine with clindamycin, SD: standard deviation.

Risk factors for small-for-gestational-age

In the univariable analysis, different treatments were not associated with SGA (Table 6-7). In the multivariable analysis adjusted for gestational period, maternal height, BMI, parasitaemia, malaria species, HIV status and gravidity combined with the previous history of pregnancy loss, different antimalarial treatments remained not associated with SGA. The risk of SGA was the highest when women had malaria in ≥ 37 weeks of gestation (aOR 1.38, 95% CI 0.66 to 2.87, $p=0.39$) compared with 14–19.9 weeks, but it was not significant and based on a small number of observations. Between 14 and 37 weeks of gestation, the risk of SGA decreased over the gestational period. Taller height (aOR 0.92 per cm, 95% CI 0.89 to 0.95, $p<0.001$) and higher BMI (aOR 0.95 per kg/m^2 , 95% CI 0.93 to 0.96, $p<0.001$) were associated with a lower risk of SGA. Higher baseline parasitaemia (aOR 1.14 per 10-fold increase, 95% CI 1.04 to 1.26, $p=0.01$) and coinfection of other malaria species (aOR 2.54, 95% CI 1.08 to 5.97, $p=0.03$) were associated with a higher risk of SGA. Compared with primigravid women, the risk of SGA was lower in G2 women with no previous pregnancy loss (aOR 0.61, 95% CI 0.49 to 0.77, $p<0.001$) and $G\geq 3$ women with no loss (aOR 0.48, 95% CI 0.39 to 0.59, $p<0.001$) or one loss (aOR 0.54, 95% CI 0.41 to 0.72, $p<0.001$), but was not different in G2 women with one loss (aOR 0.83, 95% CI 0.60 to 1.15, $p=0.26$), or $G\geq 3$ women with equal to or more than two losses (aOR 0.78, 95% CI 0.51 to 1.19, $p=0.25$). A positive HIV status was not associated with a risk of SGA (aOR 1.05, 95% CI 0.41 to 2.71, $p=0.91$).

When the risk of SGA after each treatment was assessed in each gestational period by

introducing the interaction term between treatment and gestational period (Table 6-8), the risk of SGA was significantly higher in ASSP in 28–36.9 weeks (26/41, aOR 2.73, 95% CI 1.19 to 6.24, $p=0.02$) compared with that of AL during the same period. For all other drugs, the risk was not different in any gestational periods. The first trimester (<14 weeks) and ≥ 37 weeks were not assessed because of the small numbers of women.

A sensitivity analysis was conducted considering the imprecision of EGA at a maximum of plus one week in one study. The overall pooled proportion of SGA was 38.1% (1310/3674, 95% CI 32.7 to 43.6, I^2 88%). The results of the univariable and multivariable analyses were similar to the primary analysis results (Appendix Table 6-3, 4). As in the primary analysis, the adjusted risk of SGA was consistently higher in ASSP in 28–36.9 weeks (aOR 2.91, 95% CI 1.29 to 6.55, $p=0.01$) compared with that of AL during the same period.

Table 6-7. Univariable and multivariable analyses of the risk of small-for-gestational-age

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
AL	241/973	Reference		Reference	
ASAQ	164/700	1.02 (0.79-1.32)	0.85	1.06 (0.81-1.38)	0.68
ASMQ	257/820	0.99 (0.78-1.26)	0.95	1.01 (0.78-1.29)	0.96
DP	163/716	0.86 (0.67-1.12)	0.26	0.88 (0.67-1.15)	0.34
ASSP	66/129	1.44 (0.85-2.44)	0.17	1.31 (0.76-2.26)	0.33
AS	56/154	1.38 (0.82-2.29)	0.22	1.28 (0.75-2.18)	0.37
AAP	25/55	1.99 (0.89-4.46)	0.09	1.80 (0.74-4.34)	0.19
Q	20/119	0.56 (0.31-1.04)	0.07	0.67 (0.33-1.36)	0.27
QC	15/41	1.28 (0.51-3.18)	0.60	1.04 (0.40-2.71)	0.93
Age (year)					
<20	379/1202	Reference			
20-24	332/1185	0.65 (0.54-0.79)	<0.001		
25-29	170/717	0.52 (0.42-0.66)	<0.001		
30-34	63/357	0.37 (0.27-0.50)	<0.001		
≥35	63/246	0.55 (0.40-0.76)	<0.001		
Gravidity and previous history of pregnancy loss					
G1	444/1316	Reference		Reference	
G2 with no loss	154/614	0.59 (0.47-0.74)	<0.001	0.61 (0.49-0.77)	<0.001
G≥3 with no loss	186/1011	0.40 (0.33-0.49)	<0.001	0.48 (0.39-0.59)	<0.001
G2 with 1 loss	76/225	0.83 (0.61-1.14)	0.25	0.83 (0.60-1.15)	0.26
G≥3 with 1 loss	88/387	0.46 (0.35-0.61)	<0.001	0.54 (0.41-0.72)	<0.001
G≥3 with ≥2 losses	40/117	0.77 (0.51-1.17)	0.21	0.78 (0.51-1.19)	0.25
Height (cm)	891/3347	0.95 (0.94-0.96)	<0.001	0.95 (0.93-0.96)	<0.001
BMI	891/3347	0.93 (0.90-0.95)	<0.001	0.92 (0.89-0.95)	<0.001
Fever (>37.5°C)	112/325	1.12 (0.86-1.46)	0.39		
No fever	888/3352	Reference			
Haemoglobin on day 0 (g/dL)					
	1003/3690	0.90 (0.85-0.95)	<0.001		
Parasitaemia (log ₁₀ /μL)	1007/3707	1.24 (1.13-1.36)	<0.001	1.14 (1.04-1.26)	0.01
Presence of gametocyte	36/133	0.85 (0.56-1.28)	0.43		
Without gametocyte	953/3522	Reference			
Mixed infection	14/25	2.87 (1.25-6.59)	0.01	2.54 (1.08-5.97)	0.03
Monoinfection	993/3682	Reference		Reference	
HIV infection	6/24	0.88 (0.36-2.15)	0.77	1.05 (0.41-2.71)	0.91
not confirmed	849/3105	Reference		Reference	
Gestational period					
<14 weeks	10/31	0.69 (0.30-1.57)	0.37	0.64 (0.28-1.48)	0.30
14-19.9 weeks	227/765	Reference		Reference	
20-23.9 weeks	246/914	0.81 (0.65-1.01)	0.06	0.80 (0.63-1.00)	0.06
24-27.9 weeks	219/820	0.74 (0.59-0.94)	0.01	0.80 (0.63-1.02)	0.07
28-36.9 weeks	289/1138	0.60 (0.48-0.75)	<0.001	0.70 (0.56-0.88)	0.002
≥37 weeks	16/39	1.06 (0.53-2.15)	0.86	1.38 (0.66-2.87)	0.39
Malaria transmission					
Low	277/726	Reference			
Moderate	484/2040	0.85 (0.52-1.38)	0.51		
High	246/941	0.77 (0.42-1.40)	0.39		

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, G: gravidity, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. * Adjusted for treatment.

Table 6-8. Univariable and multivariable analyses of the risk of small-for-gestational-age after different treatments in different gestational periods of pregnancy

Treatment	Proportion	Univariable		Multivariable*	
		OR (95% CI)	p-value	OR (95% CI)	p-value
14-19.9 weeks					
AL	64/230	Reference		Reference	
ASAQ	45/163	1.15 (0.71-1.85)	0.57	1.11 (0.68-1.80)	0.68
ASMQ	43/107	0.99 (0.59-1.66)	0.96	1.03 (0.61-1.75)	0.91
DP	37/152	0.85 (0.52-1.40)	0.52	0.85 (0.51-1.41)	0.53
ASSP	11/25	0.77 (0.30-1.98)	0.59	0.70 (0.27-1.83)	0.46
AS	12/34	1.05 (0.45-2.45)	0.91	1.09 (0.45-2.61)	0.85
AAP	5/12	1.58 (0.40-6.26)	0.51	1.61 (0.38-6.74)	0.52
Q	8/35	0.56 (0.22-1.43)	0.23	0.69 (0.25-1.86)	0.46
QC	2/7	0.78 (0.13-4.72)	0.78	0.56 (0.08-3.65)	0.54
20-23.9 weeks					
AL	68/250	Reference		Reference	
ASAQ	36/164	0.82 (0.51-1.34)	0.44	0.89 (0.54-1.47)	0.65
ASMQ	65/209	0.83 (0.55-1.28)	0.40	0.86 (0.56-1.34)	0.51
DP	46/183	0.96 (0.61-1.52)	0.86	0.96 (0.60-1.53)	0.85
ASSP	13/34	0.85 (0.36-2.01)	0.71	0.74 (0.30-1.79)	0.50
AS	11/25	1.73 (0.69-4.36)	0.24	1.49 (0.57-3.86)	0.41
AAP	1/10	0.23 (0.03-2.02)	0.18	0.27 (0.03-2.50)	0.25
Q	4/34	0.29 (0.09-0.93)	0.04	0.35 (0.10-1.16)	0.09
QC	2/5	1.49 (0.21-10.41)	0.69	1.63 (0.22-11.82)	0.63
24-27.9 weeks					
AL	50/206	Reference		Reference	
ASAQ	47/193	1.14 (0.71-1.84)	0.59	1.17 (0.72-1.92)	0.52
ASMQ	61/198	1.04 (0.66-1.64)	0.88	0.99 (0.62-1.59)	0.96
DP	29/138	0.83 (0.49-1.43)	0.51	0.82 (0.47-1.43)	0.49
ASSP	16/27	2.13 (0.84-5.39)	0.11	1.80 (0.69-4.68)	0.23
AS	4/16	0.90 (0.26-3.15)	0.87	0.56 (0.15-2.11)	0.39
AAP	5/10	2.40 (0.57-10.16)	0.23	1.63 (0.36-7.38)	0.53
Q	4/22	0.67 (0.20-2.23)	0.51	0.86 (0.24-3.05)	0.82
QC	3/10	1.21 (0.26-5.75)	0.81	0.93 (0.19-4.67)	0.93
28-36.9 weeks					
AL	53/267	Reference		Reference	
ASAQ	36/180	1.03 (0.63-1.70)	0.90	1.07 (0.64-1.79)	0.79
ASMQ	88/301	1.23 (0.82-1.86)	0.32	1.23 (0.80-1.88)	0.34
DP	47/236	0.89 (0.56-1.41)	0.61	0.88 (0.55-1.42)	0.60
ASSP	26/41	2.84 (1.27-6.35)	0.01	2.73 (1.19-6.24)	0.02
AS	22/59	2.33 (1.12-4.81)	0.02	1.99 (0.94-4.21)	0.07
AAP	8/17	2.62 (0.77-8.87)	0.12	2.19 (0.62-7.77)	0.22
Q	3/22	0.63 (0.17-2.34)	0.49	0.69 (0.18-2.71)	0.60
QC	6/15	2.61 (0.73-9.39)	0.14	1.78 (0.47-6.74)	0.39

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin.

* Adjusted for height, BMI, parasitaemia on log₁₀ scale, malaria species, HIV status and gravidity combined with previous history of pregnancy loss.

Placental malaria

Overall summary and baseline characteristics

Among 3,033 women in four studies that assessed placental histopathology, 441 (17.6%, 95% CI 12.1 to 23.8, I^2 94%.) women were categorized as no infection, 49 (1.6%, 95% CI 0.8 to 2.5, I^2 60%.) acute infection, 1,816 (57.5%, 95% CI 53.6 to 61.5, I^2 77%.) past infection and 727 (21.6%, 95% CI 15.9 to 27.8, I^2 93%.) chronic infection. Overall positivity of malaria pigment was 80.1% (2543/3033, 95% CI 73.5 to 85.9, I^2 84%), and the risk factors for deposition of malaria pigment (i.e. past and chronic infection) were investigated.

Six antimalarial treatments were included in this analysis: AL (n=893), ASAQ (n=649), ASMQ (n=668), DP (n=658), AS (n=85) and quinine (n=80) (Table 6-9). Median age was 22 (interquartile range 19–27), and median parity was 1 (interquartile range 0–2). The included malaria episodes were mostly in the second (69.5%, 2107/3031) or third trimester (30.4%, 921/3031). The majority of the episodes (99.5%, 3017/3033) were *P. falciparum* mono-infections and the remainder were mixed infections. The geometric mean parasite density was 895 (range 5–292,360) asexual parasites / μ L. Only 6.6% (199/3032) of women were febrile at presentation (>37.5 °C). After treatment, 74.6% (2227/2987) had no recurrences within 63 days, 3.3% (98/2987) had recrudescence, 20.0% (596/2987) had reinfection and 2.2% (66/2987) had a falciparum malaria recurrence without PCR information.

Table 6-9. Baseline characteristics of pregnant women who were assessed for placental malaria

Characteristic	All		AL		ASAQ		ASMQ		DP		AS		Q	
	N	% (N)	N	% (N)	N	% (N)	N	% (N)	N	% (N)	N	% (N)	N	% (N)
	Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)	
Interval from malaria to delivery (week)	3033	14.1 (6.1)	893	14.3 (6.2)	649	14.7 (5.8)	668	13.5 (5.7)	658	14.0 (6.2)	85	13.8 (8.0)	80	14.6 (6.7)
Age group <20	3033	34.7 (1051)	893	37.6 (336)	649	31.1 (202)	668	31.9 (213)	658	40.4 (266)	85	15.3 (13)	80	26.3 (21)
20-24		30.3 (920)		29.1 (260)		31.7 (206)		30.1 (201)		30.4 (200)		22.4 (19)		42.5 (34)
25-29		18.7 (567)		18.4 (164)		20.0 (130)		19.3 (129)		16.7 (110)		18.8 (16)		22.5 (18)
30-34		9.8 (296)		9.0 (80)		10.6 (69)		11.1 (74)		8.1 (53)		18.8 (16)		5.0 (4)
>35		6.6 (199)		5.9 (53)		6.5 (42)		7.6 (51)		4.4 (29)		24.7 (21)		3.8 (3)
Parity 0	3030	43.3 (1311)	891	43.5 (388)	648	40.9 (265)	668	42.1 (281)	658	49.1 (323)	85	24.7 (21)	80	41.3 (33)
1		19.6 (595)		19.0 (169)		20.5 (133)		17.7 (118)		21.1 (139)		17.6 (15)		26.3 (21)
≥2		37.1 (1124)		37.5 (334)		38.6 (250)		40.3 (269)		29.8 (196)		57.6 (49)		32.5 (26)
Height (cm)	2845	156.6 (6.7)	785	156.2 (6.6)	649	158.0 (6.6)	668	157.2 (7.2)	658	155.7 (6.2)	85	151.6 (5.7)	0	
BMI	2845	22.3 (3.0)	785	21.7 (2.4)	649	22.5 (3.3)	668	22.5 (3.2)	658	22.7 (2.9)	85	21.6 (2.5)	0	
Body temperature (°C)	3032	36.7 (0.6)	893	36.7 (0.6)	649	36.7 (0.6)	667	36.7 (0.5)	658	36.5 (0.5)	85	37.0 (1.1)	80	37.1 (0.6)
Haemoglobin on day 0 (g/dL)	3024	10.1 (1.4)	885	10.2 (1.4)	649	10.1 (1.3)	668	10.1 (1.3)	658	10.1 (1.3)	85	9.6 (1.5)	79	10.5 (1.6)
Parasitaemia (log ₁₀ /μL)	3033	3.0 (0.8)	893	3.1 (0.8)	649	2.8 (0.8)	668	2.9 (0.8)	658	2.9 (0.8)	85	3.5 (1.0)	80	3.3 (0.9)
Presence of gametocyte	3028	3.0 (91)	890	3.1 (28)	649	3.2 (21)	668	0.9 (6)	658	3.0 (20)	83	8.4 (7)	80	11.3 (9)
Mixed infection	3033	0.5 (16)	893	0.8 (7)	649	0.0 (0)	668	0.0 (0)	658	0.0 (0)	85	10.6 (9)	80	0.0 (0)
HIV infection	2315	1.1 (25)	700	1.4 (10)	479	0.6 (3)	497	0.0 (0)	577	1.2 (7)	0		62	8.1 (5)
Treatment outcome														
No recurrence	2987	74.6 (2227)	880	60.1 (529)	645	81.2 (524)	658	72.3 (476)	642	87.7 (563)	84	82.1 (69)	78	84.6 (66)
Recrudescence		3.3 (98)		5.6 (49)		1.7 (11)		3.6 (24)		0.9 (6)		8.3 (7)		1.3 (1)
Reinfection		20.0 (596)		32.2 (283)		15.2 (98)		21.6 (142)		9.7 (62)		8.3 (7)		5.1 (4)
No PCR		2.2 (66)		2.2 (19)		1.9 (12)		2.4 (16)		1.7 (11)		1.2 (1)		9.0 (7)
MAP category														
Low	3033	8.3 (252)	893	13.5 (121)	649	0.0 (0)	668	0.0 (0)	658	0.0 (0)	85	100.0 (85)	80	57.5 (46)
Moderate		61.9 (1877)		58.0 (518)		54.1 (351)		54.5 (364)		92.7 (610)		0.0 (0)		42.5 (34)
High		29.8 (904)		28.4 (254)		45.9 (298)		45.5 (304)		7.3 (48)		0.0 (0)		0.0 (0)

AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, DP: Dihydroartemisinin-piperaquine,, Q: Quinine monotherapy, SD: standard deviation.

Table 6-10. Univariable and multivariable analyses of the risk of malaria pigment in the placenta by treatment

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
AL	733/880	Reference		Reference	
ASAQ	544/645	0.93 (0.67-1.29)	0.68	0.91 (0.64-1.30)	0.61
ASMQ	560/658	0.80 (0.57-1.12)	0.20	0.79 (0.55-1.14)	0.21
DP	559/642	0.96 (0.67-1.36)	0.81	0.93 (0.63-1.36)	0.69
AS	56/84	1.63 (0.86-3.10)	0.13	1.73 (0.84-3.55)	0.14
Q	53/78	1.05 (0.55-2.00)	0.89	0.89 (0.44-1.80)	0.74
Interval from malaria to delivery (week)	2505/2987	0.98 (0.96-0.99)	0.01	0.97 (0.95-0.99)	0.002
Age (year)	2505/2987	0.89 (0.88-0.91)	<0.001	0.93 (0.91-0.95)	<0.001
Parity					
0	1199/1292	Reference		Reference	
1	498/580	0.50 (0.36-0.69)	<0.001	0.83 (0.58-1.17)	0.29
≥2	807/1112	0.20 (0.16-0.26)	<0.001	0.60 (0.41-0.86)	0.01
Height (cm)	2383/2806	0.98 (0.96-0.99)	0.01		
BMI	2383/2806	0.95 (0.91-0.98)	0.002		
Body temperature (°C)	2504/2986	1.34 (1.13-1.60)	0.001	1.26 (1.04-1.53)	0.02
Haemoglobin on day 0 (g/dL)	2500/2978	0.65 (0.60-0.71)	<0.001	0.72 (0.66-0.79)	<0.001
Parasitaemia (log ₁₀ /μL)	2505/2987	2.06 (1.78-2.38)	<0.001	1.67 (1.42-1.96)	<0.001
Presence of gametocyte	84/91	3.51 (1.58-7.82)	0.002	3.60 (1.58-8.23)	0.002
Without gametocyte	2417/2891	Reference		Reference	
Mixed infection	12/16	1.67 (0.51-5.42)	0.39		
Monoinfection	2493/2971	Reference			
HIV infection	18/24	1.10 (0.41-2.93)	0.85		
Negative	2035/2414	Reference			
Malaria transmission					
Low	159/249	Reference		Reference	
Moderate	1588/1838	0.84 (0.41-1.71)	0.63	1.04 (0.47-2.30)	0.92
High	758/900	0.97 (0.39-2.39)	0.94	1.20 (0.43-3.35)	0.73

AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, OR: odds ratio, Q: Quinine monotherapy. * Adjusted for treatment.

Risk factors for placental malaria

The first model was developed to assess the impact of different treatments. In both the univariable and multivariable analyses, the risk of malaria pigment in the placenta was not different for different treatments compared with that for AL (Table 6-10).

In the multivariable analysis, shorter interval from malaria episode to delivery (aOR 0.97 per week, 95% CI 0.95 to 0.99, p=0.002), lower age (aOR 0.93 per year, 95% CI 0.91 to 0.95, p<0.001), higher body temperature (aOR 1.26 per °C, 95% CI 1.04 to 1.53, p=0.02),

Table 6-11. Univariable and multivariable analyses of the risk of malaria pigment in the placenta by parity in different malaria transmission intensities

Characteristic	Proportion	Univariable*		Multivariable‡	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Low transmission					
Parity 0	57/81	Reference		Reference	
1	34/50	0.93 (0.43-2.00)	0.84	1.21 (0.52-2.79)	0.66
≥2	68/118	0.59 (0.32-1.10)	0.09	1.32 (0.65-2.69)	0.45
Moderate transmission					
Parity 0	878/934	Reference		Reference	
1	302/349	0.43 (0.29-0.66)	<0.001	0.73 (0.47-1.13)	0.16
≥2	408/553	0.17 (0.12-0.24)	<0.001	0.52 (0.34-0.80)	0.003
High transmission					
Parity 0	264/277	Reference		Reference	
1	162/181	0.44 (0.21-0.91)	0.03	0.84 (0.39-1.79)	0.64
≥2	331/441	0.14 (0.08-0.26)	<0.001	0.50 (0.26-0.98)	0.04

AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, OR: odds ratio, PCR: polymerase chain reaction, Q: Quinine monotherapy. * Adjusted for treatment. †Adjusted for treatment, interval from malaria to delivery, age, body temperature, haemoglobin on day 0, parasitaemia and presence of gametocyte.

lower haemoglobin level (aOR 0.72 per g/dL, 95% CI 0.65 to 0.79, $p<0.001$), higher baseline parasitaemia (aOR 1.67 per 10-fold increase, 95% CI 1.42 to 1.96, $p<0.001$) and presence of gametocytaemia (aOR 3.60, 95% CI 1.58 to 8.23, $p=0.002$) were associated with a higher risk of the presence of malaria pigment in the placenta. When the effect of parity was analysed by different malaria transmission intensities, the adjusted risk of placental malaria in pregnant women with ≥ 3 parities was lower than nulliparous women in moderate and high endemic areas, but not in low endemic areas (Table 6-11).

Another model was developed to assess the impact of treatment outcome (Table 6-12). In the univariable analysis, the risks of placental malaria were higher in pregnant women who had a *P. falciparum* recurrence of any types (PCR-uncorrected recurrence) within 63 days (OR 3.08, 95% CI 2.26 to 4.20, $p<0.001$). There was no difference among the three types of recurrence: recrudescence (OR 3.57, 95% CI 1.68 to 7.60); reinfection (OR 3.42, 95% CI 2.38 to 4.94); recurrence without PCR (OR 1.51 95% CI 0.76 to 3.03).

Table 6-12. Univariable and multivariable analyses of the risk of malaria pigment in the placenta by treatment outcome

Variable	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment outcome					
No recurrence	1799/2227	Reference		Reference	
Recrudescence	90/98	3.57 (1.68-7.60)	0.001	3.65 (1.53-8.70)	0.004
Reinfection	560/596	3.42 (2.38-4.94)	<0.001	2.56 (1.75-3.76)	<0.001
Recurrence without PCR	56/66	1.51 (0.76-3.03)	0.24	1.36 (0.63-2.92)	0.43
Interval from malaria to delivery (week)	2505/2987	0.97 (0.96-0.99)	0.003	0.97 (0.95-0.99)	0.001
Age (year)	2505/2987	0.90 (0.88-0.91)	<0.001	0.93 (0.91-0.95)	<0.001
Parity					
0	1199/1292	Reference		Reference	
1	498/580	0.52 (0.38-0.72)	<0.001	0.86 (0.61-1.22)	0.40
≥2	807/1112	0.22 (0.17-0.29)	<0.001	0.63 (0.44-0.92)	0.02
Height (cm)	2383/2806	0.98 (0.96-0.99)	0.01		
BMI	2383/2806	0.95 (0.92-0.98)	0.004		
Body temperature (°C)	2504/2986	1.31 (1.10-1.56)	0.003	1.25 (1.03-1.52)	0.02
Haemoglobin on day 0 (g/dL)	2500/2978	0.66 (0.61-0.71)	<0.001	0.72 (0.66-0.78)	<0.001
Parasitaemia (log ₁₀ /μL)	2505/2987	1.92 (1.65-2.22)	<0.001	1.58 (1.35-1.86)	<0.001
Presence of gametocyte	84/91	3.57 (1.60-7.96)	0.002	3.83 (1.66-8.83)	0.002
Without gametocyte	2417/2891	Reference		Reference	
Mixed infection	12/16	1.62 (0.50-5.30)	0.42		
Monoinfection	2493/2971	Reference			
HIV infection	18/24	1.18 (0.45-3.10)	0.74		
Negative / not tested	2487/2963	Reference			
Malaria transmission					
Low	159/249	Reference		Reference	
Moderate	1588/1838	0.93 (0.45-1.92)	0.85	1.14 (0.51-2.56)	0.75
High	758/900	0.95 (0.40-2.25)	0.91	1.24 (0.46-3.36)	0.67

AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, OR: odds ratio, Q: Quinine monotherapy. * Adjusted for treatment outcome.

In the multivariable model adjusted for other risk factors, the risks of placental malaria were higher in pregnant women who had a *P. falciparum* recurrence of any types in 63 days (aOR 2.46, 95% CI 1.77 to 3.43, $p < 0.001$). There was no difference among the three types of recurrence: recrudescence (aOR 3.65, 95% CI 1.53 to 8.70); reinfection (aOR 2.56, 95% CI 1.75 to 3.76); recurrence without PCR (aOR 1.36, 95% CI 0.63 to 2.92). The estimates of other variables were similar to the previous analysis assessing different treatments.

6.4 Discussion

This meta-analysis demonstrated that among four commonly used ACTs, namely AL, ASAQ, ASMQ and DP, the adjusted risks of stillbirth, PTB, SGA and placental malaria (deposition of malaria pigment in the placenta) were not different when they were used for treating uncomplicated falciparum malaria in the second or third trimester of pregnancy. The results for the other treatments need careful interpretation because of the small number of women included in the analyses, but generally, they were associated with similar risks to AL, ASAQ, ASMQ and DP.

One exception arose with the risk of SGA after ASSP, involving data contributed from only two studies. One study, which contributed 90% of the included ASSP data, did not report SGA but did describe a smaller mean birthweight (and a higher proportion of LBW) of ASSP than ASMQ in the original report.²⁵⁷ This finding was based on a small number of women; studies from other settings are needed to confirm this finding, although it is unlikely that studies on ASSP will be conducted considering the high prevalence of resistance markers for SP in both India (where ASSP is still used) and sub-Saharan Africa.²⁸⁷ The prevalence of molecular resistance to SP in the study area (Northern India) was high at the study period,³⁸⁸ which means that the apparent high parasitological efficacy was due to artesunate and there might have been less (or no) additional effects from SP as a partner drug. SGA can be a more sensitive indicator of a poorly effective drug than LBW as it measures growth restriction in newborns that weigh more than or equal to 2,500 g, making this an important finding of the

analysis.

Higher parasitaemia at baseline was associated with higher risks of stillbirth, SGA and placental malaria even after adjustment for gravidity/parity (except stillbirth, $p=0.06$).

This finding implies that early detection based on regular and active screening has the potential to improve pregnancy outcomes.

The very low rate of pregnancy loss from miscarriage is a function of: the natural history of miscarriage, which decreases considerably after the end of the first trimester; and enrolment in pregnancy clinical trials, which have systematically excluded women in the first trimester of pregnancy. The low rate of stillbirth (1.7%) among pregnancies in women treated with highly effective ACTs is notable, even considering that the women must have received improved acute care while the risk of pregnancy loss is high (as shown in Chapter 8), together with more frequent active clinical and parasitological follow-ups in the study settings. The data suggesting up to 1 in 5 stillbirths in sub-Saharan Africa can be attributed to *P. falciparum* malaria infection in pregnancy were derived predominantly from cross-sectional surveys, retrospective analysis or cohort studies, before widespread uptake of early detection and treatment with ACTs for pregnant women.¹¹ In this meta-analysis, although with or without significance, the risk of stillbirth was lower in women without previous history of pregnancy loss than primigravid women³⁸⁹ and higher in underweight ($\text{BMI} < 18.5 \text{ kg/m}^2$)³⁹⁰ or overweight ($\text{BMI} > 30 \text{ kg/m}^2$) women,^{389,391} which is consistent with previous findings.

The prevalence of SGA in this pooled analysis was higher than regional estimates.³⁹²

This could be explained by the fact that all women included had at least one malaria infection leading to a higher risk of SGA,³¹ but it also highlights that highly effective preventive measures are required to lower the risk of SGA. The prevalence of PTB in this pooled analysis was similar to the general background population,³⁹³ implying that highly effective drugs against uncomplicated falciparum malaria alongside improved acute care can lower the immediate risk of PTB to some extent.

Treated malaria infection in the early second trimester (before 20 weeks) as well as late third trimester (after 37 weeks) was associated with the highest risk of SGA, which was also shown previously.^{31,394,395} Development of placenta is considered to be affected by malaria in early pregnancy,^{395,396} and the fetal weight gain increased towards the delivery.¹⁶⁵ In contrast, the risk of PTB by malaria infection increases towards the delivery in the second and third trimester as was shown in an observational cohort study comparing women with or without malaria infection.³¹ Malaria infection increases the immediate risk of preterm delivery despite the effective treatment in the third trimester, thus close monitoring will be required. Although with a small number of observations, the current study implied that treated malaria infection in the first trimester was a risk of PTB, which possibly can be mediated by impairing development of placenta.⁹⁸

A recent systematic review reported that a previous history of stillbirth was associated with a higher risk of PTB and SGA.³⁹⁷ Higher parity has a known association with a lower risk of SGA, but the effect on PTB is less obvious.^{398,399} These findings are consistent

with ours. A previous cohort study in Canada, which included 184,000 births, reported that the risk of PTB was higher in <20 and >35 year olds than 20–34 year olds, with a nadir in 30–34 year olds.⁴⁰⁰ Similar trends of higher risks of PTB, but not SGA, in <18 and ≥ 35 year-old women were also reported in a meta-analysis of data from LMICs.³⁹⁹ In our analyses, the risks of PTB were lower in 20–35 year olds than <20 year olds, but similar in ≥ 35 year olds. Age was not associated with SGA. Shorter height and lower pre-pregnancy body weight (or BMI) are reported to be associated with LBW, SGA and PTB.^{347,401–404} Shorter height and lower BMI (at enrolment) were independently associated with SGA and PTB in our meta-analysis.

Placental malaria is reported to be associated with higher risks of LBW and PTB.^{27,382–384,405,406} PTB is more likely to be caused by active infection at delivery (i.e. acute and chronic infection of the placenta), whereas chronic infection is associated with a higher risk of LBW or IUGR.^{27,383,384}

The current IPD meta-analysis demonstrated that recurrence of falciparum malaria within 63 days (PCR-uncorrected treatment failure) is associated with a higher risk of placental malaria (deposition of malaria pigment), but the risk was not different among different treatments. Treatment failure (both recrudescence and reinfection) and the number of malaria episodes (patent parasitaemia) were associated with a higher risk of placental malaria as was separately suggested in univariable analysis in two previous studies,^{304,405} which are included in this meta-analysis. The risks of placental malaria were not different between different types of treatment failure by PCR, but this could have been due to the small number

of observations of recrudescence and recurrence without PCR, and also because data on malaria infection after the study period (~63 days) were not available although recrudescence after 63 days may not be negligible in pregnancy as was shown in Chapter 5.

The risk of placental malaria is considered to be affected by gravidity, age, frequency of parasitaemia and parasite density,^{27,407} which was confirmed in this meta-analysis (with the exception of frequency of parasitaemia, which could not be assessed). Additionally, higher body temperature before treatment and gametocytaemia were associated with a higher risk of placental malaria. These two characteristics can be surrogates of the severity of the infection. Inflammatory cytokines causing fever (e.g. TNF- α) are reported to be associated with a higher parasite burden in the placenta.^{27,408} The presence of gametocytes and lower haemoglobin can reflect a more extended period of infection before treatment,^{22,355} although haemoglobin in pregnancy can be affected by gestational age or antenatal care. A longer interval between the treated malaria episode and delivery was associated with a lower risk of placental malaria, which has also been shown in previous studies.^{313,405,409} These support an earlier finding from a low endemic area that malaria pigment in the placenta can be cleared over time after prompt and successful treatment.^{316,326,410}

The strength of this meta-analysis is the use of good quality data at the individual level, collected in prospective trials with the largest number of pregnant women ever to be amassed to assess the impact of treatment of falciparum malaria in pregnancy. The finding is supported by the robustness of the data as known anthropometric and baseline characteristics

were found to be associated with the risks of stillbirth, PTB and SGA, in this pooled data set. In this meta-analysis, 91.5 % (4120/4503) of the women were from eight studies that used ultrasound to estimate EGA allowing a better characterization of gestation for analysis of PTB and SGA. With IPD, standardized definitions for both exposures and outcomes were applied. Individual studies used different cut-offs or definitions as described in Chapter 3-III,²⁸⁸ thus aggregated data meta-analysis was difficult. SGA, rather than LBW or birthweight, is a more reliable measurement as the surrogate of fetal growth. Malaria episodes at different periods in pregnancy can affect pregnancy outcomes with varying magnitude,³¹ which was taken into account in this meta-analysis by splitting pregnancy period into four different risk periods.

The main limitation of this meta-analysis is the presence of some confounders in each included study. Although important confounders were commonly assessed (e.g. age, height, BMI, parity or gravidity, HIV status) and adjusted in our meta-analyses, it is possible that some potential confounders remained unadjusted. For example, the number of malaria episodes in the pregnancy was reported to be associated with an increased risk of SGA^{31,349} or placental malaria.⁴⁰⁵ However, most studies enrolled women in their second or third trimester and only followed up for 28–63 days; therefore, the history of malaria and antimalarial use including IPTp before and after the study period was not always available (or not systematically collected). Status of chronic diseases, smoking, marital status and literacy were reported to be associated with pregnancy outcomes,^{347,389,393,399} but not available in most studies.²⁸⁸ Obstetrically, a previous history of SGA or PTB is associated with subsequent

pregnancy outcomes³⁹⁷ but was not available, and previous history of pregnancy loss (stillbirth and miscarriage) was only roughly estimated by parity and gravidity in this meta-analysis. BMI was assessed using body weight at enrolment rather than pre-pregnancy weight, but a low BMI will be significant regardless of gestation of pregnancy. It was not possible to consider gestational weight gain, which is associated with fetal development and PTB.⁴¹¹ Our pooled data only include malaria treatment episodes, not comparators confirmed to be without malaria; thus, it was not possible to assess the risks of outcomes after treated malaria episodes compared with those without malaria. For comparability of outcomes to other studies, infants with congenital abnormality and twins were excluded from our analyses. This exclusion would underestimate the true burden of stillbirth, PTB and SGA, and may reduce the effect size of comparisons but not the direction of associations. The number of twins and congenital abnormalities was small and was not different among different antimalarial treatments; and less than one percent of the pregnant women included in this pooled analysis were enrolled in the first trimester, which significantly reduced the risk of congenital abnormality by external factors such as antimalarials. Congenital abnormality in stillborns was presumably underreported but recommended procedures such as autopsy were not mentioned, or maybe even possible, in any of the study sites.^{412,413} This situation should be improved in future treatment trials. Nonetheless, past meta-analyses on pregnancy outcomes also had to make a pragmatic decision on which variables could be adjusted,^{13,399} and variables that were commonly assessed were adjusted in this meta-analysis.

EGA at birth is one of the most significant factors impacting pregnancy outcomes.^{325,339} In this meta-analysis, one study did not have EGA at delivery but provided EGA at the time of the malaria episode in weeks rather than in weeks and days. This ambiguity of a maximum of seven days resulted in differences in the prevalence of SGA and PTB and changed some of the analysis results that had a small number of observations. Recent estimates of PTB in Asia and sub-Saharan Africa are 10.4% (uncertainty interval 8.7 to 11.9) and 12.0% (8.6 to 16.7), respectively,³⁹³ which are similar to the prevalence in this pooled analysis. A previous study reported that the prevalence of SGA using the INTERGROWTH-21st standard growth chart was estimated to be 21.6% (uncertainty range 14.2–37.7) in Southeast Asia, 34.2 % (22.2–51.3) in South Asia and 16.5% (8.7–25.1) in sub-Saharan Africa.³⁹² Considering all pregnant women included in our pooled analysis had at least one malaria episode, an elevated risk of SGA would be expected³¹ and the true prevalence of SGA would be somewhere between our best (32.3%) and worst case scenario (38.1%). These findings highlight the importance of accurate estimation of gestational age and the difficulty of pooling results based on different estimation methodologies with different accuracy.

Pregnancy outcomes analysed here were the secondary outcomes in our IPD meta-analysis on treatment efficacy. Thus, there are some studies without PCR-corrected efficacy (i.e. studies with only PCR-uncorrected efficacy) that could be included if the primary objective is to assess pregnancy outcomes after different treatments. However, most of these

excluded studies were conducted in the 1990s, and only three studies (totalling 271 women) used ultrasound to estimate gestational age.^{205,213,243} In one of them, the proportion of LBW (birthweight <2,500 g) was more frequently observed after DP than ASAQ (13.2% [12/91] vs. 4.2% [3/72], $p=0.05$), although the mean birthweight and the proportion of PTB (<37 weeks) were not different.²⁴³ In our meta-analysis, the risk of SGA was not different between DP and ASAQ, and inclusion of the three studies without PCR-corrected efficacy is unlikely to change our findings considering the relative size of this IPD meta-analysis.

Finally, the number of women who were enrolled in their first trimester was low. Although the safety of artemisinin-derivatives use in the first trimester has been reported to be assured based on observational cohort studies,^{32,33} not enough data are available to compare different ACTs. As the adverse impact of first trimester malaria infection on fetal development was implied in the current study and has also been reported in other studies,^{98,414} future ACT treatment studies should not exclude first trimester women.

6.5 Conclusions

This meta-analysis pooling data from some 4,500 women from both Asia and sub-Saharan Africa demonstrated highly reassuring pregnancy outcome results. The risks of late miscarriage and stillbirth were low; and late miscarriage, stillbirth, PTB, SGA and placental malaria were not different between the most commonly used ACTs (i.e. AL, ASAQ, ASMQ and DP) in the second and third trimesters. Accurate assessment of EGA, malaria episodes beyond the current study framework (28–63 days), thorough reporting of congenital

abnormality, including abnormality in stillbirth, is applicable to improvement in future studies. Early detection and treatment with highly effective ACTs will result in improved pregnancy outcomes but highly effective prevention is required to reduce the very high rate of SGA, which has significant future effects and economic burden for individuals and for countries where malaria is endemic.

Chapter 7. Safe cardiotoxic profile of four antimalarials for pregnant women with falciparum or vivax malaria on the Thailand-Myanmar border

7.1 Introduction

Quinoline antimalarials are known to have pharmacological activity on the cardiovascular system. For example, quinidine, the dextrorotatory diastereomer of quinine, is mainly used as a class 1a antiarrhythmic rather than as an antimalarial, although its antimalarial activity is higher than quinine.^{415,416} Another example is halofantrine. This drug is no longer used because of the marked drug-induced prolongation of QT interval in the therapeutic dose,⁴¹⁷ associated with sudden cardiac deaths.^{415,418,419} Other quinoline antimalarials that are currently used, namely chloroquine, amodiaquine, mefloquine, lumefantrine and piperazine are reported to have some effects on ion channels in myocardial cells.⁴¹⁵ Therefore, the potential cardiovascular effect, mainly measured as the prolongation of QT interval on ECG with or without being corrected by heart rate (QT/QTc prolongation) as a biomarker,⁴²⁰ has been of clinical and research interest.

Previous studies conducted in malaria patients and also in healthy adult volunteers found that the clinical safety of currently used antimalarials is reassuring, although some degree of drug-induced QT/QTc prolongation was reported.^{415,420,421} The magnitude of drug-induced QT/QTc prolongation is reported to be highest in chloroquine, followed by piperazine and amodiaquine, and lowest in lumefantrine and mefloquine.⁴²¹

Pregnant women are a susceptible subgroup of malaria patients who require special attention regarding cardiotoxicity because of the physiological and pharmacokinetic / pharmacodynamic differences due to pregnancy itself.^{158,249,352} Changes in sex hormones

during pregnancy can affect the QT interval.⁴²² Furthermore, the effect on QT prolongation is reported to be consistently higher in women than in men.^{415,423} Few studies using ECG have been conducted in pregnancy in the past. The effect on QT prolongation in pregnant women treated with chloroquine for malaria or autoimmune diseases is not studied so much,^{421,424,425} although chloroquine has been widely accepted to be safe in pregnancy. Previous ECG studies showed that the assessed drugs (AL, ASAQ, AAP, DP and mefloquine monotherapy) were not associated with clinically important cardiac abnormal findings in pregnant women when they are used as either treatment or prevention.^{192,208,219,247,250,258,267,270-272,275,426} However, details of the QT changes, especially when the drug was used as treatment for parasitaemia in pregnant women (rather than being used as prevention for otherwise healthy pregnant women), were scarce.

The difficulty in assessing the magnitude of drug effects on the QT/QTc interval is that malaria infection itself affects the QT/QTc interval.⁴¹⁵ Therefore, in patients with malaria parasitaemia, differences in the interval before and after drug administration may not be entirely attributable to the effect of drugs. Comparing different treatments will be a better way to assess the impact of different drugs.

This chapter describes the results of a study comparing the effect of four different antimalarials (AL, ASMQ, DP and chloroquine) on the QT/QTc interval when these drugs were used as treatment for uncomplicated malaria in pregnancy among women on the Thailand-Myanmar border.

7.2 Methods

Study site and eligibility criteria

This study was a part of an RCT in pregnancy conducted in 2010–2016 on the Thailand-Myanmar border (NCT01054248).²³⁶ Details of this RCT have not been published as of July 2019. Briefly, pregnant women were screened for malaria parasites by blood smear at the first antenatal consultation and then every two weeks. Inclusion criteria were: age 18–45 years, gestational age of >13 weeks by ultrasound with a viable fetus, microscopically confirmed uncomplicated malaria of any species with a parasitaemia of $\geq 5/500$ white blood cells, and no signs of labour. Patients with severe malaria, hyperparasitaemia ($\geq 4\%$), severe anaemia (haematocrit $< 20\%$) or known history of chronic diseases were excluded. Majority of the women were Karen ethnicity.

After giving informed consent in their own language, pregnant women were randomized to take either AL, ASMQ or DP. The treatment allocation was concealed using sealed opaque envelopes. A computer-generated randomization of 1:1:1 in blocks of 15 was used. This RCT was open label: only the reader of the ECG was blinded for the treatment allocation.

Drugs were given under full supervision for all doses. AL was given at a higher dose and for a longer period than the current standard: five tablets of Coartem[®] (20/120 mg artemether/lumefantrine) were given twice a day for four days regardless of body weight, with 100 mL of chocolate milk each time. ASMQ was given once daily for three days as either a

loose combination of artesunate (50 mg/tablet) plus mefloquine (250 mg/tablet), or a fixed-dose combination (artesunate/mefloquine 100/220 mg) depending on availability. For the loose combination, the dose was rounded to the nearest quarter of a tablet for both drugs based on body weight (4 mg/kg artesunate and 8 mg/kg mefloquine). For the fixed-dose combination, two tablets were given each day to all women (with body weight >29kg). DP was given based on body weight (2.4 mg/kg dihydroartemisinin and 20 mg/kg piperazine) once daily for three days. Standard fixed-dose tablets containing 40/320 mg of DHA/PPQ were used, and the dose was rounded up to the nearest half of a tablet. Chloroquine (10 mg/kg on day 0 and 1, and 5 mg/kg on day 2) was used for the recurrence of non-falciparum parasitaemia during follow-up. One tablet contained 250 mg chloroquine phosphate, and the dose was rounded up to the nearest quarter of a tablet.

After treatment, women were followed up weekly for clinical, obstetric and parasitological assessment until delivery or for 63 days, whichever was later.

ECG assessment

A standard 12-lead ECG (NihonKohden ECG-1250K, Tokyo, Japan) was measured at baseline before drug administration (day 0), at 4–6 hours following the last dose (defined as day peak), and on day 7 or after (day 7). ECG was assessed in a supine position after a short rest in the same position. ECGs were only assessed in around half the patients (259/511) enrolled in this study, mainly depending on the temporal availability of ECG machines at the

study sites.

One assessor, who was blinded for the treatment allocation, read the ECGs fully manually. Another assessor independently read the ECGs of the first 180 patients. Any discrepancies were checked by a second look. The end of the T-wave was defined as the point of intersection between the isoelectric line and the tangent line to the steepest downslope of the T-wave. If the U-wave was fused with the T-wave and its height was more than half of that of the T-wave, the U-wave was included for measurement of the QT.⁴²⁷ In total six complexes were measured using at least four different leads, and the median of these six complexes was taken.⁴²⁸ The minimum precision of reading was 0.5 mm, which corresponded to 10 ms at a speed of 50 mm/sec. Heart rate produced by the ECG machine was used to calculate the average RR interval.⁴²⁸ All concomitant medications used during the ECG assessment period were recorded. Drugs known to be associated with QT-prolongation⁴²⁹ were considered in the analysis.

Statistical analysis

Proportions of the study population with QT prolongation on day 0, day peak and day 7 were summarized by treatment, using both uncorrected QT (QTu) and QT corrected by the Fridericia method (QTcF).⁴³⁰ QT prolongation was defined either by the absolute value of QTu or QTcF (>480 ms or >500 ms), or by the difference in QTu or QTcF from day 0 (increase >30 ms or >60 ms).^{421,431}

To compare the QT intervals with different heart rates, a correction method specifically for this study population was derived using the data on day 0 (hereinafter referred to as QTcP).⁴³² Either QTcF or QTcP that was less related to heart rate was used for later comparisons. As a convention, ms was used as the unit for both QTcF and QTcP. As the cut-off for QTcP was not established, QTcP was not used for checking the QT/QTc prolongation defined above.

Linear mixed-effects models were used for the analyses. For interpretation, coefficients of linear regression were expressed as the mean difference. Repeated measures analyses were conducted for QTc at different time points. The model included an interaction term between treatment and time as a forced variable but without treatment, assuming that there was no difference in the baseline QTc.⁴³³ Other variables were included one by one in a univariable analysis. All variables with $p < 0.05$ in the univariable analysis were put together in the first multivariable model, and the variable with the largest p-value was excluded each time until the p-value reached < 0.05 (backward elimination).⁴³⁴ The Wald test was used to select covariates. Finally, all excluded variables were put in the multivariable model one by one to check whether there were any significant variables or confounding factors. Variables that changed the coefficients of treatment by more than 10% were kept in the multivariable model regardless of the p-value if clinically considered as a confounder.⁴³⁵ To avoid any cluster-level confounding, within-cluster effect and between-cluster effect were evaluated separately to see whether they were significantly different. If variables were highly correlated, only one

variable was included in the multivariable model, based on clinical importance. Parasitaemia included the total asexual parasite load of any species of malaria. Electrolytes were not tested, thus not included in the analysis. Symptoms that are known to be (or are potentially) associated with QT prolongation were assessed as covariates. Random intercepts were used for within-person correlation and within-episode correlation if any was statistically significant ($p < 0.05$ by likelihood ratio test).⁴³³ Normality of outcome and the residuals of the final model were assessed visually and by revised D'Agostino test.⁴³⁶

Two types of sensitivity analyses were conducted including all variables in the final multivariable model: a model excluding episodes with any concomitant drugs known to be associated with QT/QTc prolongation used; and a model allowing the difference in QTc on day 0.⁴³³

7.3 Results

Baseline characteristics

From 279 malaria episodes in 259 pregnant women, 754 ECG records were assessed: on day 0 ($n=273$), on day peak ($n=256$) and day 7 ($n=225$). The recording speed of ECG was 50 mm/sec in 98.7% (744/754) of the records. Some records were excluded from the analyses: ECG assessed after drug administration on day 0 ($n=2$); <6 leads assessed on day 0 ($n=2$), on day peak ($n=2$) or on day 7 ($n=3$); poor quality on day 0 ($n=1$). Appropriate ECGs on all three days were available in 205 episodes.

The number of episodes treated by AL, ASMQ, DP, and chloroquine was 86, 82, 88,

and 21 respectively. Two episodes of artesunate-clindamycin were assessed, but only on day 0; thus, drug effect was not analysed. Twenty patients had two episodes where ECGs were assessed: the median interval between two episodes was 67 (range 27–133) days.

The mean age was 25.6 (standard deviation [SD] 6.9, range 18–45) and the mean gravidity was 2.9 (SD 2.0, maximum 10). The mean EGA on day 0 was 26.0 weeks (SD 8.3, range 7.0–40.1). There were 79 *P. falciparum* mono-infections, 194 *P. vivax* mono-infections, 5 coinfections of *P. falciparum* and *P. vivax*, and 1 *P. malariae* mono-infection. Only 30.2% (84/278) were febrile (body temperature >37.5°C).

There were no apparent differences among the three randomized treatment groups (Table 7-1). Chloroquine was used exclusively for recurrences of *P. vivax* mono-infection, with fewer symptoms. There were no apparent differences between those who were assessed by ECG and those who were not enrolled in the RCT (Table 7-2).

Clinical outcomes

On day peak, 15.7% (40/254) of the patients whose ECG was assessed were still parasitaemic. No patients were febrile (among 197 women assessed). On day 7, all patients (n=226) were afebrile, and only one patient was still parasitaemic (1/500 white blood cells) in the DP group. ECG was not assessed for this patient on day 7.

There were neither maternal deaths nor cases with life-threatening arrhythmias (i.e. ventricular tachycardia, ventricular fibrillation or Torsade de Pointes).

Table 7-1. Baseline characteristics on day 0 of pregnant women who were assessed by ECG

Characteristic	All		AL		ASMQ		DP		CHQ	
	N	Mean (SD) / % (N)	N	Mean (SD) / % (N)	N	Mean (SD) / % (N)	N	Mean (SD) / % (N)	N	Mean (SD) / % (N)
Age (years)	259*	25.6 (6.9)	86	25.1 (7.1)	82	25.8 (6.6)	88	25.5 (6.9)	21	25.7 (7.6)
Gravidity	259*	2.9 (2.0)	86	2.9 (2.2)	82	3.0 (2.1)	88	2.7 (1.8)	21	3.1 (2.0)
Height (cm)	259*	151.0 (5.6)	86	151.0 (6.1)	82	151.4 (4.9)	88	150.9 (5.9)	21	150.8 (5.2)
Smoking	259*	20.8% (54)	86	23.3% (20)	82	18.3% (15)	88	19.3% (17)	21	28.6% (6)
Gestational age (weeks)	279	26.0 (8.3)	86	25.7 (8.6)	82	26.5 (8.9)	88	24.6 (7.8)	21	29.8 (5.3)
Weight (kg)	276	52.3 (8.0)	86	51.2 (7.8)	82	53.1 (7.6)	88	51.8 (8.3)	20	55.5 (8.0)
Fever (>37.5°C)	278	30.2% (84)	86	33.7% (29)	82	34.1% (28)	88	27.3% (24)	21	14.3% (3)
Heart rate (/min)	273	93.6 (16.2)	84	96.2 (17.2)	80	95.6 (16.0)	88	91.0 (15.7)	19	86.8 (12.6)
Haematocrit (%)	279	32.4 (3.7)	86	32.3 (3.3)	82	32.8 (4.3)	88	32.3 (3.8)	21	31.9 (2.7)
Malaria species	279		86		82		88		21	
falciparum monoinfection		28.3% (79)		30.2% (26)		35.4% (29)		25.0% (22)		0.0% (0)
vivax monoinfection		69.5% (194)		68.6% (59)		62.2% (51)		71.6% (63)		100.0% (21)
falciparum + vivax		1.8% (5)		1.2% (1)		2.4% (2)		2.3% (2)		0.0% (0)
malariae monoinfection		0.4% (1)		0.0% (0)		0.0% (0)		1.1% (1)		0.0% (0)
Asexual parasite load	279	10262.9 (21418.2)	86	10304.4 (19886.1)	82	13090.7 (25924.4)	88	9621.2 (20502.7)	21	2400.8 (6403.9)
Presence of gametocyte	279	48.4% (135)	86	47.7% (41)	82	40.2% (33)	88	52.3% (46)	21	66.7% (14)
Anorexia	279	38.0% (106)	86	38.4% (33)	82	40.2% (33)	88	37.5% (33)	21	23.8% (5)
Nausea	279	31.5% (88)	86	36.0% (31)	82	37.8% (31)	88	29.5% (26)	21	0.0% (0)
Vomiting	279	16.1% (45)	86	12.8% (11)	82	24.4% (20)	88	14.8% (13)	21	0.0% (0)
Dizziness	279	63.1% (176)	86	64.0% (55)	82	61.0% (50)	88	68.2% (60)	21	47.6% (10)
Diarrhoea	279	2.2% (6)	86	3.5% (3)	82	0.0% (0)	88	3.4% (3)	21	0.0% (0)
Palpitation	279	31.5% (88)	86	33.7% (29)	82	30.5% (25)	88	37.5% (33)	21	0.0% (0)
Fatigue	279	53.8% (150)	86	50.0% (43)	82	56.1% (46)	88	55.7% (49)	21	47.6% (10)

AL: artemether-lumefantrine, ASMQ: artesunate-mefloquine, CHQ: chloroquine, DP: dihydroartemisinin-piperaquine, SD: standard deviation. * Only the first episode was included.

Table 7-2. Baseline characteristics comparing those who were assessed by ECG and those who were not

Characteristic	ECG assessed (ACTs)		ECG not-assessed (ACTs)		ECG assessed (CHQ)		ECG not-assessed (CHQ)	
	N	Mean (SD) / % (N)	N	Mean (SD) / % (N)	N	Mean (SD) / % (N)	N	Mean (SD) / % (N)
Age (years)	256	25.5 (6.9)	255	26.2 (8.1)	18	25.1 (7.6)	31	25.5 (6.9)
Gravidity	256	2.9 (2.0)	255	3.1 (2.5)	18	3.1 (2.0)	31	2.7 (1.9)
Height (cm)	256	151.1 (5.6)	255	150.6 (8.2)	18	150.3 (5.4)	31	149.0 (19.0)
Smoking	256	20.3% (52)	255	19.2% (49)	18	22.2% (4)	31	16.1% (5)
Gestational age (weeks)	256	25.6 (8.4)	255	25.9 (7.9)	18	28.7 (6.1)	31	24.5 (10.4)
Weight (kg)	256	52.0 (7.9)	255	51.5 (7.3)	17	55.1 (7.4)	31	50.7 (8.7)
Fever (>37.5°C)	256	31.6% (81)	255	23.1% (59)	18	16.7% (3)	31	22.6% (7)
Pulse rate (/beats per min)	254	91.3 (12.3)	246	89.8 (10.8)	18	84.3 (9.2)	31	89.9 (15.2)
Haematocrit (%)	256	32.5 (3.8)	255	31.8 (4.2)	18	31.8 (2.6)	31	33.1 (2.9)
Malaria species	256		255		18		31	
falciparum monoinfection		30.1% (77)		25.5% (65)		0.0% (0)		0.0% (0)
vivax monoinfection		67.6% (173)		70.6% (180)		100.0% (18)		100.0% (31)
falciparum + vivax		2.0% (5)		3.9% (10)		0.0% (0)		0.0% (0)
malariae monoinfection		0.4% (1)		0.2% (0)		0.0% (0)		0.0% (0)
Asexual parasite load	256	10962.0 (22157.2)	255	11257.0 (25512.7)	18	2696.0 (6895.7)	31	6129.9 (17947.1)
Presence of gametocyte	256	46.9% (120)	255	53.7% (137)	18	72.2% (13)	31	74.2% (23)
Anorexia	256	38.7% (99)	254	49.2% (125)	18	22.2% (4)	30	13.3% (4)
Nausea	256	34.4% (88)	255	31.4% (80)	18	0.0% (0)	30	10.0% (3)
Vomiting	256	17.2% (44)	255	19.2% (49)	18	0.0% (0)	30	6.7% (2)
Dizziness	256	64.5% (165)	255	59.6% (152)	18	50.0% (9)	30	43.3% (13)
Diarrhoea	256	2.3% (6)	255	1.6% (4)	18	0.0% (0)	30	0.0% (0)
Palpitation	256	34.0% (87)	255	30.2% (77)	18	0.0% (0)	30	13.3% (4)
Fatigue	256	53.9% (138)	255	42.0% (107)	18	50.0% (9)	30	20.0% (6)
Treatment	256		255		-		-	
AL		33.6% (86)		32.9% (84)				
ASMQ		34.4% (88)		32.9% (84)				
DP		32.0% (82)		34.1% (87)				

ACTs: artemisinin-based combination therapies, AL: artemether-lumefantrine, ASMQ: artesunate-mefloquine, CHQ: chloroquine, DP: dihydroartemisinin-piperaquine, ECG: electrocardiogram, SD: standard deviation.

Table 7-3. QT interval (uncorrected and corrected by Fridericia method) for each treatment

Measurement	All		AL		ASMQ		DP		CHQ	
	Total	Mean (SD) Range	Total	Mean (SD) Range	Total	Mean (SD) Range	Total	Mean (SD) Range	Total	Mean (SD) Range
Day 0										
Heart rate (/min)	268	93.4 (16.2) 58–159	83	96.2 (17.3) 63–159	78	95.6 (16.2) 61–137	86	90.3 (15.2) 58–132	19	86.8 (12.6) 61–112
QT uncorrected	268	334.3 (27.4) 250.0–415.0	83	330.4 (30.5) 250.0–390.0	78	332.6 (26.4) 275.0–415.0	86	337.6 (26.0) 280.0–395.0	19	342.4 (21.6) 300.0–380.0
QTc Fridericia	268	384.8 (20.4) 322.7–436.3	83	383.8 (21.7) 322.7–428.3	78	385.9 (21.2) 335.2–431.9	86	384.5 (19.3) 338.2–436.3	19	385.6 (17.5) 348.5–423.4
Day peak										
Heart rate (/min)	254	78.6 (11.8) 48–120	82	78.7 (13.0) 48–111	77	79.9 (12.8) 57–120	79	77.7 (10.1) 59–107	16	76.8 (8.3) 59–90
QT uncorrected	254	367.1 (28.1) 285.0–445.0	82	361.5 (28.9) 290.0–435.0	77	359.8 (27.5) 285.0–420.0	79	377.8 (25.5) 300.0–445.0	16	377.8 (21.7) 335.0–410.0
QTc Fridericia	254	399.6 (21.1) 342.6–461.9	82	393.1 (18.9) 352.3–438.2	77	393.6 (20.8) 342.6–447.0	79	410.2 (19.9) 363.7–461.9	16	409.0 (15.2) 362.5–425.3
Day 7										
Heart rate (/min)	226	86.2 (12.9) 52–124	71	86.9 (13.3) 52–124	68	83.1 (12.4) 58–112	67	86.9 (13.0) 65–123	20	92.2 (9.8) 75–109
QT uncorrected	226	346.0 (26.7) 285.0–420.0	71	346.3 (28.9) 290.0–420.0	68	352.2 (26.9) 285.0–410.0	67	341.5 (26.4) 290.0–400.0	20	339.5 (12.0) 320.0–360.0
QTc Fridericia	226	388.3 (19.1) 335.7–429.3	71	389.3 (18.2) 349.6–429.3	68	390.5 (20.2) 335.7–428.4	67	384.3 (19.6) 336.9–423.7	20	391.0 (15.5) 350.8–416.4
Day peak – day 0										
QT >30 ms	245	64.5% (158)	79	62.0% (49)	73	53.4% (39)	77	76.6% (59)	16	68.8% (11)
QT >60 ms	245	19.6% (48)	79	12.7% (10)	73	21.9% (16)	77	24.7% (19)	16	18.8% (3)
QTc Fridericia >30 ms	245	23.3% (57)	79	10.1% (8)	73	16.4% (12)	77	40.3% (31)	16	37.5% (6)
QTc Fridericia >60 ms	245	1.6% (4)	79	0.0% (0)	73	0.0% (0)	77	5.2% (4)	16	0.0% (0)

AL: artemether-lumefantrine, ASMQ: artesunate-mefloquine, CHQ; chloroquine, DP: dihydroartemisinin-piperaquine, QTc Fridericia: QT interval corrected by Fridericia method, SD: standard deviation.

QTcF

The mean QTu and QTcF on day 0 were 334.3 ms (SD 27.4) and 384.8 ms (SD 20.4), respectively (Table 7-3). There was no apparent difference in QTcF on day 0 among the treatment groups.

No patients had QTu >480 ms or QTcF >480 ms at any time. The maximum QTu and QTcF were 445 and 461.9 ms, respectively. Both of these were observed on day peak of DP in two different patients, and concomitant medications were not used for them.

When compared with the baseline, a QTcF increase of >30 ms was observed on day peak in 10.1% (8/79) of AL, 16.4% (12/73) of ASMQ, 40.4% (31/77) of DP and 37.5% (6/16) of chloroquine (Table 7-3). A QTcF increase of >60 ms was observed only in DP (5.2%, 4/77) with a maximum of 68 ms.

Bradycardia (heart rate <60 /min) on day peak was observed in 13 patients: 9.8% (8/82) of AL, 2.6% (2/78) of ASMQ, 2.5% (2/79) of DP and 5.9% (1/17) of chloroquine. Of the symptoms that might be associated with the bradycardia, only two of the 13 patients complained of dizziness (one each in DP and ASMQ). None of them had vomited.

QTc by population correction

Using the day 0 data, the population correction formula obtained was $QTcP = QTu/RR^{0.381}$. QTcP was no longer associated with heart rate by linear regression analysis ($p=0.84$) while QTcF was associated with heart rate ($p=0.007$) on day 0 (Figure 7-1). This

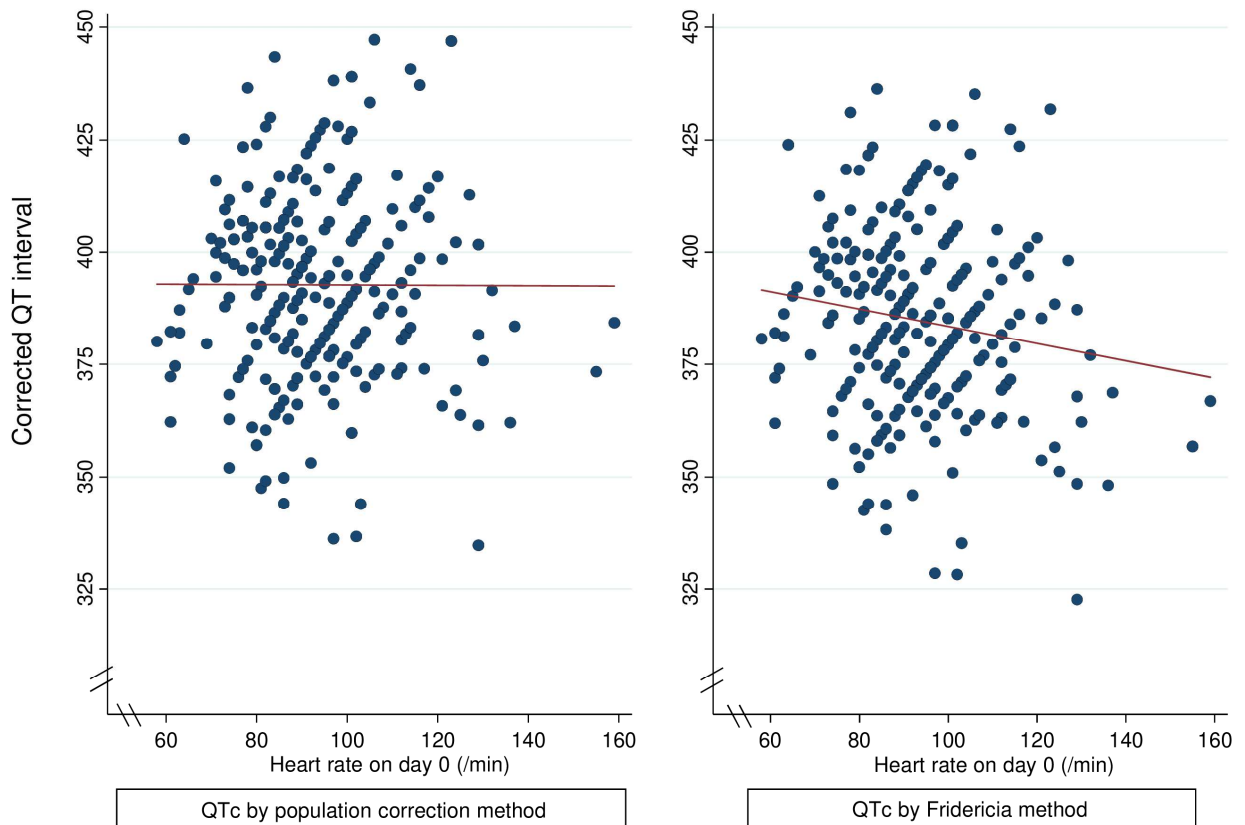


Figure 7-1. Comparison between corrected QT interval (QTc) by Fridericia correction method and population correction method

Linear relationship remains after the Fridericia correction, but not after population correction ($QT/RR^{0.381}$).

relationship of no association between QTcP and heart rate was also the same for day peak and day 7, thus QTcP was used for further analyses.

Factors associated with QTcP

In the univariable analysis, QTcP on day peak was longer in DP (mean difference 17.49 ms, 95% CI 12.37 to 22.62, $p < 0.001$) and chloroquine (mean difference 13.62 ms, 95% CI 5.41 to 21.82, $p = 0.001$) than AL, but no different in ASMQ (mean difference -0.37 ms, 95% CI -5.54 to 4.81, $p = 0.89$). On day 7, there was no difference among the treatment groups ($p = 0.40$).

Table 7-4. Factors associated with QT corrected by population correction (QTcP) by multi-level univariable and multivariable linear regression

Variable	Univariable			Multivariable		
	N	Coefficient (95% CI)	p-value	N	Coefficient (95% CI)	p-value
Treatment on different time (interaction)						
Day Peak						
AL	70	Reference	-	70	Reference	-
ASMQ	74	-0.37 (-5.54–4.81)	0.89	76	2.45 (-4.20–9.10)	0.47
DP	79	17.49 (12.37–22.62)	<0.001	79	17.84 (11.58–24.10)	<0.001
CHQ	15	13.62 (5.41–21.82)	0.001	14	18.31 (8.78–27.84)	<0.001
Day 7						
AL	64	Reference	-	64	Reference	-
ASMQ	66	-0.05 (-5.54–5.44)	0.99	67	-0.05 (-5.59–5.48)	0.99
DP	62	-4.06 (-9.53–1.42)	0.15	62	-5.11 (-10.70–0.48)	0.07
CHQ	20	-0.13 (-7.72–7.45)	0.97	20	1.07 (-6.64–8.77)	0.79
Day (for AL)						
0	83	Reference	-	83	Reference	-
Peak	64	6.28 (2.49–10.06)	0.001	64	6.78 (1.36–12.19)	0.01
7	70	3.57 (-0.44–7.57)	0.081	70	5.64 (0.76–10.52)	0.02
Other variables						
Age (year)	748	0.53 (0.25–0.81)	<0.001	720	0.53 (0.24–0.81)	<0.001
Gravidity	748	1.76 (0.80–2.73)	<0.001			
Height (cm)	748	0.06 (-0.29–0.42)	0.72			
Smoking	154/748	0.78 (-4.21–5.77)	0.76			
EGA (weeks)	748	-0.43 (-0.65–0.21)	<0.001	720	-0.40 (-0.62–0.18)	<0.001
Weight (kg)	745	0.004 (-0.25–0.26)	0.97			
Body temperature (°C)	679	-1.47 (-4.07–1.13)	0.27			
Heart rate (/min)	748	0.07 (-0.03–0.16)	0.18			
Haematocrit (%)	584	-0.33 (-0.78–0.12)	0.15			
Malaria species						
negative	438	Reference	-			
falciparum monoinfection	110	0.09 (-5.58–5.77)	0.97			
vivax monoinfection	192	-1.35 (-7.35–4.66)	0.66			
falciparum + vivax	5	-3.71 (-18.42–11.00)	0.62			
malariae monoinfection	1	0.47 (-30.77–31.70)	0.98			
Asexual parasitaemia (1000/uL)	746	0.12 (0.03–0.21)	0.007	720	0.11 (0.02–0.19)	0.02
Gametocytaemia	135/747			135/720		
Between effect		16.63 (6.17–27.10)	0.002		13.53 (3.17–23.90)	0.01
Within effect		-0.79 (-4.77–3.19)	0.70		-1.08 (-5.06–2.91)	0.60
Anorexia	135/721					
Between effect		8.80 (1.69–15.92)	0.02			
Within effect		-2.10 (-6.27–2.08)	0.33			
Vomit	55/723	5.23 (0.41–10.06)	0.03			
Diarrhoea	5/722	27.49 (12.25–42.73)	<0.001	5/720	23.16 (8.18–38.14)	0.002
Time ECG assessed						
0:00–5:59	39	2.31 (-4.28–8.91)	0.49	36	3.13 (-3.68–9.93)	0.37
6:00–11:59	313	-1.69 (-4.78–1.40)	0.29	296	-0.32 (-3.47–2.83)	0.84
12:00–17:59	309	Reference	-	302	Reference	-
18:00–23:59	87	-2.07 (-6.74–2.60)	0.39	86	-2.85 (-7.49–1.79)	0.23

AL: artemether-lumefantrine, ASMQ: artesunate-mefloquine, CHQ: chloroquine, CI: confidence interval, DP: dihydroartemisinin-piperaquine, ECG: electrocardiogram. EGA: estimated gestational age. p-value by Wald test. Random-slope models were used for accounting for within-person correlation. Day and interaction between day and treatment were included in the models as forced variables. Multivariable model includes all the variables listed in the column.

Higher age, higher gravidity, higher parasitaemia load, vomiting and diarrhoea were significantly associated with a longer QTcP (Table 7-4). Higher EGA was associated with a lower QTcP. Those who had gametocytaemia were associated with a longer QTcP than those who did not, although the appearance (or the disappearance) of gametocytaemia in the same person on different days did not affect QTcP. Similarly, those who had anorexia were associated with a longer QTcP than those who did not, although the development of (or the recovery from) anorexia in the same person did not affect QTcP.

In the multivariable analysis, QTcP on day peak was longer in DP (adjusted mean difference 17.84 ms, 95% CI 11.58 to 24.10, $p < 0.001$) and chloroquine (adjusted mean difference 18.31 ms, 95% CI 8.78 to 27.84, $p < 0.001$) than AL, but not different in ASMQ (adjusted mean difference 2.45 ms, 95% CI -4.20 to 9.10, $p = 0.47$). There was no difference between DP and chloroquine ($p = 0.91$). On day peak, QTcP was longer than day 0 in all treatment groups: the adjusted mean difference from day 0 was 6.78 ms (95% CI 1.36 to 12.19) in AL, 9.23 ms (95% CI 3.86 to 14.59) in ASMQ, 24.62 ms (95% CI 20.13 to 29.10) in DP and 25.09 ms (95% CI 16.66 to 33.52) in chloroquine.

On day 7, there was no difference among the treatment groups ($p = 0.17$). QTcP on day 7 was longer than day 0 in AL and ASMQ, but there was no difference from day 0 in DP and chloroquine (Figure 7-2): the adjusted mean difference from day 0 was 5.64 ms (95% CI 0.76 to 10.52) in AL, 5.59 ms (95% CI 0.90 to 10.27) in ASMQ, 0.53 ms (95% CI -4.47 to 5.53) in DP and 6.71 ms (95% CI -0.64 to 14.05) in chloroquine.

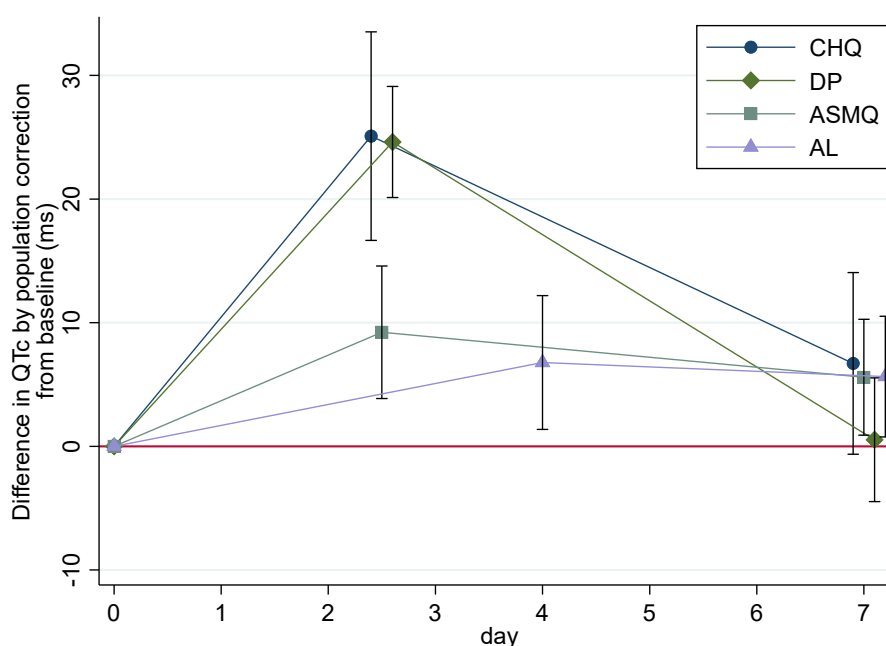


Figure 7-2. Difference in adjusted QTc by population correction method from baseline over time by treatment

Five other variables were associated with QTcP: age (adjusted mean difference 0.53 ms/year, 95% CI 0.24 to 0.81, $p < 0.001$), EGA (adjusted mean difference -0.40 ms/week, 95% CI -0.62 to -0.18, $p < 0.001$), asexual parasitaemia load (adjusted mean difference 0.11 ms/1000/ μ L, 95% CI 0.02 to 0.19, $p = 0.02$), presence of gametocytes (adjusted mean difference between those who had gametocytaemia and those who did not 13.53 ms, 95% CI 3.17 to 23.90, $p = 0.01$) and diarrhoea (adjusted mean difference 23.16 ms, 95% CI 8.18 to 38.14, $p = 0.002$).

As the time of day when ECG was assessed was a confounder for treatment effect, it was included in the final model. Although not statistically significant, QTcP was longer when the ECG was assessed between midnight and 6.00 AM. Anorexia and vomiting became not significant after adjusting for other variables, and gravidity was omitted because of the

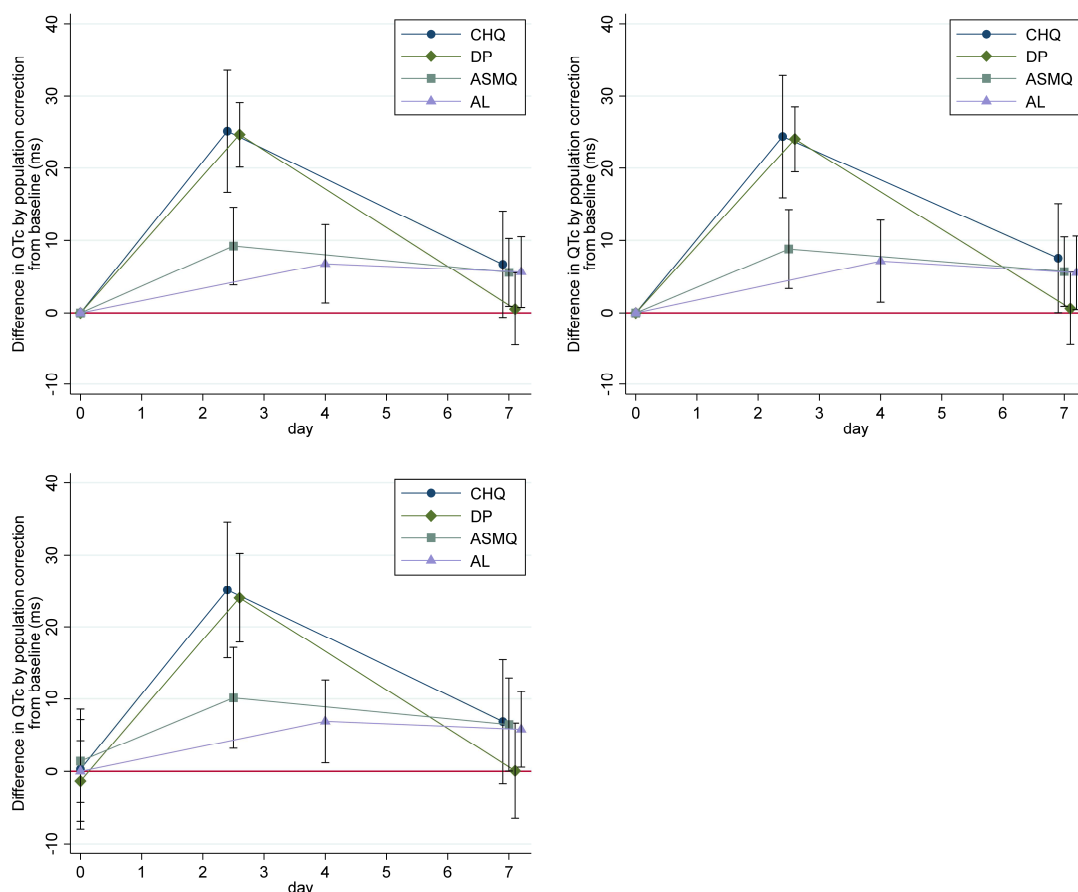


Figure 7-3. Adjusted QTc by population correction method over time by treatment in the final model and two sensitivity analysis models

The main model adjusting for the baseline (top left, the same as Figure 7-2), a model excluding those who used concomitant medication (top right), and a model allowing the baseline difference (bottom)

correlation with age. Random intercepts for episode-level clustering were not significant ($p=0.32$). Thus, only random intercepts for patient-level clustering were used. Normality of QTcP, level-1 residuals and level-2 residuals were adequate (Appendix Figure 7-1 and 7-2).

Sensitivity analyses

Among the concomitant medications used in the study period, azithromycin ($n=7$) and ciprofloxacin ($n=8$) were the only two drugs identified that could potentially affect the QT interval. In a sensitivity analysis excluding patients treated with these two medications and

also those who received blood transfusions, the conclusions were unchanged (Figure 7-3).

As the chloroquine group was not randomized and used for recurrent infections, QTcP on day 0 could be different from other drugs. To avoid over-adjustment by assuming the same QTcP on day 0, a sensitivity analysis was conducted using a model that allowed a difference in the baseline QTcP on day 0. In this sensitivity analysis, the point estimates were very similar to the primary model (Figure 7-3). There was no significant difference in the QTcP on day 0 among the treatment groups with or without adjusting for other covariates.

7.4 Discussion

Quinoline antimalarials are known to have cardiotoxic effects potentially leading to drug-induced QT/QTc prolongation. Piperaquine, a quinoline, is currently used in the form of DP, which is a relatively new ACT with the most promising efficacy and the longest post-treatment prophylaxis. Therefore, the cardiotoxicity of DP is of great clinical interest: one potentially drug-related sudden death after DP has been reported in the literature.⁴³⁷ However, the safety of these drugs during pregnancy has not been thoroughly assessed. Eight cohorts have assessed ECG after antimalarial use in pregnancy: mefloquine monotherapy (total 80 pregnant women in two cohorts),^{250,258} quinine monotherapy (total 152 pregnant women in one cohort),¹⁹² AL (total 203 pregnant women in two cohorts),^{192,208,219} DP (total 492 pregnant women in five cohorts),^{267,270-272,426,438,439} ASAQ (total 83 pregnant women in one cohort)²⁴⁷ and AAP (total 22 pregnant women in one cohort).²⁷⁵ One ongoing study is specifically assessing the cardiotoxicity of DP in pregnancy as IPTp (i.e. enrolling participants regardless

of the presence of parasitaemia).³⁰⁵ No clinically relevant abnormalities were reported in any of these studies, although details of QT prolongation in pregnant patients with parasitaemia were scarce.

The current study shows that DP and chloroquine were similarly associated with longer QT/QTc on day peak than AL or ASMQ when they were used as treatment for pregnant patients. The adjusted mean increases on day peak compared with AL were 17.84 (95% CI 11.58 to 24.10) ms for DP and 18.31 (95% CI 8.78 to 27.84) ms for chloroquine.

The findings reported here remain consistent with previously reported results of antimalarials in pregnant women. In a study of IPTp with DP, both QTcF and QTc corrected by Bazett methods (QTcB) at 3–4 hours following the last dose, increased by mean 17 ms from the baseline.²⁷⁰ Another study assessed the effect of DP on ECG in 16 pregnant women (including two women with asymptomatic parasitaemia): QTcB was longer at 4 hours following the administration of DP than that at the baseline with median 13.5 ms (interquartile range 5–26 ms).²⁶⁷ These reported differences in QT interval after DP were smaller than the observed difference in QT interval on day peak from day 0 in the current study, but similar to the difference between DP and AL on day peak. Considering that the majority of women in these two studies were aparasitaemic, this could be due to the effect of malaria itself, which is discussed below.

In non-pregnant populations, DP and chloroquine are reported to have similar effects on QT/QTc: in Thai male and female healthy adult volunteers the peak QTcF prolongation in

the first 24 hours was 7.08 ms (2.16 to 22.1) in DP and 6.32 (-1.45 to 12.3) in chloroquine.^{440,441} In AL, a small increase in QTcF was also reported in a previous study in healthy adult volunteers (7.45 ms at 68-hour after standard three-day regimen),⁴²¹ which is similar to our findings, although the total dose of lumefantrine was 66% higher in our study. The effect of mefloquine is also reported to be similar to that of AL.^{421,442}

For all drugs, the QT/QTc on day 7 was slightly longer than day 0 (without statistical significance for chloroquine and DP). This finding can be explained by shortened QT/QTc on day 0 resulting from increased sympathetic tone due to malaria infection.⁴¹⁵ This effect of the autonomic nerve system on QT/QTc is reported to be independent of heart rate.⁴⁴³

Some variables were shown to be associated with QT/QTc in this study. This is consistent with previous studies that show that higher age and diarrhoea causing hypokalaemia are associated with a longer QT/QTc,^{444,445} although the biochemistry was not assessed in our study. Higher gestational age was associated with a shorter QT/QTc as previously reported.⁴³⁸ This finding may be due to the level of progesterone (or the ratio of progesterone to estradiol), which increases towards the end of pregnancy.^{422,446} Although the QT/QTc interval in pregnancy can be influenced by other factors altogether,⁴⁴⁷ an overall lower risk of cardiac events during pregnancy is reported among women with hereditary long-QT syndromes.⁴⁴⁸ A higher parasitaemia load and the presence of gametocytes were found to be associated with a longer QTcP. Although these two parasitological factors can be explained as a surrogate of the severity or the duration of infection, further ECG studies are needed in

patients infected with malaria. Higher parasitaemia was reported to be associated with a longer QTcB in children.⁴⁴⁹

The time of day when ECG was assessed was found to be a confounding factor. In this study, QTcP was longer when ECG was assessed between midnight and early morning (0–6AM) although it was not statistically significant. This finding on the diurnal change in QT was consistent with previous studies.⁴⁵⁰⁻⁴⁵² It was important to take account of this diurnal variation in QT interval in this study because more patients in AL were assessed after midnight on day peak because AL was the only drug administered twice a day.

QTcF was associated with heart rate in our study population. QTcP was developed specifically for this pregnant Karen population. The QTcP derived from this study population ($QT/RR^{0.381}$) was similar to the model ($QT/RR^{0.4}$) that was derived from non-pregnant populations in the same region in the past.⁴⁵³ The problem with the population correction method is, however, that there is no established threshold for increased risk. Therefore, QTcF and uncorrected QT were used for comparison with the established thresholds. Nonetheless, in order to compare drug effects, QTcP is believed to be a better measurement for comparing different records with different heart rate.

There are some limitations to this study. Firstly, randomization was only done for AL, ASMQ and DP. Chloroquine was used for *P. vivax* recurrence, so some baseline characteristics, such as parasitaemia load and symptoms, were not comparable with the other three drugs where allocation was randomized. The sensitivity analysis allowing difference on

day 0, however, reached the same conclusion. Importantly, there was no difference in baseline QT/QTc interval among these four drugs. Therefore, our conclusion on the drug effect, which was measured as the difference in QTcP from baseline, will not change.

Secondly, the tangent method of defining QT/QTc interval, which was used in this study, is reported in shorter intervals than the differential threshold method by around 25 ms.⁴⁵⁴ There is, however, no consensus on which method should be used.⁴²⁰ In our study, the maximum QTcF recorded was 461 ms. Thus, even considering possible systematic shortening by applying the tangent method, it is unlikely that any patients in this study could have exceeded the known threshold at 500ms. The main outcome, the difference in QT/QTc (i.e. internal comparisons), is expected to be less affected by the definition of the end of the T-wave.

7.5 Conclusions

Quinoline antimalarials are known to be associated with QT/QTc prolongation; however, evidence of their safety profile for treating malaria in pregnant women has not been reported previously. Our results indicate that the impact of DP on QT prolongation was similar to that of chloroquine. Although both DP and chloroquine were associated with a similarly longer QTc interval on day peak than AL or ASMQ, the recorded longest QTc interval did not exceed the known threshold for increased risk for fatal arrhythmia. The drug effect on QT/QTc was not significant on day 7. Our study, thus, demonstrated that currently used antimalarials (namely AL, ASMQ, DP and chloroquine) are used safely in pregnancy.

Chapter 8. Risk factors for adverse maternal and fetal outcomes following severe malaria in Southeast Asia

8.1 Introduction

The predominant mortality due to malaria worldwide is from severe falciparum malaria (severe malaria),^{9,455} and mortality is less frequently observed in *P. falciparum* when it is uncomplicated, or uncomplicated and hyperparasitaemic.³⁷² Other malaria species contribute to mortality, but none surpass the nearly 100% mortality of untreated *P. falciparum* cerebral malaria.¹⁴⁰ If treated promptly with intravenous artesunate, mortality from severe malaria can be reduced to 10–20% in the general population.^{456–458} Since 2006, intravenous artesunate has been recommended by WHO as the first-line treatment for all patients with severe malaria, regardless of species and including pregnant women in all trimesters.¹⁴⁰ The priority in treating severe malaria in pregnancy is to save the life of the mother but increased risks of adverse fetal and neonatal outcomes are also reported.⁴⁵⁹

In pregnancy, the risk of developing severe malaria is estimated to be 2–3 times higher than in non-pregnant populations,^{460,461} along with higher mortality^{462,463} estimated to be as high as 50%.^{12,140} However, the Southeast Asian Quinine Artesunate Malarial trial (SEAQUAMAT), a cornerstone multicentre RCT on the treatment of severe malaria, reported that mortality in pregnancy, 10% (5/49), was lower than in non-pregnant adults, 19% (65/337).⁴⁵⁶ An epidemiological study conducted in the early 1990s on the Thailand-Myanmar border reported that while severe malaria was 3.0 (95% CI 1.4 to 6.2) times more common in pregnant than non-pregnant women, the mortality was similar (3.3% versus 6.1%, respectively).⁴⁶⁰ In India, there was no difference in the mortality between pregnant and non-

Box 8-1. WHO definition of severe malaria

Reprinted from Guidelines for the treatment of malaria. Third edition, World Health Organization, Page 73-4, Copyright (2015).¹⁴⁰

- Impaired consciousness: A Glasgow coma score <11 in adults or a Blantyre coma score <3 in children
- Prostration: Generalized weakness so that the person is unable to sit, stand or walk without assistance
- Multiple convulsions: More than two episodes within 24 h
- Acidosis: A base deficit of >8 mEq/L or, if not available, a plasma bicarbonate level of <15 mmol/L or venous plasma lactate ≥5 mmol/L. Severe acidosis manifests clinically as respiratory distress (rapid, deep, laboured breathing).
- Hypoglycaemia: Blood or plasma glucose <2.2 mmol/L (<40 mg/dL)
- Severe malarial anaemia: Haemoglobin concentration ≤5 g/dL or a haematocrit of ≤15% in children <12 years of age (<7 g/dL and <20%, respectively, in adults) with a parasite count >10,000/μL
- Renal impairment: Plasma or serum creatinine >265 μmol/L (3 mg/dL) or blood urea >20 mmol/L
- Jaundice: Plasma or serum bilirubin >50 μmol/L (3 mg/dL) with a parasite count >100 000/ μL
- Pulmonary oedema: Radiologically confirmed or oxygen saturation <92% on room air with a respiratory rate >30/min, often with chest indrawing and crepitations on auscultation
- Significant bleeding: Including recurrent or prolonged bleeding from the nose, gums or venepuncture sites; haematemesis or melaena
- Shock: Compensated shock is defined as capillary refill ≥3 s or temperature gradient on leg (mid to proximal limb), but no hypotension. Decompensated shock is defined as systolic blood pressure <70 mmHg in children or <90 mmHg in adults, with evidence of impaired perfusion (cool peripheries or prolonged capillary refill).
- Hyperparasitaemia: *P. falciparum* parasitaemia >10%

pregnant groups (39% versus 33%, respectively).⁴⁶⁴

WHO defines severe malaria by six clinical and six laboratory findings (Box 8-1).¹⁴⁰

This clinical working definition of severe malaria is a generalized, pragmatic tool that can be applied in resource-limited settings and is very much intended to prevent delay in treatment, particularly in children, which can be fatal.

However, these generalized severe malaria criteria may unintentionally identify

different sub-groups of risk when applied across all populations as signs and symptoms of severe malaria vary with age⁴⁵⁵ and possibly pregnancy status. Application of these criteria to patient groups with different backgrounds could explain the differences in reported 'mortality from severe malaria'.

Among the severe criteria, severe anaemia is on the list principally because it is one of the most common presentations of the disease in young children,^{9,25} who bear the brunt of severe malaria mortality.^{19,455} However, anaemia in the tropics can have many other causes²⁵ and anaemia is prevalent in pregnant women even those not in the tropics, and in those without malaria.⁹⁹ Pregnant women who are already anaemic may easily shift towards a severe anaemia category.²⁵³ Severe anaemia is reported as being among the most common presenting signs/symptoms of severe malaria in pregnant women: in Indonesia 76% of pregnant women with severe malaria had severe anaemia;²⁰⁴ on the Thailand-Myanmar border severe anaemia was significantly more common in pregnant women (70%) than non-pregnant women of child-bearing age (28%) as a severe malaria criterion;⁴⁶⁰ and in a similar comparison in severe malaria patients in India, 20% versus 4%.⁴⁶³ Severe malaria cases with severe anaemia alone are reported to be associated with lower mortality in general in Africa and Asia.^{140,460,465-467} To complicate the picture further, the physiology of pregnancy calls for separate definitions of anaemia across trimesters.

Like severe anaemia, hyperparasitaemia is more common in pregnant patients.⁹ Hyperparasitaemia without any other severe signs (uncomplicated hyperparasitaemia) is

currently categorized as severe malaria according to the WHO definition. Although higher parasitaemia is generally associated with severe clinical manifestations, it can vary:^{9,25} previous studies show that uncomplicated hyperparasitaemia can be successfully treated with oral ACTs without mortality,^{372,468} although more extended administration of artemisinin derivatives is required.^{371,372} The proportion of uncomplicated hyperparasitaemia patients will also affect the overall mortality of severe malaria, particularly when it is an isolated (only one fulfilled) criterion. The point at which hyperparasitaemia becomes problematic is debatable and differs significantly depending on background immunity, with lower cut-off points for lower transmission settings (10% in high transmission and 4% in low transmission).⁴⁵⁵ Whether the cut-off point for the definition of hyperparasitaemia in pregnancy *per se* requires lowering has not been studied.

Risk factors associated with *P. falciparum* maternal mortality have been afforded little attention as the data remain difficult to accumulate, with the last published data being the SEAQUAMAT study.⁴⁵⁹ Several severity scores and risk factors have been proposed for adults,^{458,462,469} but these were not specific for pregnancy. For this reason, this report retrospectively reviewed case notes of WHO severe falciparum malaria in pregnancy collected since 1989 at SMRU on the Thailand-Myanmar border.

This report aims to describe maternal and fetal outcomes following severe falciparum malaria in pregnancy and to identify the risk factors that predict higher maternal mortality.

8.2 Methods

Study populations

SMRU has been providing primary health care and antenatal care on the Thailand-Myanmar border since 1986. Pregnant women are followed up every 1–2 weeks at ANCs. At every visit, a blood smear is screened for malaria parasites. Women with parasitaemia are treated with effective antimalarial drugs regardless of symptomatology. As malaria has decreased in the area, malaria screening intensity has likewise decreased and become more focused on screening in the risk period (the first three antenatal visits), in the event of fever, when there is a history of malaria in pregnancy or in women diagnosed with malaria in pregnancy. This shift took place in 2010 among refugees and in 2017 in migrant clinics. Haematocrit was examined in all pregnant women with parasitaemia. The prevalence of HIV and syphilis were both <0.5% in this population in 2012–2013.⁴⁷⁰

Data extraction

An audit of routine clinical records from the period 1989 to 2017 was conducted. Baseline clinical information on malaria and pregnancy were recorded prospectively and extracted retrospectively. Only pregnant women with severe falciparum malaria, according to the WHO definition,¹⁴⁰ were included.

Table 8-1. Definition of the malaria diagnostic groups at SMRU

Diagnostic group	Definition
1. Uncomplicated malaria	- Presence of peripheral parasitaemia <4% - No signs of clinical severity (able to sit and drink without assistance*) - Patients with severe anaemia but do not fulfil the criteria below are included here
2. Uncomplicated hyperparasitaemia	- Presence of peripheral parasitaemia $\geq 4\%$ - No signs of clinical severity (able to sit and drink without assistance*)
3. Severe malaria	- Presence of peripheral parasitaemia (at any density) - Requires assistance to sit and drink* - Signs of severity except anaemia and hyperparasitaemia

*rapid assessment for cerebral involvement and prostration

Management of the patients

At SMRU, a combination of clinical assessment, malaria smear and haematocrit, was used to diagnose uncomplicated malaria, uncomplicated hyperparasitaemia or severe malaria (Table 8-1). Treatment varied for these three diagnostic groups and in pregnancy treatment has been routinely updated reflecting the most recent evidence (Table 8-2). In this setting, parasitaemic pregnant women with only severe anaemia (defined here as the uncomplicated severe anaemia group) or hyperparasitaemia (defined here as the uncomplicated hyperparasitaemia group) without dysfunction of any other vital organs were treated differently from severe patients with ≥ 1 of the other severe malaria criteria (defined here as the SMRU severe group).

Blood transfusion was considered if the haematocrit was less than 20% (haemoglobin ≤ 6 g/dL) in symptomatic women or for hypovolemic shock. Patients who developed acute kidney injury (anuria), respiratory failure or non-responding shock were transferred to a general hospital in Thailand for possible renal replacement therapy or cardiopulmonary

Table 8-2. Treatment for severe malaria, uncomplicated hyperparasitaemia and severe anaemia at SMRU over time

Start year	Treatment	
First-line initial treatment for severe malaria in pregnancy†		
1989	Q IV: 20mg/kg (H0) over 4 hours followed by 10mg/kg over 2 hours every 8 hours	
1996	Q IV: 20mg/kg (H0) over 4 hours followed by 10mg/kg over 2 hours every 8 hours Or AM IM: 3.2 mg/kg (H0) followed by 1.6 mg/kg every 24 hours (or 8-12 hourly in 1996–7)	
2000	AS IV + (from 2006) clindamycin p.o. H0: 2.4 mg, H12 and H24 1.2mg/kg and then 1.2 mg/kg every 24 hours Or AM IM or Q IV if AS IV is unavailable	
2013	AS IV + clindamycin p.o. 2.4mg/kg at H0, H12, H24 and then every 24 hours Suspected AS resistance or severe malaria with hyperparasitaemia (≥4% RBC) Q IV + AS IV	
First-line for uncomplicated hyperparasitaemia (≥4% RBC)		
1986	Q IV: 20mg/kg (H0) over 4 hours followed by 10mg/kg over 2 hours every 8 hours	
1994	AS p.o. 4mg/kg (D0), 2mg/kg (D1–2) and 1mg/kg (D3–6) (total dose 12 mg/kg)†	
1997	AS p.o. 4mg/kg (D0), 2mg/kg (D1–4) and 1mg/kg (D5–6) (total dose 16mg/kg) †	
1999	AS p.o. 4mg/kg (D0), 2mg/kg (D1–6) (total dose 16mg/kg) †	
2012	AS p.o. 4mg/kg (D0), 2mg/kg (D1–6) with DP p.o. 3 days (D4–6)*	
2013	AS p.o. 4 mg/kg (D0–2) followed by DP p.o. 5 days (D2–6)*	
2016	Any ACT extended regimen (for 7 days)*	
First-line for uncomplicated malaria#		
1989	All trimesters	Q7 or or MFQ p.o. (25mg/kg single dose)
1994	1 st trimester 2 nd & 3 rd trimester	Q7 1 st episode: Q7 or MFQ p.o. (25mg/kg single dose) ≥2 nd episode: AS p.o. (2mg/kg D0–D4, 1mg/kg D5–6)
1996	1 st trimester 2 nd & 3 rd trimester	Q7 AS p.o. (2mg/kg D0–D4, 1mg/kg D5–6) or MAS
2006	1 st trimester 2 nd & 3 rd trimester	Q p.o. + clindamycin for 7 days AS p.o. + clindamycin for 7 days
2017	All trimesters	Available ACT

AS: artesunate. AC: artesunate+clindamycin. D: day. DP: dihydroartemisinin+piperaquine. H: hour. IM: intramuscular. IV: intravenous. MAS: mefloquine+artesunate. MFQ: mefloquine. p.o.: per os. Q: quinine. RBC: red blood cells.

Clindamycin was given orally 5mg/kg three times a day for 7 days.

MAS: mefloquine 25 mg/kg in total + artesunate 4mg/kg for three days

Q7: Q p.o.: 10mg/kg three times a day for 7 days.

A rescue dose (1.2 mg/kg) was given if parasitaemia increased above the 95% of the usual parasite clearance.

‡ Treatment was switched to oral drug once tolerated. Total 12–16 mg/kg (-2012) or 16–18 mg/kg (2013-) of AS or AM was given over 7 days.

† A partner drug (e.g. MFQ, or atovaquone-proguanil if available) was used whenever possible.

*First dose was given by IV or IM (2.4mg/kg) to those with asexual parasitaemia of $>20\%$ RBC.

#From 1995 to 2017, 5 RCTs on treatment of uncomplicated malaria in pregnancy were conducted with drugs including: AS+MFQ, Q+clindamycin, Artemether-lumefantrine, AS+atovaquone-proguanil, DP, AS, AC

support, but transfer was rare.

Parasite count, haematocrit, blood glucose and urine dipstick were assessed on site. Parasite count was assessed every 4–6 hours until negative twice except for cases with only severe anaemia, for whom it was assessed daily. Haematocrit was assessed every 24 hours. Blood glucose was assessed every 4 hours if the patient was unconscious. Other biochemistry, arterial blood gas analysis and chest X-ray were not available on site.

As in other low resource settings, some laboratory severe malaria criteria could not be routinely assessed: raised bilirubin level (jaundice), renal failure (anuria) and respiratory failure were diagnosed clinically. Metabolic acidosis was not assessed.

Gestational age (EGA) was assessed by either ultrasound (from 2001), Dubowitz newborn examination or fundal height.³²⁵ The majority of births occurred at SMRU clinics with skilled birth attendants. Details of delivery and pregnancy outcomes were recorded for pregnancies including home births. Birthweight was measured with electronic Seca medical scales with a precision of 10 g.

Statistical analysis

Baseline characteristics were summarized at the episode level stratified by the three diagnostic groups as defined above.

Our outcomes of interest were 1) maternal mortality and 2) pregnancy outcomes (fetal mortality and morbidity). For those who had multiple episodes of severe malaria, only

the final severe episodes were used for assessing pregnancy outcomes. For maternal mortality, each episode was regarded as unique. Overall maternal mortality was calculated as a proportion assuming that all maternal deaths were captured (i.e. patients who were lost to follow-up after treatment completion and discharge were assumed to be alive). Confidence intervals of proportions were calculated by the Wilson method,²⁹⁸ and proportions were compared by Fisher's exact test. As a sensitivity analysis, 28-day mortality was assessed using the Kaplan-Meier product limit estimate accounting for the lost-to-follow-up.

To find the risk factors for maternal death, baseline characteristics and the WHO severe criteria were assessed by meta-analysis methods pooling SMRU and SEAQUAMAT cohorts. The SEAQUAMAT study was a randomized control trial that compared intravenous artesunate and intravenous quinine for severe falciparum malaria, and was conducted in Bangladesh, India, Indonesia and Myanmar, in 2003–2005. Pregnancy was not an exclusion criterion in that study. Details of the SEAQUAMAT cohort have been described elsewhere.⁴⁵⁶

Risk ratios for binary variables were pooled using the fixed effects Mantel-Haenszel method. When there were no events (i.e. maternal deaths) observed in one or both comparison groups, a continuity correction was made by adding the reciprocal of the opposite group size, as proposed by Sweeting *et al* for pooling rare outcomes with group size imbalances.⁴⁷¹ As a sensitivity analysis, different constraints to the total sum of continuity corrections were applied.⁴⁷¹ When one of the comparison groups did not have any pregnant women, that cohort was excluded from the pooled analysis. For continuous variables, firstly the odds ratio was

calculated with univariable logistic regression and then pooled by meta-analysis methods.

Heterogeneity was measured by I^2 .²⁹⁹ If there was a significant heterogeneity (i.e. $p < 0.05$), the random effects model developed by DerSimonian & Laird⁴⁷² was used. Because of the small sample size (and the small number of outcomes), multivariable analyses were not conducted.

To assess maternal mortality, patients were categorized into three groups based on the WHO severe malaria criteria: cerebral malaria, severe malaria without coma, and uncomplicated severe anaemia or uncomplicated hyperparasitaemia. The discriminative ability of the diagnostic grouping was assessed by c-statistic, which was derived from a receiver operating characteristic curve by the non-parametric method.⁴⁷³

Analyses on pregnancy outcomes excluded those who died and those who were lost to follow-up before delivery. Fetal loss was categorized to miscarriage (< 28 weeks) or stillbirth (≥ 28 weeks).^{177,178} PTB was defined as live birth from ≥ 28 weeks but before 37 complete weeks (37 0/7 weeks).¹⁷⁹ Live-born singleton babies without congenital abnormality were included in the analyses of birthweight. SGA was defined as birthweight $< 10\%$ using the INTERGROWTH-21st international standard accounting for gestational age at delivery and sex.¹⁶⁵ LBW defined as $< 2,500$ g¹⁷⁹ was included only for comparison with older literature. As a sensitivity analysis, assessment of birthweight was also conducted but only for those whose birthweight was measured within three days of the date of birth. The postpartum period begins at delivery and extends to 42 days (6 weeks).¹⁷⁶

8.3 Results

Increasing ANC and declining malaria in pregnancy at SMRU over time

Between 1989 and 2017 there were 114 episodes in 112 pregnant women that fulfilled the WHO severe malaria criteria at SMRU, with the last case in 2015. In 1989–2015, the number of pregnant women followed up at the SMRU ANCs increased while the number of malaria cases in pregnancy in both refugees and migrants trended downwards (Figure 8-1). Annual malaria incidence in pregnancy decreased over the years from 0.54 (in 1989) to 0 (in 2014/2015) malaria episodes/pregnant woman in refugees and from 0.91 (in 1999) to 0.002 (in 2015) in migrants (Appendix Figure 8-1).

In particular, after 2010, both the number and the incidence of malaria in pregnancy decreased dramatically. The proportion of uncomplicated hyperparasitaemia appeared to increase over the years except for the last three years (2010–2012 in refugees and 2013–2015 in migrants) when malaria itself became rare (Appendix Figure 8-1).

Baseline characteristics of the SMRU cohort

At SMRU, the most common severe criteria were hyperparasitaemia (51%, 55/107) and severe anaemia (45%, 46/103) followed by coma (21%, 23/112), although most of the patients with severe anaemia and hyperparasitaemia did not fulfil any other severe malaria criteria (i.e. in either the uncomplicated severe anaemia group or the uncomplicated hyperparasitaemia group) (Table 8-3). A total of 45 episodes were the uncomplicated

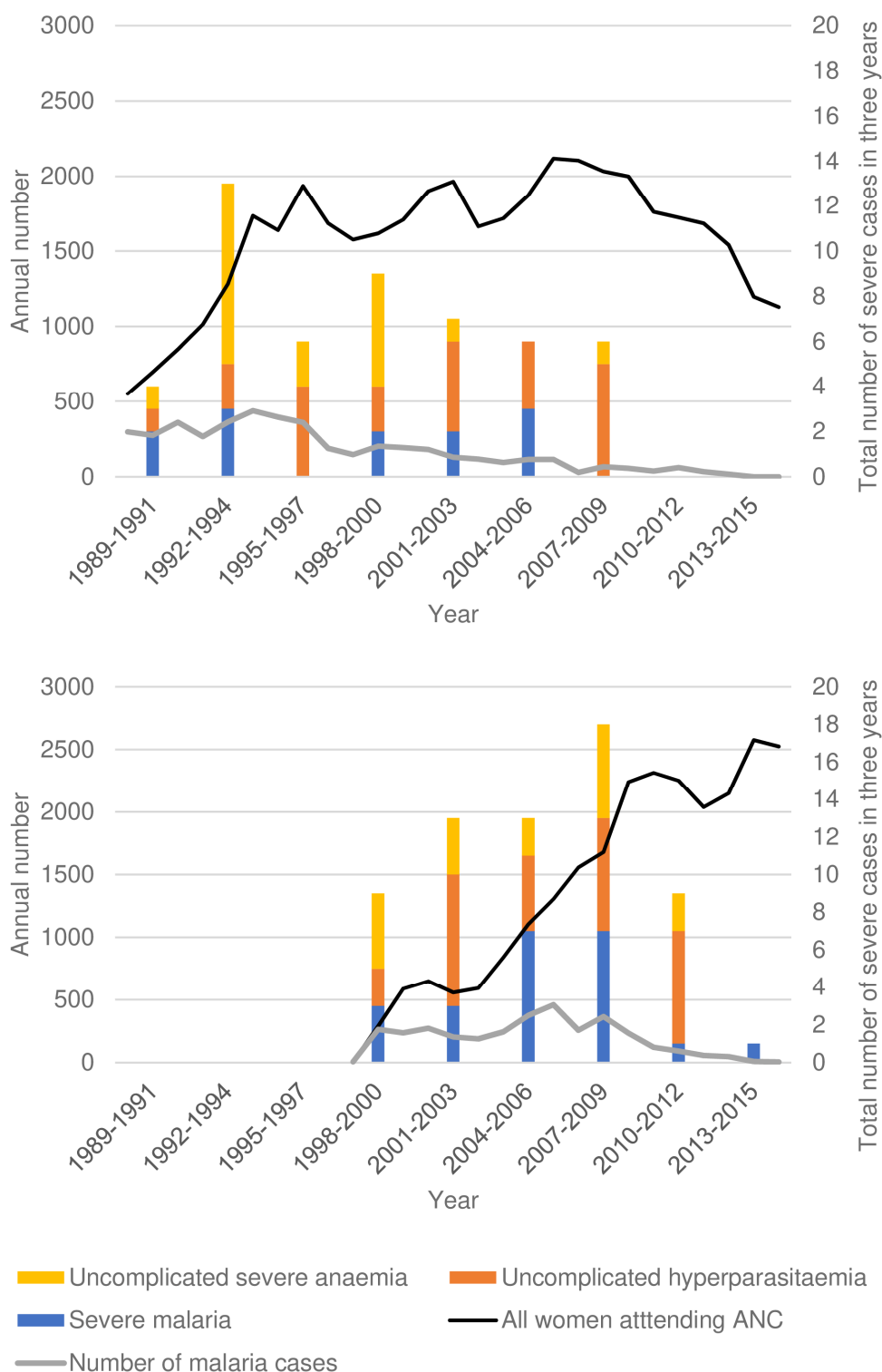


Figure 8-1. Number of pregnant women who attended SMRU ANC, malaria cases in pregnancy and severe malaria in pregnancy in refugees (top) and migrants (bottom)
The annual number of all pregnant women and malaria cases in pregnancy are shown by lines (left axis). The number of severe cases were summed up every three years and are shown in bar (right axis).

Table 8-3. Proportion of patients by severe criterion

WHO severe malaria criterion	SMRU (n=114)	SEAQUAMAT (n=51)
Coma	21% (23/112)	33% (17/51)
Severe anaemia	45% (46/103)	44% (21/48)
Severe anaemia only	33% (34/103)	13% (6/48)
Renal failure	6% (5/90)	16% (8/51)
Respiratory insufficiency	7% (7/100)	18% (9/51)
Convulsion	2% (2/100)	6% (3/51)
Severe metabolic acidosis	Not assessed	14% (3/21)
Jaundice	6% (6/100)	41% (21/51)
Hypoglycemia	8% (4/53)	2% (1/47)
Hypotension	2% (2/101)	4% (2/49)
Hyperparasitaemia	51% (55/107)	24% (12/49)
Hyperparasitaemia only	42% (45/107)	Not included (0/49)*
Prostration	10% (10/101)	Not assessed
Severe bleeding	Not assessed	Not assessed

The denominator is the number of patients assessed or information available.

- SEAQUAMATA study enrolled patients by rapid diagnostic test and clinical symptoms, therefore those who fulfill only hyperparasitaemia was not included in the study.
- The proportions of missing information are as following: coma (2%); severe anaemia (10%); renal failure (21%); respiratory insufficiency (12%); convulsion (12%); jaundice (12%); hypoglycemia (54%); hypotension (11%); hyperparasitaemia (6%); prostration (11%).

hyperparasitaemia, 34 episodes were the uncomplicated severe anaemia group, and 34 episodes were the SMRU severe group. One patient with both hyperparasitaemia and severe anaemia was categorized into the uncomplicated hyperparasitaemia group. More than half (64%, 73/114) of the WHO severe episodes occurred in those who came to the ANC for the first time or after >14 days of absence.

The SMRU severe group was slightly older (median 27 years, interquartile range 18–34), gestation was more advanced (median EGA 31.4 weeks, interquartile range 22.7–34.2) and a higher proportion had gametocytaemia than the other two diagnostic categories (Table 8-4). Three severe episodes occurred during the postpartum period; all of them delivered at home. Lower haematocrit in the uncomplicated severe anaemia group and higher baseline

parasitaemia in the uncomplicated hyperparasitaemia group were expected observations because of the diagnostic categories. The other characteristics were comparable among these three groups.

There was a shift of treatment from quinine to artemisinin derivatives over time according to the local guideline as described above. Intravenous (IV) quinine was used until 1999, and IV artesunate was used after 2000. Intramuscular artemether was used only tentatively until 2000, after which IV artesunate was securely available. Oral artemisinin-derivatives were used from 1994. ASMQ (n=7) was only used for three years (1994–1997). AC (n=24) use started in 2001 and replaced artesunate monotherapy (n=25). One episode enrolled in a study was treated with DP. Oral quinine (n=10) was used until 1999, and only three cases were treated with oral quinine after 2000: all three cases were in the early second trimester with severe anaemia only.

All patients in the severe diagnostic category were treated parenterally. Women with uncomplicated hyperparasitaemia were treated parenterally in 22% (10/46) of cases and rescue parenteral treatment was given in a further 20% (9/46). In the uncomplicated severe anaemia group, three patients (9%, 3/34) were treated parenterally.

Table 8-4. Background of pregnant women with the WHO criteria for severe malaria

Characteristic	SMRU severe (n=34)	Uncomplicated hyperparasitaemia (n=46)	Uncomplicated severe anaemia (n=34)	SEAQUAMAT (n=51)
Age (years)	27 [18-34]	25 [19-30]	24.5 [20-33]	24 [20-30]
Trimester				
first	12% (4/34)	17% (8/46)	15% (5/34)	6% (3/51)
second	27% (10/34)	39% (18/46)	50% (17/34)	51% (26/51)
third	55% (17/34)	43% (29/46)	35% (12/34)	43% (22/51)
postpartum	9% (3/34)*	0	0	0
EGA (week)	31.4 [22.7-34.2]	25.4 [18.1-32.3]	24.4 [17.6-30.2]	26 [20-32]
Parity	2 [0-3]	1 [0-2]	1 [0-3]	No data
Gravidity	3 [1-5]	2 [1-4]	2 [1-4]	No data
Body weight (kg)	50 [45-54] (n=24)	49.5 [44-52]	46 [43-50] (n=33)	50 [44-54]
Fever >37.5 °C	54% (13/24)	42% (18/43)	52% (17/33)	67% (30/45)
Day of fever	3 [3-5] (n=25)	3 [2-4] (n=45)	4 [1-5]	5 [3-7] (n=49)
Species				
Pf mono	90% (28/31)	91% (42/46)	88% (30/34)	No data
Pf + Pv	10% (3/31)	9% (4/46)	12% (4/34)	
Parasitaemia (/μL)	196,690 [112,538-372,655] (n=27)	462,522 [413,978- 529,027]	42,666 [16,052-64,433]	29,390 [80-113,040] (n=49)
Gametocyaemia	48% (13/27)	22% (10/46)	21% (7/34)	No data
Haematocrit (%)	24 [18-29] (n=24)	29 [25.4-33.4] (n=44)	17 [16-18]	22 [17-25] (n=45)
No previous ANC visits	29% (10/34)	30% (14/46)	35% (12/34)	No data
Days since last negative smear	14 [10-24] (n=22)	12.5 [9-26.5] (n=32)	18 [7-35] (n=22)	No data
Treatment				
IV artesunate	73% (22/30)	20% (9/46)	3% (1/34)	47% (23/49)
IM artemether	13% (4/30)	0	0	0
IV quinine	13% (4/30)	2% (1/46)	6% (2/34)	53% (26/49)
Oral ABT	0	57% (26/46)	65% (22/34)	0
ABT + rescue IV	0	20% (9/46)	0	0
Oral quinine	0	2% (1/46)	26% (9/34)	0

ABT: artemisinin-based therapy (either artesunate monotherapy or artemisinin-based combination therapy), ANC: antenatal care, EGA: estimated gestational age, IM: intramuscular, IV: intravenous. Median [interquartile range] is shown. The number of patients assessed is shown in round brackets if not all were assessed.

* Postpartum day 2, 2, 24.

Description of the SEAQUAMAT pregnancy cohort

In the SEAQUAMAT study, 51 pregnant patients were included, all with parasitaemia. The most common severe criterion was severe anaemia (44%, 21/48) followed by jaundice (41%, 21/51) and coma (33%, 17/51) (Table 8-3). Six women (13%, 6/48) only had severe anaemia. Age and EGA were lower than the SMRU severe diagnostic category but similar to the uncomplicated hyperparasitaemia and severe anaemia categories (Table 8-4). More patients were febrile (67%, 30/45), but mean parasitaemia was lower in the SEAQUAMAT than the SMRU cohort. In the SEAQUAMAT study, patients were randomly assigned to either IV quinine (n=26) and IV artesunate (n=23).

Maternal mortality

There were ten maternal deaths (9%, 10/114) in total at SMRU. The maternal mortality ratio (MMR) attributable to malaria during this period was 20 per 100,000 live births (10/49996). MMR (three-year blocks) was highest at the beginning of this period (1989–1991) at 102 (95%CI 28 to 371) per 100,000 live births and decreased to 0 (95%CI 0 to 49) per 100,000 live births in 2013–2015 (Appendix Figure 8-2). Most of the deaths (70%, 7/10) occurred within 24 hours of admission. No deaths were observed after five days. The overall 28-day mortality was 9.5% (95%CI 5.3 to 17.0%) by the Kaplan-Meier estimator after taking account of those who were lost to follow-up. Mortality was only observed in the severe diagnostic category, thus 29% (10/34) of this group died.

In the SMRU severe group, mortality was 9% (2/22) after IV artesunate, 25% (1/4) after intramuscular artemether, and 75% (3/4) after IV quinine. All deaths from IV artesunate and intramuscular artemether occurred within 24 hours, while two patients died on day 4 on IV quinine. Mortality was 0% in the uncomplicated severe anaemia group and the uncomplicated hyperparasitaemia group, justifying the local guideline of using oral treatment for most cases (84%, 67/80).

Blood transfusion was commonly required for a diagnosis of severe (67%, 20/30) and uncomplicated malaria with severe anaemia (93%, 27/29), but less often in the uncomplicated hyperparasitaemia diagnostic category (38%, 17/45).

In the SEAQUAMAT cohort, maternal mortality was 10% (5/51). It was 9% (2/23) after IV artesunate and 12% (3/26) after IV quinine.

Factors associated with maternal death

Factors associated with maternal death were assessed pooling the SMRU cohort and the SEAQUAMAT cohort (Table 8-5, 8-6).

Among the baseline characteristics, none were associated with maternal death. Maternal mortality was 5% (95% CI 0.9 to 23.6%, 1/20) in the first trimester, 8% (95% CI 3.9 to 17.2%, 6/71) in the second trimester, 10% (95% CI 4.9 to 19.0%, 7/71) in the third trimester and 33% (95% CI 6.9 to 79.2%, 1/3) in the postpartum period. EGA as a continuous variable was not linearly associated with the risk of maternal death. The presence of

Table 8-5. Proportion and pooled risk ratio for maternal death by baseline characteristics and WHO severe criterion

Characteristic	Maternal death		Pooled risk ratio	p-value
	Symptom (+)	Symptom (-)		
Fever >37.5 °C	6% (5/78)	6% (4/67)	0.97 (0.25–3.70)	0.96
Presence of gametocytes†	10% (3/30)	3% (2/77)	3.85 (0.68–21.91)	0.13
Presence of schizonts†	7% (1/15)	4% (4/92)	1.53 (0.18–12.80)	0.69
Mixed infection†	0% (0/11)	7% (7/100)	0.01 (0.00–4532647)	0.66
Coma	33% (13/40)	2% (2/123)	18.23 (4.63–71.73)	<0.001
Severe anaemia	9% (6/67)	4% (3/84)	2.52 (0.66–9.66)	0.18
Severe anaemia only	0% (0/40)	8% (9/111)	0.02 (0.00–176.82)	0.39
Renal failure	31% (4/13)	2% (3/128)	7.52 (2.24–25.29)	0.001
Respiratory insufficiency	31% (5/16)	4% (6/135)	6.00 (0.24–152.18)*	0.28*
Convulsion	20% (1/5)	7% (10/146)	2.47 (0.39–15.62)	0.34
Metabolic acidosis‡	33% (1/3)	6% (1/18)	6.00 (0.50–72.21)	0.16
Jaundice	11% (3/27)	6% (7/124)	1.44 (0.40–5.17)	0.58
Hypoglycemia	40% (2/5)	6% (6/95)	7.77 (1.59–37.99)	0.01
Hypotension	25% (1/4)	5% (8/146)	3.99 (0.62–25.58)	0.14
Hyperparasitaemia	6% (4/67)	7% (6/89)	1.09 (0.34–3.49)	0.88
Hyperparasitaemia only†	0% (0/45)	9% (5/57)	0.01 (0.00–167.34)	0.36
Prostration†	10% (1/10)	4% (4/91)	2.28 (0.28–18.43)	0.44
Treatment				
IV artesunate	7% (4/55)	(omitted)	(omitted)	
IM artemether	25% (1/4)			
IV quinine	18% (6/33)			
Oral quinine	0% (0/10)			
Oral ABT	0% (0/57)			
Study site				
Bangladesh	25% (3/12)	(omitted)	(omitted)	
India	0% (0/1)			
Indonesia	0% (0/10)			
Myanmar	7% (2/28)			
SMRU	9% (10/114)			

Risk ratio and p-value are based on fixed effects model (no marks) or random effects model (*). Variables assessed only in the SMRU cohort (†) or the SEAQUAMAT cohort (§). ABT: artemisinin-based treatment. IM: intramuscular. IV: intravenous.

gametocytes and the presence of schizonts were associated with higher risks of maternal

death, and mixed infection with a lower risk, but none of them were statistically significant.

Baseline parasitaemia was not associated with maternal death even when the uncomplicated

Table 8-6. Pooled odds ratio for maternal death by continuous baseline characteristics

Characteristic	Average (SD)		Pooled odds ratio (/one unit)	p-value
	Survivor	Non-survivor		
Age (year)	25.2 (6.58)	27.5 (7.92)	1.05 (0.97–1.14)	0.22
Body weight (kg)	49.1 (7.90)	48.7 (9.07)	0.94 (0.73–1.23)*	0.67*
EGA (week)	25.3 (8.75)	28.0 (8.65)	1.01 (0.81–1.25)*	0.96*
Parity†	1.7 (2.10)	1.9 (1.79)	1.05 (0.78–1.41)	0.76
Day of fever (day)	5.2 (9.75)	6.67 (3.64)	1.01 (0.95–1.07)	0.81
Parasitaemia (log ₁₀ (#/μL))	11.6 (1.89)	10.7 (3.24)	0.89 (0.66–1.20)	0.44

Risk ratio and p-value are based on fixed effects model (no marks) or random effects model (*).

Variables assessed only in the SMRU cohort (†) or the SEAQUAMAT cohort (§).

ABT: artemisinin-based treatment. EGA: estimated gestational age. IM: intramuscular. IV: intravenous. SD: standard deviation.

hyperparasitaemia group and the uncomplicated severe anaemia group from the SMRU cohort were excluded.

All the WHO severe criteria excluding hyperparasitaemia showed a higher risk of maternal death. Among them, three criteria were significantly associated with maternal death: coma (pooled risk ratio [RR] 18.23, 95% CI 4.63 to 71.73, $p < 0.001$), renal failure (pooled RR 7.52, 95% CI 2.24 to 25.29, $p = 0.001$) and hypoglycaemia (pooled RR 7.77, 95% CI 1.59 to 37.99, $p = 0.01$). Patients with only severe anaemia or uncomplicated hyperparasitaemia showed a lower risk of maternal death but without statistical significance. Different continuity corrections did not change the conclusion (Appendix Table 8-1). Heterogeneities of the two cohorts are shown in Appendix Table 8-2.

Mortality after IV artesunate and IV quinine was 7% (4/55) and 18% (6/33), respectively. Mortality was higher in Bangladesh (25%, 3/9) in the SEAQUAMAT cohort,

Table 8-7. Risk of maternal death by diagnostic group

	All (n=165)	SMRU cohort (n=114)	SEAQUAMAT cohort (n=51)
Mortality			
Cerebral malaria	33% (13/40)	39% (9/23)	24% (4/17)
Severe malaria without coma	5% (2/39)	9% (1/11)	4% (1/28)
Only severe anaemia and/or hyperparasitaemia	0% (0/86)	0% (0/80)	0% (0/6)
p-value	<0.001	<0.001	0.10
C-statistics	0.88 (0.82–0.92)	0.92 (0.86–0.96)	0.77 (0.63–0.87)

than in other sites, but this was without statistical significance.

Maternal mortality was assessed for the pre-specified diagnostic groups: cerebral malaria, severe malaria without coma and those who had only severe anaemia and/or hyperparasitaemia. Overall, mortality was highest in cerebral malaria (33%, 13/40), followed by severe malaria without coma (5%, 2/39). There was no maternal death in 86 patients who had only severe anaemia and/or hyperparasitaemia. The same trend was observed when the SMRU cohort and the SEAQUAMAT cohort were analysed separately (Table 8-7).

Pregnancy outcomes

In the SMRU cohort, pregnancy outcomes were available in 80% (82/102) of patients excluding those who died before delivery (n=7) and postpartum episodes (n=3) (Table 8-8). For those who had multiple episodes (n=2), only the last severe episode was included.

Fetal losses occurred in 11% (9/82) of cases: seven miscarriages and two stillbirths. The proportion of miscarriage among those who had severe malaria before the gestational age of 28 weeks was 17% (7/42) and highest in the SMRU severe group (25%, 2/8). Stillbirth was

only observed in the SMRU severe group 12% of cases (2/17). Fetal losses occurred in the first week of severe malaria in 67% (6/9) of cases. If seven pregnant women who died before delivery (two before the gestational age of 28 weeks and five after 28 weeks) were included in the calculation, the amount of fetal loss was 18% (16/89). In the SMRU severe group, the proportion of miscarriage and stillbirth, including in pregnant women who died, were 40% (4/10) and 32% (7/22), respectively.

All live-born babies were singletons, and there were no babies with a congenital abnormality (n=73). Median EGA at delivery was 38.7 (interquartile range 37.5–40) weeks (n=73), and 21% (15/73) were preterm (i.e. <37 weeks). Median birthweight was 2,780 (interquartile range 2,500–3,100) g, and 19% (11/57) were categorized as LBW. The proportion of SGA was 38% (26/69). These figures were similar when only those whose birthweight had been assessed within three days of birth were included.

In the SMRU cohort the severe diagnostic group had a higher risk of fetal loss (21%, 4/19, p=0.20), PTB (33%, 5/15, p=0.28), and SGA (54%, 7/13, p=0.21) than the other diagnostic groups, but without statistical significance. This tendency was unchanged if the analysis was restricted to those whose birthweight was measured within three days of birth.

In the SEAQUAMAT study, pregnancy outcomes were not assessed since the patients were not followed-up until delivery.

Table 8-8. Summary of maternal and fetal outcomes in SMRU cohort

	All	SMRU severe	Uncomplicated hyperparasitaemia	Uncomplicated severe anaemia
Delivery outcomes assessed	80% (82/102)	86% (19/22)	80% (37/46)	76% (26/34)
Fetal loss	11% (9/82)	21% (4/19)	8% (3/37)	8% (2/26)
<28 weeks	17% (7/42)	25% (2/8)	15% (3/20)	14% (2/14)
≥28 weeks	3% (2/75)	12% (2/17)	0% (0/34)	0% (0/24)
Day to fetal loss	-	0, 1, 4, 7	0, 3, 54	23, 30
Estimated gestational age at delivery*	38.7 [37.5–40.0] (n=73)	38.2 [33.2–39.5] (n=15)	38.9 [38.1–40.0] (n=34)	39.1 [37.3–40.4] (n=24)
Preterm delivery (<37 weeks)*	21% (15/73)	33% (5/15)	18% (6/34)	17% (4/24)
Sex (boy)*	50% (37/72)	47% (7/15)	47% (16/34)	57% (13/23)
Birthweight†	2780 [2500–3100] (n=57)	2600 [2300–3000] (n=9)	2765 [2630–3075] (n=28)	2900 [2460–3150] (n=20)
SGA	38% (26/69)	54% (7/13)	30% (10/33)	39% (9/23)
Z-score*	-0.97 [-1.5 – -0.21] (n=69)	-1.3 [-1.8 – -0.14] (n=13)	-0.91 [-1.4 – -0.21] (n=33)	-1.1 [-1.8 – -0.24] (n=23)
LBW (<2500g) ‡	19% (11/57)	33% (3/9)	11% (3/28)	25% (5/20)
Congenital abnormality	0/73	0/15	0/34	0/24
Mode of delivery*				
NVD	90% (66/73)	80% (12/15)	91% (31/34)	96% (23/24)
Breech	1% (1/73)	7% (1/15)	0	0
C-section	4% (3/73)	7% (1/15)	6% (2/34)	0
Forceps	1% (1/73)	7% (1/15)	0	0
No information	3% (2/73)	0	3% (1/34)	4% (1/24)
Only include birthweight weighted within 3 days from birth				
Birthweight†	2760 [2500–3100] (n=38)	2600 [2200–3100] (n=7)	2730 [2600–3050] (n=22)	3000 [2420–3100] (n=9)
SGA	41% (18/44)	55% (6/11)	39% (9/23)	30% (3/10)
Z-score*	-1.1 [-1.7 – -0.53] (n=44)	-1.3 [-2.1 – -0.14] (n=11)	-0.97 [-1.6 – -0.62] (n=23)	-0.88 [-1.8 – -0.55] (n=10)
LBW (<2500g) ‡	21% (8/38)	43% (3/7)	9% (2/22)	33% (3/9)

C-section: caesarean section. LBW: low birth weight. NVD: normal vaginal delivery. SGA: Small for gestational age

Fetal outcomes excludes those whose mother died before delivery and postpartum episodes. Only the last episodes were included from those who had multiple episodes. INTERBROWTH-21st international standard growth chart was used for SGA (defined as <10%) and Z-score of birthweight for gestational age. * Only live born singleton included. ‡ Only live born full-term singleton included.

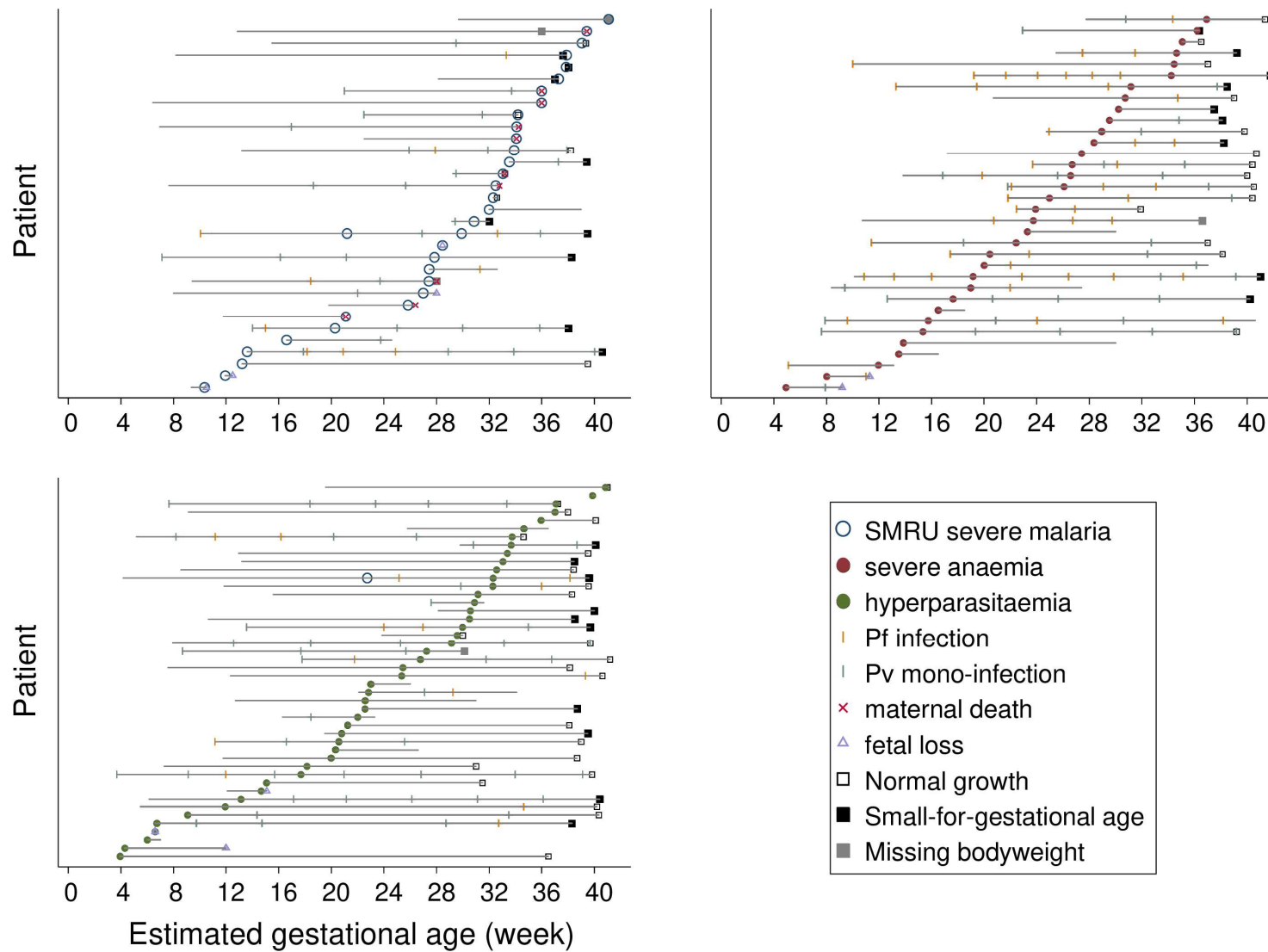


Figure 8-2. Timings of malaria episodes, maternal and fetal outcomes. Each line starts at the first antenatal clinic visit and ends at the last antenatal clinic visit (either maternal death, the end of pregnancy, or lost-to-follow-up.)

Timing of the adverse outcomes

The timings of malaria episodes and maternal and fetal events in each patient are summarized in Figure 8-2. Adverse events within seven days of the severe malaria episode, which was assumed to be more directly related to the severe malaria episode, occurred more commonly in the SMRU severe diagnostic group. In this severe group, among 27 episodes that occurred before 37 weeks, 59% (16/27) had an adverse outcome in the following week (nine maternal deaths, four fetal losses without maternal deaths and three PTBs). In the uncomplicated hyperparasitaemia group, 42 episodes occurred before 37 weeks. Excluding one patient who was lost to follow-up <7 days, 10% (4/41) had an adverse outcome within a week (two fetal losses and two PTBs). In the uncomplicated severe anaemia group, 34 episodes occurred before 37 weeks. Only one (3%, 1/34) had an adverse outcome within a week (PTB at 36.3 gestational weeks).

8.4 Discussion

This analysis endorses use of the WHO severe malaria criteria, which is essential to provide early life-saving IV therapy for patients with severe malaria. This analysis also portrays vastly different mortality outcomes depending on how the WHO criteria are interpreted. At SMRU, the overall mortality in pregnant women who met any WHO criteria for severe malaria was 9%; however, it was three times higher (29%) when pregnant women with only severe anaemia or only hyperparasitaemia, and without signs of any other organ

dysfunction, were excluded (i.e. retain only the SMRU severe diagnostic group). Indeed, the highest mortality (39%) was observed when cerebral malaria cases were included. This confirms that different application of severe criteria can be a contributing factor to the discrepancies observed in malaria-related mortality in pregnancy in the international literature.

This descriptive analysis revealed that the presence of any of the WHO severe criteria showed higher risks of maternal mortality, which was significant for coma, renal failure and hypoglycaemia in pregnant women. Another important finding is that patients with only severe anaemia and/or hyperparasitaemia were at lower risk of mortality as reported two decades ago,⁴⁶⁰ particularly when blood transfusion is available for anaemia. At SMRU these two diagnostic groups were mostly treated orally with close clinical and parasitological monitoring, without any maternal mortalities.

Maternal deaths in the SMRU cohort occurred within 24 hours of IV artesunate while two out of three maternal deaths occurred after 24 hours on IV quinine. This observation agrees with a previous report that effective antimalarial drugs can improve mortality only after 24 hours.⁴⁵⁶ Deaths that occurred in the first 24 hours and had received IV artesunate, had presented too late in the disease process – emphasizing the low threshold for providing IV treatment to suspected severe cases. In this cohort, 64% of severe episodes occurred in those who came to ANC for the first time or after >14 days of absence, suggesting access issues. Encouraging women to come to ANC early in the first trimester and improving regular

attendance may reduce the incidence of severe malaria. Barriers to ANC attendance need to be identified and overcome.

The overall proportion of fetal mortality by miscarriage and stillbirth was 17% (7/42) and 3% (2/75), respectively; with proportions of 25% (2/8) and 12% (2/17), respectively in the SMRU severe diagnostic group. In this setting, the prevalence of miscarriage and stillbirth among those who had not had malaria during pregnancy was reported to be 19.2% (3198/16668) and 1.2% (153/13158), respectively.¹⁵⁴

Severe malaria in pregnancy was associated with an increased risk of PTB and SGA. The overall prevalence of PTB was 21% (15/73), which is higher than the 12% (989/8,221, $p=0.03$) found in those who had malaria in pregnancy and 9.3% (3882/4,1839, $p=0.003$) in those who did not have malaria in this setting.³¹ Compared with those who had malaria in pregnancy, the proportion of PTB was significantly higher in the SMRU severe group (33%, 5/15, $p=0.03$), but not in the uncomplicated hyperparasitaemia group (18%, 6/34, $p=0.29$) or the uncomplicated severe anaemia group (17%, 4/24, $p=0.52$).

The proportion of SGA (38%, 26/69) was higher than 25% (2040/8221, $p=0.02$) in those who had malaria in pregnancy and 19% (7965/41839, $p<0.001$) in those who did not have malaria in this setting.³¹ Compared with those who had malaria in pregnancy, the proportion of SGA was significantly higher in the SMRU severe group (54%, 7/13, $p=0.02$), but not in the uncomplicated hyperparasitaemia group (30%, 10/33, $p=0.43$) or the uncomplicated severe anaemia group (39%, 9/23, $p=0.14$).

Immediate effects on both mother and fetus were seen predominantly in the SMRU severe diagnostic group, but not in the uncomplicated hyperparasitaemia or the severe anaemia groups. Fetal losses occurred within the first week of diagnosis in 67% (6/9) of cases. All three fetal losses after the first week following severe malaria were in the first trimester (i.e. <13 weeks), during which other causes of miscarriage would need to be considered. Overall the data support a previous study where severe malaria was observed to increase the immediate risk of fetal loss.¹⁹⁶

This descriptive analysis has several limitations. Firstly, some of the severe malaria criteria had missing information. However, these missing criteria requires laboratory investigation and are frequently unavailable at the primary healthcare level in malaria-endemic countries. The results of this study have practical utility for settings where most malaria is diagnosed and treated, i.e. in rural and remote settings. For the categorization into three diagnostic groups, it is very unlikely that severe systemic conditions were present but not recorded in the clinically stable groups (i.e. the uncomplicated hyperparasitaemia group and the uncomplicated severe anaemia group), who were all admitted for treatment.

Secondly, the treatment was not controlled for maternal death. This was because the small sample size did not allow multivariable adjustment and patients with only severe anaemia and/or hyperparasitaemia were treated differently (based on the known lower risk). Hyperparasitaemia needs longer treatment with artemisinin derivatives,³⁷¹ but can be successfully treated with oral treatment with little adverse maternal and fetal outcome as

shown in this study. The cut-off of hyperparasitaemia requiring IV treatment needs further evaluation for pregnant women. There was no apparent difference in the distribution of severe criteria in the quinine and artesunate groups. Therefore, the assessment of risk factors will not be affected. Recently, poor responses to IV artesunate for severe malaria were reported in this area.¹³⁷ In this cohort, however, the last maternal death occurred in 2009 (2010 in the SEAQUAMAT study), before the emergence of artemisinin-resistance and also before wide scale efforts to eliminate malaria in this area.

Finally, both the SMRU and the SEAQUAMAT cohorts involved a small number of pregnant women from Southeast or South Asia, although this is the largest combined cohort of severe falciparum malaria in pregnancy in the literature as far as we are aware.⁴⁵⁹ Further assessment in sub-Saharan Africa is required considering the different prevalence of severe criteria. Data from these countries, where maternal mortality in general is underreported, are sparse.¹

In the current situation of declining malaria, the risk of developing severe malaria may increase. In pregnancy, severe malaria causes mortality in both mother and fetus. This report demonstrated that the different application of the WHO severe criteria to pregnant women can cause a non-negligible difference in the summary mortality from severe malaria. Severe anaemia and hyperparasitaemia were relatively common in pregnant women and do not necessarily require IV treatment if the patient is otherwise clinically stable. Adverse maternal and fetal outcomes occurred mostly in the SMRU severe group, and particularly

during the first week after treatment. Revision of the definition and treatment for pregnant populations should be considered. These findings will be useful for improving clinical management and the comparability of further epidemiological research on severe malaria in pregnancy. The findings from this study have focused on *P. falciparum* but the WHO criteria for severe malaria have been used for any species of *Plasmodium*, and there is a significant research gap on the spectrum of disease severity for other species.

8.5 Conclusions

Maternal mortality from malaria varies greatly in the literature but this may relate in part to the application of the WHO severe malaria criteria. Increased risks of adverse maternal, fetal and neonatal outcomes were mainly observed in severe malaria patients but not in those who only had hyperparasitaemia and/or severe anaemia. Supervised oral, not intravenous, treatment with a diagnosis of uncomplicated hyperparasitaemia and/or uncomplicated malaria with severe anaemia results in a similar risk of adverse outcomes to uncomplicated malaria infections in pregnancy. Cerebral malaria was associated with the highest risk of maternal death, most of which occurred within 24 hours of treatment. Greater malaria control efforts in the non-pregnant population resulted in a significant decline in malaria in pregnancy and hence mortality from severe malaria. This should not deter efforts to provide access to ANC and reduce barriers to early diagnosis and treatment for pregnant women.

Chapter 9. Discussion

Part of this chapter has been published in a peer-reviewed journal and is freely available under the Creative Commons Attribution licence as follows. Information included in this chapter has been updated since publication.

Saito M, Gilder ME, Nosten F, Guérin PJ, McGready R. Methodology of assessment and reporting of safety in anti-malarial treatment efficacy studies of uncomplicated falciparum malaria in pregnancy: a systematic literature review. *Malar J.* 2017;16:491.

Saito M, Gilder ME, Nosten F, McGready R, Guérin PJ. Systematic literature review and meta-analysis of the efficacy of artemisinin-based and quinine-based treatments for uncomplicated falciparum malaria in pregnancy: methodological challenges. *Malar J.* 2017;16:488.

9.1 Summary of the research and implications

Throughout this thesis, a knowledge gap on the treatment of falciparum malaria in pregnancy (Box 9-1) has been revealed. Studies on antimalarial efficacy in pregnancy are disproportionately few considering the impact of this parasitic infection on the mother, fetus and infant. They have been included in only 3–6% of the studies on antimalarial treatment efficacy in the past,^{474,475} leading to a delay in the use of newer antimalarials that have been proven to be safe and more efficacious in non-pregnant populations.³ This continuation of old and less efficacious treatment for pregnant women has put the mother and fetus at further risk over the last 20 years. Despite the longevity of the use of quinine and its purported safety in pregnancy, very few data were available to support the current WHO recommendation for this treatment in pregnant women.

The IPD meta-analysis in this thesis (Chapters 4 and 6), together with previous research on the safety of artemisinins in the first trimester,^{32,33} provides compelling evidence that quinine monotherapy should be withdrawn from the recommendations and be replaced with ACTs for all pregnant women who have uncomplicated falciparum malaria (Table 9-1). Quinine-based treatment is not favoured because of its lower efficacy (when it is not combined with clindamycin), lower adherence, and a higher risk of gametocyte development (i.e. a higher chance for parasites to be transmitted). While the efficacy improves with the combination of quinine and clindamycin, in reality few sites make use of this evidence.

Box 9-1. Summary of the findings in this thesis

Summary of findings

Knowledge gap in antimalarial drug studies in pregnancy

1. Efficacy studies in pregnancy are not only limited in number but deploy varied definitions because of lack of standard study design for pregnant women
2. Precise and accurate estimation of gestational age is essential to assess birth outcomes
3. Longer follow-up is required to assess treatment efficacy in pregnant women

Efficacy of antimalarials in pregnancy

1. Quinine monotherapy is inferior to ACTs because of lower efficacy, higher risks of gametocyte development and adverse symptoms
2. Adding clindamycin to quinine can improve efficacy, but does not overcome the other disadvantages of quinine monotherapy
3. Slightly lower efficacy of AL may require further investigation on dose optimization for pregnant women

Safety of antimalarials in pregnancy

1. None of the highly effective ACTs presented a more favourable profile for reducing stillbirth, preterm birth, or small-for-gestational age than others
2. DP and chloroquine were similarly associated with longer QT/QTc interval on day peak than AL or ASMQ, but no drugs exceeded the known threshold for increased risk of fatal arrhythmia
3. Severe malaria is a risk of adverse maternal, fetal and neonatal outcomes but pregnant women who only have hyperparasitaemia or severe anaemia can be treated safely with ACTs

ACTs, given as treatment for uncomplicated falciparum malaria, are highly effective and safe for both mother and fetus (Chapter 4 and 6). However, AL, the most widely used ACT, may not necessarily be the best drug for pregnant women. The efficacy of AL was found to be slightly lower than other ACTs, but this needs further confirmation. This lower efficacy can be more problematic when other risk factors are present such as nulliparous status or areas of low malaria transmission. These can both be associated with lower immunity and are an indication of the need to increase attention to clinical care for these women. It may be necessary to tailor dosing (particularly of AL) for pregnant women. Although there seem to be

Table 9-1. Summary of evidence profile comparing artemisinin-based treatment (ABT) and quinine-based treatment (QBT) following GRADE scale¹⁶⁹

Quality assessment							Number of women		Effect of QBT / ABT	Quality	Importance
No of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	ABT	QBT	Relative (95% CI)		
PCR-corrected treatment failure											
1 (Ch. 4)	IPD meta-analysis	Not serious ^a	Not serious	Not serious	Not serious	Large effect	68/1278 (5.3%) (AL)	31/244 (12.7%) (Q)	aHR 6.11 (2.57 to 14.54)	High	Critical
Placental malaria											
1 (Ch. 6)	IPD meta-analysis	Serious ^b	Not serious	Serious ^b	Not serious		743/893 (83.2%) (AL)	55/82 (67.1%) (Q)	aHR 0.87 (0.43 to 1.75)	Moderate	Important
Gametocyte development after treatment											
1. (Ch. 4)	IPD meta-analysis	Not serious ^a	Not serious	Not serious	Not serious	Large effect	34/4153 (0.8%)	17/233 (7.3%)	aHR 13.22 (4.60 to 37.97)	High	Important
Tinnitus											
1 (Ch. 4)	IPD meta-analysis	Very serious ^a	Not serious	Not serious	Not serious	Large effect	41/1150 (3.6%) (AL)	165/228 (72.4%) (Q)	aOR 227.88 (72.81 to 713.25)	High	Critical
Vomiting											
1 (Ch. 4)	IPD meta-analysis	Very serious ^a	Not serious	Not serious	Not serious	Large effect	33/1223 (2.7%) (AL)	39/227 (17.2%) (Q)	aOR 9.40 (4.58 to 19.28)	High	Critical
Nausea											
1 (Ch. 4)	IPD meta-analysis	Very serious ^a	Not serious	Not serious	Not serious	Large effect	75/1225 (6.1%) (AL)	74/227 (32.65) (Q)	aOR 6.88 (3.53 to 13.42)	High	Important

Table 9-1 continued.

Quality assessment							Number of women		Effect of QBT / ABT	Quality	Importance
No of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	ABT	QBT	Relative (95% CI)		
Abdominal pain											
1 (Ch. 4)	IPD meta-analysis	Very serious ^a	Not serious	Not serious	Not serious		99/1177 (8.4%) (AL)	51/228 (22.4%) (Q)	aOR 1.80 (1.05 to 3.10)	Moderate	Important
Anorexia											
1 (Ch. 4)	IPD meta-analysis	Very serious ^a	Not serious	Not serious	Not serious		116/1228 (9.4%) (AL)	55/227 (24.2%) (Q)	aOR 3.8 (1.78 to 8.49)	Moderate	Important
Headache											
1 (Ch. 4)	IPD meta-analysis	Very serious ^a	Not serious	Serious ^c	Not serious		273/1229 (22.2%) (AL)	36/228 (15.8%) (Q)	aOR 0.74 (0.37 to 1.46)	Moderate	Important
Dizziness											
1 (Ch. 4)	IPD meta-analysis	Very serious ^a	Not serious	Not serious	Not serious	Large effect	102/1251 (8.2%) (AL)	137/228 (60.1%) (Q)	aOR 9.65 (5.62 to 16.58)	High	Important
Hypoglycaemia											
1	RCT ¹⁹⁸	Very serious ^{a,d,e}	Not serious	Not serious	Not serious	Large effect	3/28 (10.7%) (ASMQ)	21/29 (72.4%) (Q)	RR 6.76 (2.27 to 20.15)	Low	Critical

Table 9-1 continued.

Quality assessment						Number of women		Effect of QBT / ABT	Quality	Importance	
No of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	ABT	QBT			Relative (95% CI)
Miscarriage (pregnancy ended at or before 28 weeks of gestation) after antimalarial use in the first trimester											
1	IPD meta-analysis ³³	Not serious ^a	Not serious	Not serious	Not serious		37/671 (5.5%)	96/945 (10.2%)	aHR 1.37 (0.83 to 2.27)	Moderate	Critical
Stillbirth (fetal losses at 28 weeks or more of gestation) after antimalarial use in the second or third trimester											
1 (Ch. 6)	IPD meta-analysis	Not serious ^a	Not serious	serious ^b	Not serious		19/1087 (1.7%) (AL)	2/184 (1.1%) (Q)	aHR 0.71 (0.12 to 4.28)	Moderate	Critical
Congenital abnormality (antimalarial use in the first trimester)											
1	IPD meta-analysis ³³	Not serious ^{a, g}	Not serious	Not serious	Not serious		9/551 (1.6%)	10/741 (1.3%) (Q)	Crude RR 0.83 (0.34 to 2.02)	Moderate	Critical
Congenital abnormality (antimalarial use in the second or third trimester)											
1 (Ch. 6)	IPD meta-analysis	Not serious ^{a, g}	Not serious	Serious ^b	Not serious		54/4137 (1.3%)	4/245 (1.6%)	Crude RR 1.25 (0.46 to 3.43)	Moderate	Critical
Small for gestational age											
1 (Ch. 6)	IPD meta-analysis	Not serious ^{a, f}	Not serious	Serious ^b	Not serious		235/953 (24.7%) (AL)	19/113 (16.8%) (Q)	aOR 0.69 (0.32 to 1.47)	Low	Critical
Moderate to late preterm birth (32 to less than 37 weeks)											
1 (Ch. 6)	IPD meta-analysis	Not serious ^{a, f}	Not serious	Serious ^b	Not serious		155/ 1028 (15.1%) (AL)	15/167 (9.0%) (Q)	aOR 1.23 (0.56 to 2.67)	Low	Critical

ABT: artemisinin-based treatment, aHR: adjusted hazard ratio, AL: artemether-lumefantrine, aOR: adjusted Odds ratio, ASMQ: artesunate-mefloquine, Ch. Chapter, CI: Confidence interval, QBT: quinine-based treatment, Q: quinine monotherapy, RCT: randomized control trial, R: risk ratio.

a. Open-label studies b. Recurrence of malaria infection, which can affect the outcome, is not taken into account. c. This is probably due to malaria itself, rather than drug. Different policy on the antipyretic use was employed, which could have affected the outcome. d. Concealment of allocation is not clear. e. Randomisation is not clear. f. Estimation of gestational age may not be precise. g. Only external (major) abnormality was assessed.

High Further research is very unlikely to change our confidence in the estimate of effect.

Moderate Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low Any estimate of effect is very uncertain.

some variabilities between different populations, the blood concentration of AL is consistently lower in pregnant women,²³⁴ which can reduce efficacy as reported in the IPD meta-analysis (Chapter 4). Both ASAQ and ASMQ were highly effective, although both drugs had slightly higher risks of adverse symptoms as was previously known from studies in the general population.¹¹⁵ DP is effective and safe, with the longest post-treatment prophylactic period as was confirmed in our analysis of the time to recrudescence (Chapter 5). The only concern regarding DP was the potential cardiotoxicity,⁴¹⁵ but one of the current DPhil chapters (Chapter 7) confirmed that, for treating pregnant women with uncomplicated malaria, DP is as safe as chloroquine, which has been used safely in pregnancy for decades. The adjusted risk of treatment failure of ASSP was even higher than AL in the IPD meta-analysis, although not statistically significant. Considering the global prevalence of molecular markers of resistance to SP,²⁸⁷ ASSP should be withdrawn from the recommendation. Owing to resistance to SP, even if partial, ASSP functions as a monotherapy of artesunate, leaving the exposed parasites with a higher chance of developing resistance to artesunate. The use of ASSP in the third trimester was associated with a higher risk of SGA, which might support the possibility that the SP did not work as well as other partner drugs. SGA might be a more sensitive way to assess antimalarial impact, in addition to its role in predicting the long-term consequences for the child. The diagnosis of SGA, however, relies on accurate gestational age assessment in pregnancy.

The efficacy of antimalarial drugs thus needs to be monitored for pregnant women.

However, the currently recommended length of follow-up is insufficient. The results of this thesis (Chapter 5) imply that AL requires a follow-up of 63 days, longer than the current recommendation of 28 days, to capture enough treatment failure to determine true efficacy. A shorter follow-up overestimates parasitological efficacy, which leaves pregnant women at a higher risk of recurrence and promotes the development of resistant strains of malaria parasites.

Resistance of *P. falciparum* to artemisinin and partner drugs is a global concern.^{107,133,476} However, pregnant women may not be the best sentinel population for the purpose of monitoring drug resistance because they constitute a much smaller population (thus it is not easy to conduct a large trial) and treatment failure puts both mother and fetus at risk. Only drugs that are shown to be effective in non-pregnant populations should be tested on pregnant women. The purpose of antimalarial efficacy studies in pregnancy should be to choose the safest and most tolerable drug amongst highly effective drugs (i.e. >95% PCR-corrected treatment success) or to explore dose optimization for pregnant women.

Most of the direct mortality of mother and fetus is caused by severe malaria. It was revealed that pregnant women who had falciparum malaria with severe anaemia or hyperparasitaemia but without any other organ dysfunction (uncomplicated hyperparasitaemia and uncomplicated malaria with severe anaemia) did not necessarily require intravenous treatment (Chapter 8). Pregnant women with either uncomplicated hyperparasitaemia or uncomplicated malaria with severe anaemia can be successfully treated with oral ACTs

without complications. Most severe malaria episodes occurred when women were not followed up regularly or were identified at their first ANC visit, with the clinical condition mostly likely prompting the patient to seek care. As no antimalarial, including intravenous artesunate, can reduce mortality in the first 24 hours,⁴⁵⁶ it is important to focus on control programs that prevent pregnant women from developing severe malaria in the first place. Promoting early attendance at ANC from the first trimester, as well as regular attendance at ANC with prophylaxis or routine screening for malaria, can reduce the incidence of, and mortality from, severe malaria. Higher parasitaemia at baseline is associated with higher risks of treatment failure, stillbirth and SGA. This finding implies that, in low transmission settings, the risk of treatment failure, stillbirth and SGA can be reduced by regular systematic screening of peripheral smears at ANC visits, which may detect parasitaemia at a lower level than by passive screening where the patient presents because of symptoms.

This thesis paid particular attention to the treatment of falciparum malaria in pregnancy. However, this does not mean that prevention is less important. Rather, effective prevention of uncomplicated and severe malaria in pregnancy is essential. Two study findings reinforce the importance of prevention: the high prevalence of SGA among pregnant women with uncomplicated malaria; and the high risk of adverse outcomes within one week of severe malaria. Both indicate that effective treatment does not completely prevent the adverse outcomes for the mother and fetus, although it can limit the effects. Nonetheless, no prevention is 100% effective, even without considering the difficulty in implementation (or

uptake) or the lack of chemoprophylaxis recommendations in early pregnancy (i.e. the first trimester) when placentation takes place. Furthermore, in low endemic areas, there is no recommendation on how to protect pregnant women from falciparum malaria and with a diminishing number of effective antimalarials for treatment, none are recommended for prophylaxis. Treatment for pregnant women is thus necessary and should be studied alongside prevention. For both treatment and prevention, antimalarials used in pregnancy must be highly effective.

9.2 Limitations and suggestion for future work

This thesis provides a summary of the antimalarial treatments for falciparum malaria in pregnancy. There were several issues that could not be assessed, partly because of the nature of secondary data analysis. These have been highlighted and can be included and explored in future studies.

Firstly, haematological changes should be evaluated. Anaemia is an important modifiable factor that is related to maternal mortality and adverse fetal outcomes.^{477,478} The gestational changes in haematocrit and haemoglobin, and the use of haematinics should be accounted in the analyses. Frequently in tropical settings, pregnant women are routinely offered haematinics given the frequency of anaemia in pregnancy,⁴⁷⁹ but how this interacts with malaria and treatment efficacy requires more in-depth analysis.

Secondly, treatment efficacy should be compared between pregnant and non-pregnant populations. The timelines and pipeline of new antimalarials have followed a consistent

pattern that puts pregnancy at the tail end, leaving a particularly vulnerable group at a disadvantage when more effective compounds are used in the non-pregnant population.^{3,5} This comparison will be helpful to understand whether there are any systematic differences that affect the treatment outcomes, including the time to recrudescence. Our findings imply that treatment efficacy with the current dosing will be lower in pregnant women, but this needs to be confirmed. If the efficacy is consistently lower in pregnant women, this will affect policy on treatment for pregnant women.

Thirdly, the cut-off that defines hyperparasitaemia in pregnant women in terms of adverse pregnancy outcomes will be interesting to explore further. This cut-off has emerged mostly from evidence gained from studies of small children, but it is possible that changes would occur in mostly adult pregnant women in the presence of variable immunity and of the placenta. An IPD meta-analysis on severe malaria in pregnancy could also be considered.

Fourthly, the impact of treatment of vivax malaria needs further attention, as this disease affects more pregnant women, living in larger areas.⁷ Original research on vivax malaria in pregnancy is scarce^{58,101,480} and reports of more resistant strains of *P.vivax* are increasing in some areas.¹⁴¹

Besides the new study questions listed above, research on treatment efficacy in pregnancy should be continued alongside the continuous effort to accumulate the evidence on safety of antimalarials in pregnancy. To support and promote future studies on antimalarial treatment in pregnancy, a set of suggestions on the study design tailored for pregnant women

(Box 9-2 and 9-3) have been made. It must be emphasized again that precise and reliable estimation of gestational age is an essential component for evaluating the pregnancy outcomes among the suggestions. It is hoped that these will be helpful for other researchers who are planning to conduct further research on this understudied vulnerable population, and that the learning points are transferable to other infectious diseases that affect pregnant women in the tropics and in lower-resource settings.

Box 9-2. Recommendations for reporting antimalarial drug safety in pregnancy in efficacy studies²⁸⁸

Report the following:

Participants' background

- History of multiple miscarriages or stillbirths
- History of preterm birth (<37 weeks)
- History of low birthweight (<2,500 g)
- Chronic comorbidity (such as hypertension, diabetes mellitus, HIV and tuberculosis) depending on prevalence in the area
- Obstetric related morbidity e.g. pre-eclampsia, eclampsia, gestational diabetes
- Presence of common infections in pregnancy, and date of diagnosis and treatment e.g. syphilis, urinary tract infections, presence of soil transmitted helminths
- Suggested risk factors include smoking status, alcohol consumption, other local social drugs, history of non-malaria febrile illnesses, concomitant medication, consanguinity, nutritional status and socioeconomic status (marital status and educational status)

Haematology

- Haematinics (iron, folate) use: dose, date
- Haematological measurements: haemoglobin/haematocrit and date

Fetal loss

- Clearly define miscarriage and stillbirth
- Differentiate between intrapartum and antepartum stillbirth

(continued)

Box 9-2 continued.

Preterm birth

- Clearly define preterm birth using standard definitions (preferably WHO)
- Differentiate between spontaneous and iatrogenic preterm birth

Anthropometric assessment of infant

- Use standard methods for measurement of birthweight, birth length, head circumference and for quality control of these measurements
- Describe minimal precision of body weight/length scale
- Control anthropometric outcomes by gestational age and sex. International standard of anthropometric outcomes is available (e.g. INTERGROWTH-21st)
- Include normal singleton live births in summary anthropometric reporting
- Separate reporting and analysis of multiple pregnancy e.g. twins
- Record time interval between birth and anthropometric measurement

Congenital abnormality

- Report details of congenital abnormality applying systematic ICD coding
- Assess cardiac auscultation repeatedly

Growth and development

- Assess growth and development of child to one year (optional)
- Use standard referenced developmental assessment (optional)

Mortality

- Report both overall mortality and malaria-related mortality
- Report maternal death
- Define perinatal mortality, early neonatal mortality and neonatal mortality. e.g. WHO definition

Box 9-3. Recommendations to determine antimalarial efficacy in uncomplicated *P. falciparum* infection in pregnancy (beyond current WHO standards for non-pregnant patients)¹⁸⁹

Report the following:

Gestational age

- Gestational age in weeks
- Method of gestational age estimation and when it was obtained
- Proportion of pregnancies with different methods of gestational age estimation (optional)
- Quality control measures (desirable)

Parity and gravidity

- Parity and gravidity

Duration of follow-up

- Consider at least 63 days follow up for any ACTs. For ASAQ, ASMQ and DP, a longer follow-up might be needed
- Continue parasitological follow-up until delivery
- Record all episodes of *P. falciparum* and non-falciparum malaria

Other antimalarials

- Document type, date of administration and supervision (or self-administration) of IPTp
- Document type, date of administration and supervision (or self-administration) of cotrimoxazole
- In an RCT supervised treatment, treat parasite reappearance in each arm with same effective regimen that should be different to the primary treatment (and preferably given under supervision)

Placental malaria and congenital malaria

- Assess placental malaria and congenital malaria as part of assessment of efficacy (desirable)
- Assess PCR genotyping for placental and congenital malaria and compare with previous malaria infections during the pregnancy (desirable)

9.3 Concluding remarks

Pregnancy is typically a joyful and important life event, with significant global importance, towards optimizing health in the next generation. At the same time, women become very vulnerable during pregnancy. Pregnant women have been neglected in medical research, but they are different from any other neglected minority groups; pregnant women are the most important population for maintaining the human species. Improvement of clinical care in pregnancy has the potential to save both the mother and the fetus and provide optimal pregnancy and birth outcomes. Research on this important, vulnerable but understudied population will directly contribute to protecting our future.

The aetiology of adverse pregnancy outcomes is multifactorial and malaria infection in pregnancy, the theme of this thesis, is highly amenable to risk modification. Despite the major interest in malaria research globally, we do not know how to provide optimal treatment for malaria in pregnancy. It is only by clinical trials with follow-up specific to this patient group and pharmacokinetic studies that account for gestational effects, that the detailed and correct assessments for this unique problem may be identified. Research on malaria in pregnancy thus contributes to a general understanding of the complex nature of infectious diseases in pregnancy and disentangling the intertwined relationship between the effects of drugs and infection in pregnancy.

Pregnancy is not just a process ending at delivery but is a period of time that forms the basis of a life over decades after the delivery. The foundation on which we stand for the

rest of our lives, needs to be firm and solid. By providing a better foundation, research contributing to a better clinical care of pregnant women is a gift to our future. I would be honoured if this small project might contribute to the improvement of the health and well-being of pregnant women and to supporting future generations in the world.

References

1. World Health Organization. Trends in Maternal Mortality: 1990 to 2015. Estimates by WHO, UNICEF, UNFPA, World Bank Group and the United Nations Population Division. Geneva: WHO Press; 2015.
2. PLoS Medicine Editors. Drug development for maternal health cannot be left to the whims of the market. *PLoS Med.* 2008;5(6):e140.
3. White NJ, McGready RM, Nosten FH. New Medicines for Tropical Diseases in Pregnancy: Catch-22. *PLoS Med.* 2008;5(6):e133.
4. Fisk NM, Atun R. Market failure and the poverty of new drugs in maternal health. *PLoS Med.* 2008;5(1):e22.
5. Eke AC, Dooley KE, Sheffield JS. Pharmacologic Research in Pregnant Women — Time to Get It Right. *N Engl J Med.* 2019;380(14):1293-5.
6. World Health Organization. World Malaria Report 2018. Geneva: WHO Press; 2018.
7. Dellicour S, Tatem AJ, Guerra CA, Snow RW, ter Kuile FO. Quantifying the number of pregnancies at risk of malaria in 2007: a demographic study. *PLoS Med.* 2010;7(1):e1000221.
8. Nosten F, McGready R, Mutabingwa T. Case management of malaria in pregnancy. *Lancet Infect Dis.* 2007;7(2):118-25.
9. White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, et al. Malaria. *Lancet.* 2014;383(9918):723-35.
10. Desai M, ter Kuile FO, Nosten F, McGready R, Asamo K, et al. Epidemiology and burden of malaria in pregnancy. *Lancet Infect Dis.* 2007;7(2):93-104.
11. Moore KA, Simpson JA, Scoullar MJL, McGready R, Fowkes FJI. Quantification of the association between malaria in pregnancy and stillbirth: a systematic review and meta-analysis. *Lancet Glob Health.* 2017;5(11):e1101-e12.
12. Rijken MJ, McGready R, Boel ME, Poespoprodjo R, Singh N, et al. Malaria in pregnancy in the Asia-Pacific region. *Lancet Infect Dis.* 2012;12(1):75-88.
13. Cates JE, Unger HW, Briand V, Fievet N, Valea I, et al. Malaria, malnutrition, and birthweight: A meta-analysis using individual participant data. *PLoS Med.* 2017;14(8):e1002373.
14. Gething PW, Patil AP, Smith DL, Guerra CA, Elyazar IR, et al. A new world malaria map: *Plasmodium falciparum* endemicity in 2010. *Malar J.* 2011;10:378.
15. Kantele A, Jokiranta TS. Review of cases with the emerging fifth human malaria parasite, *Plasmodium knowlesi*. *Clin Infect Dis.* 2011;52(11):1356-62.
16. Lubis IND, Wijaya H, Lubis M, Lubis CP, Divis PCS, et al. Contribution of *Plasmodium knowlesi* to Multispecies Human Malaria Infections in North Sumatera, Indonesia. *J Infect Dis.* 2017;215(7):1148-55.
17. William T, Jelip J, Menon J, Anderios F, Mohammad R, et al. Changing epidemiology of malaria in Sabah, Malaysia: increasing incidence of *Plasmodium knowlesi*. *Malar J.* 2014;13:390.
18. Simpson JA, Aarons L, Collins WE, Jeffery GM, White NJ. Population dynamics of untreated *Plasmodium falciparum* malaria within the adult human host during the expansion phase of the infection. *Parasitology.* 2002;124:247-63.
19. Ashley EA, Pyae Phy A, Woodrow CJ. Malaria. *Lancet.* 2018;391(10130):1608-21.
20. Gardner MJ, Hall N, Fung E, White O, Berriman M, et al. Genome sequence of the human malaria parasite

- Plasmodium falciparum*. *Nature*. 2002;419(6906):498-511.
21. Chen Q, Fernandez V, Sundstrom A, Schlichtherle M, Datta S, et al. Developmental selection of var gene expression in *Plasmodium falciparum*. *Nature*. 1998;394(6691):392-5.
 22. Price RN, Simpson JA, Nosten F, Luxemburger C, Hkirjaroen L, et al. Factors contributing to anemia after uncomplicated falciparum malaria. *Am J Trop Med Hyg*. 2001;65(5):614-22.
 23. Buffet PA, Safeukui I, Deplaine G, Brousse V, Prendki V, et al. The pathogenesis of *Plasmodium falciparum* malaria in humans: insights from splenic physiology. *Blood*. 2011;117(2):381-92.
 24. Lamikanra AA, Brown D, Potocnik A, Casals-Pascual C, Langhorne J, et al. Malarial anemia: of mice and men. *Blood*. 2007;110(1):18-28.
 25. White NJ. Anaemia and malaria. *Malar J*. 2018;17:371.
 26. Rogerson SJ, Hviid L, Duffy PE, Leke RF, Taylor DW. Malaria in pregnancy: pathogenesis and immunity. *Lancet Infect Dis*. 2007;7(2):105-17.
 27. Brabin BJ, Romagosa C, Abdelgalil S, Menéndez C, Verhoeff FH, et al. The Sick Placenta—The Role of Malaria. *Placenta*. 2004;25(5):359-78.
 28. Rogerson SJ, Meshnick S. Malaria in pregnancy: late consequences of early infections. *J Infect Dis*. 2018;DOI: 10.1093/infdis/jiy738.
 29. Fried M, Kurtis JD, Swihart B, Pond-Tor S, Barry A, et al. Systemic Inflammatory Response to Malaria during Pregnancy Is Associated with Pregnancy Loss and Preterm Delivery. *Clin Infect Dis*. 2017;65(10):1729-35.
 30. Muehlenbachs A, Mutabingwa TK, Edmonds S, Fried M, Duffy PE. Hypertension and maternal-fetal conflict during placental malaria. *PLoS Med*. 2006;3(11):e446.
 31. Moore KA, Simpson JA, Wiladphaingern J, Min AM, Pimanpanarak M, et al. Influence of the number and timing of malaria episodes during pregnancy on prematurity and small-for-gestational-age in an area of low transmission. *BMC Med*. 2017;15:117.
 32. Moore KA, Simpson JA, Paw MK, Pimanpanarak M, Wiladphaingern J, et al. Safety of artemisinins in first trimester of prospectively followed pregnancies: an observational study. *Lancet Infect Dis*. 2016;16(5):576-83.
 33. Dellicour S, Sevene E, McGready R, Tinto H, Mosha D, et al. First-trimester artemisinin derivatives and quinine treatments and the risk of adverse pregnancy outcomes in Africa and Asia: A meta-analysis of observational studies. *PLoS Med*. 2017;14(5):e1002290.
 34. Bousema T, Drakeley C. Epidemiology and Infectivity of *Plasmodium falciparum* and *Plasmodium vivax* Gametocytes in Relation to Malaria Control and Elimination. *Clin Microbiol Rev*. 2011;24(2):377-410.
 35. White NJ. Determinants of relapse periodicity in *Plasmodium vivax* malaria. *Malar J*. 2011;10:297.
 36. Shanks GD, White NJ. The activation of vivax malaria hypnozoites by infectious diseases. *Lancet Infect Dis*. 2013;13(10):900-6.
 37. Barry A, Hansen D. Naturally acquired immunity to malaria. *Parasitology*. 2016;143(2):125-8.
 38. Doolan DL, Dobaño C, Baird JK. Acquired Immunity to Malaria. *Clin Microbiol Rev*. 2009;22(1):13-36.
 39. Pinkevych M, Petravic J, Chelimo K, Kazura JW, Moormann AM, et al. The Dynamics of Naturally Acquired Immunity to *Plasmodium falciparum* Infection. *PLoS Comput Biol*. 2012;8(10):e1002729.
 40. White NJ. Assessment of the pharmacodynamic properties of antimalarial drugs in vivo. *Antimicrob Agents*

- Chemother.* 1997;41(7):1413-22.
41. Marsh K, Snow RW. Host-parasite interaction and morbidity in malaria endemic areas. *Philos Trans R Soc Lond B Biol Sci.* 1997;352(1359):1385-94.
 42. Ataide R, Ashley EA, Powell R, Chan JA, Malloy MJ, et al. Host immunity to *Plasmodium falciparum* and the assessment of emerging artemisinin resistance in a multinational cohort. *Proc Natl Acad Sci U S A.* 2017;114(13):3515-20.
 43. Rogerson SJ, Wijesinghe RS, Meshnick SR. Host immunity as a determinant of treatment outcome in *Plasmodium falciparum* malaria. *Lancet Infect Dis.* 2010;10(1):51-9.
 44. Fowkes FJ, Boeuf P, Beeson JG. Immunity to malaria in an era of declining malaria transmission. *Parasitology.* 2016;143(2):139-53.
 45. Vleugels MP, Eling WM, Rolland R, de Graaf R. Cortisol and loss of malaria immunity in human pregnancy. *Br J Obstet Gynaecol.* 1987;94(8):758-64.
 46. Racicot K, Kwon JY, Aldo P, Silasi M, Mor G. Understanding the complexity of the immune system during pregnancy. *Am J Reprod Immunol.* 2014;72(2):107-16.
 47. Robinson DP, Klein SL. Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis. *Horm Behav.* 2012;62(3):263-71.
 48. Jamieson DJ, Theiler RN, Rasmussen SA. Emerging infections and pregnancy. *Emerg Infect Dis.* 2006;12(11):1638-43.
 49. Hviid L, Salanti A. VAR2CSA and protective immunity against pregnancy-associated *Plasmodium falciparum* malaria. *Parasitology.* 2007;134(Pt 13):1871-6.
 50. Atáide R, Mayor A, Rogerson SJ. Malaria, primigravidae, and antibodies: knowledge gained and future perspectives. *Trends Parasitol.* 2014;30(2):85-94.
 51. Fried M, Duffy PE. Adherence of *Plasmodium falciparum* to chondroitin sulfate A in the human placenta. *Science.* 1996;272(5267):1502-4.
 52. Fried M, Nosten F, Brockman A, Brabin BJ, Duffy PE. Maternal antibodies block malaria. *Nature.* 1998;395(6705):851-2.
 53. Feng G, Aitken E, Yosaatmadja F, Kalilani L, Meshnick SR, et al. Antibodies to variant surface antigens of *Plasmodium falciparum*-infected erythrocytes are associated with protection from treatment failure and the development of anemia in pregnancy. *J Infect Dis.* 2009;200(2):299-306.
 54. Ndam NT, Denoeud-Ndam L, Doritchamou J, Viwami F, Salanti A, et al. Protective Antibodies against Placental Malaria and Poor Outcomes during Pregnancy, Benin. *Emerg Infect Dis.* 2015;21(5):813-23.
 55. Salanti A, Dahlback M, Turner L, Nielsen MA, Barfod L, et al. Evidence for the involvement of VAR2CSA in pregnancy-associated malaria. *J Exp Med.* 2004;200(9):1197-203.
 56. Staalsoe T, Shulman CE, Bulmer JN, Kawuondo K, Marsh K, et al. Variant surface antigen-specific IgG and protection against clinical consequences of pregnancy-associated *Plasmodium falciparum* malaria. *Lancet.* 2004;363(9405):283-9.
 57. Mayor A, Bardaji A, Macete E, Nhampossa T, Fonseca AM, et al. Changing trends in *P. falciparum* burden, immunity, and disease in pregnancy. *N Engl J Med.* 2015;373:1607-17.
 58. ter Kuile FO, Rogerson SJ. *Plasmodium vivax* Infection during Pregnancy: An Important Problem in Need of New Solutions. *Clin Infect Dis.* 2008;46(9):1382-4.

59. McLean ARD, Stanisis D, McGready R, Chotivanich K, Clapham C, et al. *P. falciparum* infection and maternofetal antibody transfer in malaria-endemic settings of varying transmission. *PLoS One*. 2017;12(10):e0186577.
60. Harrington WE, Kakuru A, Jagannathan P. Malaria in pregnancy shapes the development of foetal and infant immunity. *Parasite Immunol*. 2019;41:e12573.
61. Jagannathan P. How does malaria in pregnancy impact malaria risk in infants? *BMC Med*. 2018;16(1):212.
62. Malhotra I, Dent A, Mungai P, Wamachi A, Ouma JH, et al. Can prenatal malaria exposure produce an immune tolerant phenotype? A prospective birth cohort study in Kenya. *PLoS Med*. 2009;6(7):e1000116.
63. Broen K, Brustoski K, Engelmann I, Luty AJ. Placental *Plasmodium falciparum* infection: causes and consequences of in utero sensitization to parasite antigens. *Mol Biochem Parasitol*. 2007;151(1):1-8.
64. Brabin B. Fetal anaemia in malarious areas: its causes and significance. *Ann Trop Paediatr*. 1992;12(3):303-10.
65. Natama HM, Rovira-Vallbona E, Sorgho H, Some MA, Traore-Coulibaly M, et al. Additional Screening and Treatment of Malaria During Pregnancy Provides Further Protection Against Malaria and Nonmalarial Fevers During the First Year of Life. *J Infect Dis*. 2018;217(12):1967-76.
66. De Beaudrap P, Turyakira E, Nabasumba C, Tumwebaze B, Piola P, et al. Timing of malaria in pregnancy and impact on infant growth and morbidity: a cohort study in Uganda. *Malar J*. 2016;15:92.
67. Bulterys PL, Chao A, Dalai SC, Zink MC, Dushimimana A, et al. Placental malaria and mother-to-child transmission of human immunodeficiency virus-1 in rural Rwanda. *Am J Trop Med Hyg*. 2011;85(2):202-6.
68. Longo S, Bollani L, Decembrino L, Di Comite A, Angelini M, et al. Short-term and long-term sequelae in intrauterine growth retardation (IUGR). *J Matern Fetal Neonatal Med*. 2013;26(3):222-5.
69. Katz J, Lee AC, Kozuki N, Lawn JE, Cousens S, et al. Mortality risk in preterm and small-for-gestational-age infants in low-income and middle-income countries: a pooled country analysis. *Lancet*. 2013;382(9890):417-25.
70. Nambozi M, Tinto H, Mwapasa V, Tagbor H, Kabuya J-BB, et al. Artemisinin-based combination therapy during pregnancy: outcome of pregnancy and infant mortality: a cohort study. *Malar J*. 2019;18:105.
71. Barker DJP. Adult Consequences of Fetal Growth Restriction. *Clin Obstet Gynecol*. 2006;49(2):270-83.
72. Skogen JC, Overland S. The fetal origins of adult disease: a narrative review of the epidemiological literature. *JRSM Short Rep*. 2012;3(8):59-.
73. von Beckerath A-K, Kollmann M, Rotky-Fast C, Karpf E, Lang U, et al. Perinatal complications and long-term neurodevelopmental outcome of infants with intrauterine growth restriction. *Am J Obstet Gynecol*. 2013;208(2):130.e1-6.
74. Arcangeli T, Thilaganathan B, Hooper R, Khan KS, Bhide A. Neurodevelopmental delay in small babies at term: a systematic review. *Ultrasound Obstet Gynecol*. 2012;40(3):267-75.
75. World Health Organization. Indoor residual spraying: An operational manual for IRS for malaria transmission, control and elimination. 2nd ed. Geneva: WHO Press; 2015.
76. Sinka ME, Bangs MJ, Manguin S, Rubio-Palis Y, Chareonviriyaphap T, et al. A global map of dominant malaria vectors. *Parasit Vectors*. 2012;5(1):69.
77. Kwansomboon N, Chaumeau V, Kittiphanakun P, Cerqueira D, Corbel V, et al. Vector bionomics and malaria transmission along the Thailand-Myanmar border: a baseline entomological survey. *J Vector Ecol*.

- 2017;42(1):84-93.
78. Parker DM, Carrara VI, Pukrittayakamee S, McGready R, Nosten FH. Malaria ecology along the Thailand-Myanmar border. *Malar J*. 2015;14:388.
 79. Trung HD, Bortel WV, Sochantha T, Keokenchanh K, Briet OJ, et al. Behavioural heterogeneity of *Anopheles* species in ecologically different localities in Southeast Asia: a challenge for vector control. *Trop Med Int Health*. 2005;10(3):251-62.
 80. Landier J, Parker DM, Thu AM, Lwin KM, Delmas G, et al. Effect of generalised access to early diagnosis and treatment and targeted mass drug administration on *Plasmodium falciparum* malaria in Eastern Myanmar: an observational study of a regional elimination programme. *Lancet*. 2018;391(10133):1916-26.
 81. Kaneko A, Taleo G, Kalkoa M, Yamar S, Kobayakawa T, et al. Malaria eradication on islands. *Lancet*. 2000;356(9241):1560-4.
 82. Poirot E, Skarbinski J, Sinclair D, Kachur SP, Slutsker L, et al. Mass drug administration for malaria. *Cochrane Database Syst Rev*. 2013;12(12):CD008846-CD.
 83. Coelho CH, Doritchamou JYA, Zaidi I, Duffy PE. Advances in malaria vaccine development: report from the 2017 malaria vaccine symposium. *NPJ Vaccines*. 2017;2(1):34.
 84. World Health Organization. A strategic framework for malaria prevention and control during pregnancy in the African region. Brazzaville: WHO Regional Office for Africa; 2004.
 85. World Health Organization. Implementing Malaria in Pregnancy Programs in the Context of World Health Organization Recommendations on Antenatal Care for a Positive Pregnancy Experience. Geneva: WHO Press; 2017.
 86. World Health Organization. WHO policy brief for the implementation of intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP). Geneva: WHO Press; 2013.
 87. Desai M, Hill J, Fernandes S, Walker P, Pell C, et al. Prevention of malaria in pregnancy. *Lancet Infect Dis*. 2018;18(4):e119-32.
 88. Moore BR, Davis TME. Pharmacotherapy for the prevention of malaria in pregnant women: currently available drugs and challenges. *Expert Opin Pharmacother*. 2018;1-18.
 89. Chico RM, Dellicour S, Roman E, Mangiaterra V, Coleman J, et al. Global Call to Action: maximize the public health impact of intermittent preventive treatment of malaria in pregnancy in sub-Saharan Africa. *Malar J*. 2015;14:207.
 90. Walker PGT, Floyd J, ter Kuile F, Cairns M. Estimated impact on birth weight of scaling up intermittent preventive treatment of malaria in pregnancy given sulphadoxine-pyrimethamine resistance in Africa: A mathematical model. *PLoS Med*. 2017;14(2):e1002243.
 91. Crawley J, Hill J, Yartey J, Robalo M, Serufulira A, et al. From evidence to action? Challenges to policy change and programme delivery for malaria in pregnancy. *Lancet Infect Dis*. 2007;7(2):145-55.
 92. Henry M, Florey L, Youll S, Gutman JR. An analysis of country adoption and implementation of the 2012 WHO recommendations for intermittent preventive treatment for pregnant women in sub-Saharan Africa. *Malar J*. 2018;17:364.
 93. Yaya S, Uthman OA, Amouzou A, Bishwajit G. Use of Intermittent Preventive Treatment among Pregnant Women in Sub-Saharan Africa: Evidence from Malaria Indicator Surveys. *Trop Med Infect Dis*. 2018;3(1):18.
 94. Huynh BT, Fievet N, Gbaguidi G, Dechavanne S, Borgella S, et al. Influence of the timing of malaria

- infection during pregnancy on birth weight and on maternal anemia in Benin. *Am J Trop Med Hyg.* 2011;85(2):214-20.
95. Huynh BT, Cottrell G, Cot M, Briand V. Burden of malaria in early pregnancy: a neglected problem? *Clin Infect Dis.* 2015;60(4):598-604.
 96. Brabin BJ. An analysis of malaria in pregnancy in Africa. *Bull World Health Organ.* 1983;61(6):1005-16.
 97. Accrombessi M, Yovo E, Fievet N, Cottrell G, Agbota G, et al. Effects of Malaria in the First Trimester of Pregnancy on Poor Maternal and Birth Outcomes in Benin. *Clin Infect Dis.* 2018;DOI: 10.1093/cid/ciy73.
 98. Moeller SL, Nyengaard JR, Larsen LG, Nielsen K, Bygbjerg IC, et al. Malaria in early pregnancy impedes the development of the placental vasculature. *J Infect Dis.* 2018;DOI: 10.1093/infdis/jiy735.
 99. Nosten F, ter Kuile F, Maelankirri L, Decludt B, White NJ. Malaria during pregnancy in an area of unstable endemicity. *Trans R Soc Trop Med Hyg.* 1991;85(4):424-9.
 100. Webster J, Ansariadi, Burdam FH, Landuwulang CUR, Bruce J, et al. Evaluation of the implementation of single screening and treatment for the control of malaria in pregnancy in Eastern Indonesia: a systems effectiveness analysis. *Malar J.* 2018;17:310.
 101. Yanow SK, Gavina K, Gnidehou S, Maestre A. Impact of Malaria in Pregnancy as Latin America Approaches Elimination. *Trends Parasitol.* 2016;32(5):416-27.
 102. Tuikue-Ndam N, Deloron P. Developing vaccines to prevent malaria in pregnant women. *Expert Opin Biol Ther.* 2015;15(8):1173-82.
 103. Doritchamou J, Bertin G, Moussiliou A, Bigey P, Viwami F, et al. First-trimester *Plasmodium falciparum* infections display a typical "placental" phenotype. *J Infect Dis.* 2012;206(12):1911-9.
 104. Desai M, Gutman J, Taylor SM, Wiegand RE, Khairallah C, et al. Impact of sulfadoxine-pyrimethamine resistance on effectiveness of intermittent preventive therapy for malaria in pregnancy at clearing infections and preventing low birth weight. *Clin Infect Dis.* 2016;62:323-33.
 105. Mockenhaupt FP, Bedu-Addo G, Eggelte TA, Hommerich L, Holmberg V, et al. Rapid Increase in the Prevalence of Sulfadoxine-Pyrimethamine Resistance among *Plasmodium falciparum* Isolated from Pregnant Women in Ghana. *J Infect Dis.* 2008;198(10):1545-9.
 106. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med.* 2009;361(5):455-67.
 107. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med.* 2014;371(5):411-23.
 108. Ranson H, Lissenden N. Insecticide Resistance in African *Anopheles* Mosquitoes: A Worsening Situation that Needs Urgent Action to Maintain Malaria Control. *Trends Parasitol.* 2016;32(3):187-96.
 109. Feighner BH, Pak SI, Novakoski WL, Kelsey LL, Strickman D. Reemergence of *Plasmodium vivax* malaria in the republic of Korea. *Emerg Infect Dis.* 1998;4(2):295-7.
 110. World Health Organization. World Malaria Report 2016. Geneva: WHO Press; 2016.
 111. van Eijk AM, Larsen DA, Kayentao K, Koshy G, Slaughter DEC, et al. Effect of *Plasmodium falciparum* sulfadoxine-pyrimethamine resistance on the effectiveness of intermittent preventive therapy for malaria in pregnancy in Africa: a systematic review and meta-analysis. *Lancet Infect Dis.* 2019;19(5):546-56.
 112. Nosten F, Rogerson SJ, Beeson JG, McGready R, Mutabingwa TK, et al. Malaria in pregnancy and the endemicity spectrum: what can we learn? *Trends Parasitol.* 2004;20(9):425-32.

113. Rogerson SJ, Unger HW. Prevention and control of malaria in pregnancy - new threats, new opportunities? *Expert Rev Anti Infect Ther.* 2017;15(4):361-75.
114. Nosten F, McGready R. Intermittent presumptive treatment in pregnancy with sulfadoxine–pyrimethamine: a counter perspective. *Malar J.* 2015;14:248.
115. Saito M, Gilder ME, McGready R, Nosten F. Antimalarial drugs for treating and preventing malaria in pregnant and lactating women. *Expert Opin Drug Saf.* 2018;17(11):1129-44.
116. White NJ. Intermittent presumptive treatment for malaria. *PLoS Med.* 2005;2(1):e3.
117. Unger HW, Hansa AP, Buffet C, Hasang W, Teo A, et al. Sulphadoxine-pyrimethamine plus azithromycin may improve birth outcomes through impacts on inflammation and placental angiogenesis independent of malarial infection. *Sci Rep.* 2019;9(1):2260.
118. Taylor WR, White NJ. Antimalarial drug toxicity: a review. *Drug Saf.* 2004;27(1):25-61.
119. White NJ. Qinghaosu (artemisinin): the price of success. *Science.* 2008;320(5874):330-4.
120. Foley M, Tilley L. Quinoline Antimalarials: Mechanisms of Action and Resistance and Prospects for New Agents. *Pharmacol Ther.* 1998;79(1):55-87.
121. Wernsdorfer WH. Epidemiology of drug resistance in malaria. *Acta Trop.* 1994;56(2):143-56.
122. D'Alessandro U, Buttiens H. History and importance of antimalarial drug resistance. *Trop Med Int Health.* 2001;6(11):845-8.
123. Roper C, Pearce R, Nair S, Sharp B, Nosten F, et al. Intercontinental spread of pyrimethamine-resistant malaria. *Science.* 2004;305(5687):1124.
124. Korenromp EL, Williams BG, Gouws E, Dye C, Snow RW. Measurement of trends in childhood malaria mortality in Africa: an assessment of progress toward targets based on verbal autopsy. *Lancet Infect Dis.* 2003;3(6):349-58.
125. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *Lancet.* 2006;367(9524):1747-57.
126. Nosten F, van Vugt M, Price R, Luxemburger C, Thway KL, et al. Effects of artesunate-mefloquine combination on incidence of *Plasmodium falciparum* malaria and mefloquine resistance in western Thailand: a prospective study. *Lancet.* 2000;356(9226):297-302.
127. Alker AP, Lim P, Sem R, Shah NK, Yi P, et al. Pfm-dr1 and in vivo resistance to artesunate-mefloquine in falciparum malaria on the Cambodian-Thai border. *Am J Trop Med Hyg.* 2007;76(4):641-7.
128. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, et al. Evidence of artemisinin-resistant malaria in western Cambodia. *N Engl J Med.* 2008;359(24):2619-20.
129. Denis MB, Tsuyuoka R, Lim P, Lindegardh N, Yi P, et al. Efficacy of artemether-lumefantrine for the treatment of uncomplicated falciparum malaria in northwest Cambodia. *Trop Med Int Health.* 2006;11(12):1800-7.
130. Denis MB, Tsuyuoka R, Poravuth Y, Narann TS, Seila S, et al. Surveillance of the efficacy of artesunate and mefloquine combination for the treatment of uncomplicated falciparum malaria in Cambodia. *Trop Med Int Health.* 2006;11(9):1360-6.
131. Saralamba S, Pan-Ngum W, Maude RJ, Lee SJ, Tarning J, et al. Intrahost modeling of artemisinin resistance in *Plasmodium falciparum*. *Proc Natl Acad Sci U S A.* 2011;108(1):397-402.
132. Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, et al. A molecular marker of artemisinin-

- resistant *Plasmodium falciparum* malaria. *Nature*. 2014;505(7481):50-5.
133. White NJ. Malaria: a molecular marker of artemisinin resistance. *Lancet*. 2014;383(9927):1439-40.
 134. Phyto AP, Ashley EA, Anderson TJC, Bozdech Z, Carrara VI, et al. Declining Efficacy of Artemisinin Combination Therapy Against *P. falciparum* Malaria on the Thai-Myanmar Border (2003-2013): The Role of Parasite Genetic Factors. *Clin Infect Dis*. 2016;63(6):784-91.
 135. Imwong M, Suwannasin K, Kunasol C, Sutawong K, Mayxay M, et al. The spread of artemisinin-resistant *Plasmodium falciparum* in the Greater Mekong subregion: a molecular epidemiology observational study. *Lancet Infect Dis*. 2017;17(5):491-7.
 136. WorldWide Antimalarial Resistance Network (WWARN). The Artemisinin Molecular Surveyor. 2018. [cited 5 July 2018]. Available from: <http://www.wwarn.org/molecular-surveyor-k13>.
 137. Phyto AP, Win KK, Thu AM, Swe LL, Htike H, et al. Poor response to artesunate treatment in two patients with severe malaria on the Thai-Myanmar border. *Malar J*. 2018;17:30.
 138. Pongvongsa T, Phommasone K, Adhikari B, Henriques G, Chotivanich K, et al. The dynamic of asymptomatic *Plasmodium falciparum* infections following mass drug administrations with dihydroartemisinin-piperaquine plus a single low dose of primaquine in Savannakhet Province, Laos. *Malar J*. 2018;17:405.
 139. Maude RJ, Socheat D, Nguon C, Saroth P, Dara P, et al. Optimising strategies for *Plasmodium falciparum* malaria elimination in Cambodia: primaquine, mass drug administration and artemisinin resistance. *PLoS One*. 2012;7(5):e37166.
 140. World Health Organization. Guidelines for the treatment of malaria. Third edition. Geneva: WHO Press; 2015.
 141. Price RN, von Seidlein L, Valecha N, Nosten F, Baird JK, et al. Global extent of chloroquine-resistant *Plasmodium vivax*: a systematic review and meta-analysis. *Lancet Infect Dis*. 2014;14(10):982-91.
 142. Silamut K, Phu NH, Whitty C, Turner GDH, Louwrier K, et al. A Quantitative Analysis of the Microvascular Sequestration of Malaria Parasites in the Human Brain. *Am J Pathol*. 1999;155(2):395-410.
 143. Price RN, Nosten F, Luxemburger C, ter Kuile FO, Paiphun L, et al. Effects of artemisinin derivatives on malaria transmissibility. *Lancet*. 1996;347(9016):1654-8.
 144. World Health Organization. Methods and techniques for clinical trials on antimalarial drug efficacy: Genotyping to identify parasite populations. Geneva: WHO Press; 2008.
 145. Clark RL. Embryotoxicity of the artemisinin antimalarials and potential consequences for use in women in the first trimester. *Reprod Toxicol*. 2009;28(3):285-96.
 146. Li Q, Weina PJ. Severe embryotoxicity of artemisinin derivatives in experimental animals, but possibly safe in pregnant women. *Molecules*. 2010;15(1):40-57.
 147. White TE, Clark RL. Sensitive periods for developmental toxicity of orally administered artesunate in the rat. *Birth Defects Res B Dev Reprod Toxicol*. 2008;83(4):407-17.
 148. Clark RL, White TE, S AC, Gaunt I, Winstanley P, et al. Developmental toxicity of artesunate and an artesunate combination in the rat and rabbit. *Birth Defects Res B Dev Reprod Toxicol*. 2004;71(6):380-94.
 149. Clark RL, Arima A, Makori N, Nakata Y, Bernard F, et al. Artesunate: developmental toxicity and toxicokinetics in monkeys. *Birth Defects Res B Dev Reprod Toxicol*. 2008;83(4):418-34.
 150. Ward SA, Sevene EJ, Hastings IM, Nosten F, McGready R. Antimalarial drugs and pregnancy: safety,

- pharmacokinetics, and pharmacovigilance. *Lancet Infect Dis*. 2007;7(2):136-44.
151. Nosten F, McGready R, d'Alessandro U, Bonell A, Verhoeff F, et al. Antimalarial drugs in pregnancy: a review. *Curr Drug Saf*. 2006;1(1):1-15.
 152. Nosten F, ter Kuile F, Thwai KL, Maelankirri L, White NJ. Spiramycin does not potentiate quinine treatment of falciparum malaria in pregnancy. *Trans R Soc Trop Med Hyg*. 1993;87(3):305-6.
 153. Mosha D, Mazuguni F, Mrema S, Sevene E, Abdulla S, et al. Safety of artemether-lumefantrine exposure in first trimester of pregnancy: an observational cohort. *Malar J*. 2014;13:197.
 154. McGready R, Lee SJ, Wiladphaingern J, Ashley EA, Rijken MJ, et al. Adverse effects of falciparum and vivax malaria and the safety of antimalarial treatment in early pregnancy: a population-based study. *Lancet Infect Dis*. 2012;12(5):388-96.
 155. Dellicour S, Desai M, Aol G, Oneko M, Ouma P, et al. Risks of miscarriage and inadvertent exposure to artemisinin derivatives in the first trimester of pregnancy: a prospective cohort study in western Kenya. *Malar J*. 2015;14:461.
 156. Ballard S, Salinger A, Arguin PM, Desai M, KR. T. Updated CDC Recommendations for Using Artemether-Lumefantrine for the Treatment of Uncomplicated Malaria in Pregnant Women in the United States. *MMWR Morb Mortal Wkly Rep*. 2018;67:424-31.
 157. WHO Malaria Policy Advisory Committee Secretariat. Malaria Policy Advisory Committee to the WHO: conclusions and recommendations of eighth biannual meeting (September 2015). *Malar J*. 2016;15:117.
 158. Costantine MM. Physiologic and pharmacokinetic changes in pregnancy. *Front Pharmacol*. 2014;5:65.
 159. Pariente G, Leibson T, Carls A, Adams-Webber T, Ito S, et al. Pregnancy-Associated Changes in Pharmacokinetics: A Systematic Review. *PLoS Med*. 2016;13(11):e1002160.
 160. Riley RD, Lambert PC, Abo-Zaid G. Meta-analysis of individual participant data: rationale, conduct, and reporting. *BMJ*. 2010;340:c221.
 161. Tierney JF, Vale C, Riley R, Smith CT, Stewart L, et al. Individual Participant Data (IPD) Meta-analyses of Randomised Controlled Trials: Guidance on Their Use. *PLoS Med*. 2015;12(7):e1001855.
 162. Lambert PC, Sutton AJ, Abrams KR, Jones DR. A comparison of summary patient-level covariates in meta-regression with individual patient data meta-analysis. *J Clin Epidemiol*. 2002;55(1):86-94.
 163. WorldWide Antimalarial Resistance Network (WWARN). Malaria in pregnancy treatment efficacy study group. 2016. [cited 5 July 2018]. Available from: <http://www.wwarn.org/working-together/study-groups/malaria-pregnancy-treatment-efficacy-study-group>.
 164. WorldWide Antimalarial Resistance Network (WWARN). Terms of Submission. 2017. [cited 5 July 2018]. Available from: <http://www.wwarn.org/tools-resources/terms-submission>.
 165. Villar J, Cheikh Ismail L, Victora CG, Ohuma EO, Bertino E, et al. International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. *Lancet*. 2014;384(9946):857-68.
 166. WorldWide Antimalarial Resistance Network (WWARN). Clinical Module: Data Management and Statistical Analysis Plan Version 1.2. 2012. [cited 5 July 2018]. Available from: <http://www.wwarn.org/sites/default/files/ClinicalDMSAP.pdf>.
 167. Stewart GB, Altman DG, Askie LM, Duley L, Simmonds MC, et al. Statistical Analysis of Individual Participant Data Meta-Analyses: A Comparison of Methods and Recommendations for Practice. *PLoS One*.

- 2012;7(10):e46042.
168. Fisher DJ, Copas AJ, Tierney JF, Parmar MK. A critical review of methods for the assessment of patient-level interactions in individual participant data meta-analysis of randomized trials, and guidance for practitioners. *J Clin Epidemiol.* 2011;64(9):949-67.
 169. Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, et al. GRADE guidelines: 1. Introduction—GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol.* 2011;64(4):383-94.
 170. Carrara VI, Lwin KM, Phyo AP, Ashley E, Wiladphaingern J, et al. Malaria burden and artemisinin resistance in the mobile and migrant population on the Thai-Myanmar border, 1999-2011: an observational study. *PLoS Med.* 2013;10(3):e1001398.
 171. Carrara VI, Sirilak S, Thonglairuam J, Rojanawatsirivet C, Proux S, et al. Deployment of early diagnosis and mefloquine-artesunate treatment of falciparum malaria in Thailand: the Tak Malaria Initiative. *PLoS Med.* 2006;3(6):e183.
 172. Villegas L, McGready R, Htway M, Paw MK, Pimanpanarak M, et al. Chloroquine prophylaxis against vivax malaria in pregnancy: a randomized, double-blind, placebo-controlled trial. *Trop Med Int Health.* 2007;12(2):209-18.
 173. Hurwitz E, Johnson D, Campbell C. Resistance of *Plasmodium falciparum* malaria to sulfadoxine-pyrimethamine ('Fansidar') in a refugee camp in Thailand. *Lancet.* 1981;317(8229):1068-70.
 174. World Health Organization. Methods for surveillance of antimalarial drug efficacy. Geneva: WHO Press; 2009.
 175. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, et al. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature.* 2015;526(7572):207-11.
 176. World Health Organization. WHO Technical Consultation on Postpartum and Postnatal Care. Geneva: WHO Press; 2010.
 177. Blencowe H, Cousens S, Jassir FB, Say L, Chou D, et al. National, regional, and worldwide estimates of stillbirth rates in 2015, with trends from 2000: a systematic analysis. *Lancet Glob Health.* 2016;4(2):e98-e108.
 178. Lawn JE, Blencowe H, Waiswa P, Amouzou A, Mathers C, et al. Stillbirths: rates, risk factors, and acceleration towards 2030. *Lancet.* 2016;387(10018):587-603.
 179. World Health Organization. International statistical classification of diseases and related health problems. 11th revision. Geneva: WHO Press; 2018.
 180. World Health Organization. WHO recommendations on interventions to improve preterm birth outcomes. Geneva: WHO Press; 2015.
 181. WHO Expert Committee on Physical Status. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. *World Health Organ Tech Rep Ser.* 1995;854:1-452.
 182. Schlaudecker EP, Munoz FM, Bardají A, Boghossian NS, Khalil A, et al. Small for gestational age: Case definition & guidelines for data collection, analysis, and presentation of maternal immunisation safety data. *Vaccine.* 2017;35:6518-28.
 183. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Geneva: WHO Press; 2011.
 184. Rogerson SJ, Desai M, Mayor A, Sicuri E, Taylor SM, et al. Burden, pathology, and costs of malaria in

- pregnancy: new developments for an old problem. *Lancet Infect Dis*. 2018;18(4):e107-e18.
185. Gomes C, Boareto AC, Dalsenter PR. Clinical and non-clinical safety of artemisinin derivatives in pregnancy. *Reprod Toxicol*. 2016;65:194-203.
 186. Kloprogge F, Jullien V, Piola P, Dhorda M, Muwanga S, et al. Population pharmacokinetics of quinine in pregnant women with uncomplicated *Plasmodium falciparum* malaria in Uganda. *J Antimicrob Chemother*. 2014;69(11):3033-40.
 187. Tarning J, Kloprogge F, Dhorda M, Jullien V, Nosten F, et al. Pharmacokinetic properties of artemether, dihydroartemisinin, lumefantrine, and quinine in pregnant women with uncomplicated *Plasmodium falciparum* malaria in Uganda. *Antimicrob Agents Chemother*. 2013;57(10):5096-103.
 188. McGready R, Cho T, Samuel, Villegas L, Brockman A, et al. Randomized comparison of quinine-clindamycin versus artesunate in the treatment of falciparum malaria in pregnancy. *Trans R Soc Trop Med Hyg*. 2001;95(6):651-6.
 189. Saito M, Gilder ME, Nosten F, McGready R, Guérin PJ. Systematic literature review and meta-analysis of the efficacy of artemisinin-based and quinine-based treatments for uncomplicated falciparum malaria in pregnancy: methodological challenges. *Malar J*. 2017;16:488.
 190. McGready R, Brockman A, Cho T, Cho D, van Vugt M, et al. Randomized comparison of mefloquine-artesunate versus quinine in the treatment of multidrug-resistant falciparum malaria in pregnancy. *Trans R Soc Trop Med Hyg*. 2000;94(6):689-93.
 191. McGready R, Thwai KL, Cho T, Samuel, Looareesuwan S, et al. The effects of quinine and chloroquine antimalarial treatments in the first trimester of pregnancy. *Trans R Soc Trop Med Hyg*. 2002;96(2):180-4.
 192. Piola P, Nabasumba C, Turyakira E, Dhorda M, Lindegardh N, et al. Efficacy and safety of artemether-lumefantrine compared with quinine in pregnant women with uncomplicated *Plasmodium falciparum* malaria: an open-label, randomised, non-inferiority trial. *Lancet Infect Dis*. 2010;10(11):762-9.
 193. Adam I, Ibrahim MH, A/elbasit IA, Elbashir MI. Low-dose quinine for treatment of chloroquine-resistant falciparum malaria in Sudanese pregnant women. *East Mediterr Health J*. 2004;10(4/5):554-9.
 194. McGready R, Ashley EA, Moo E, Cho T, Barends M, et al. A randomized comparison of artesunate-atovaquone-proguanil versus quinine in treatment for uncomplicated falciparum malaria during pregnancy. *J Infect Dis*. 2005;192(5):846-53.
 195. McGready R, Nosten F. The Thai-Burmese border: drug studies of *Plasmodium falciparum* in pregnancy. *Ann Trop Med Parasitol*. 1999;93 Suppl 1:S19-23.
 196. Looareesuwan S, Phillips RE, White NJ, Kietinun S, Karbwang J, et al. Quinine and severe falciparum malaria in late pregnancy. *Lancet*. 1985;2(8445):4-8.
 197. White NJ, Warrell DA, Chanthavanich P, Looareesuwan S, Warrell MJ, et al. Severe Hypoglycemia and Hyperinsulinemia in Falciparum Malaria. *N Engl J Med*. 1983;309(2):61-6.
 198. Bounyasong S. Randomized trial of artesunate and mefloquine in comparison with quinine sulfate to treat *P. falciparum* malaria pregnant women. *J Med Assoc Thai*. 2001;84(9):1289-99.
 199. Clark RL. Animal Embryotoxicity Studies of Key Non-Artemisinin Antimalarials and Use in Women in the First Trimester. *Birth Defects Res*. 2017;109(14):1075-126.
 200. Manyando C, Mkandawire R, Puma L, Sinkala M, Mpabalwani E, et al. Safety of artemether-lumefantrine in pregnant women with malaria: results of a prospective cohort study in Zambia. *Malar J*.

- 2010;9:249.
201. Rulisa S, Kaligirwa N, Agaba S, Karema C, Mens PF, et al. Pharmacovigilance of artemether-lumefantrine in pregnant women followed until delivery in Rwanda. *Malar J.* 2012;11:225.
 202. Tinto H, Sevene E, Dellicour S, Calip GS, d'Alessandro U, et al. Assessment of the safety of antimalarial drug use during early pregnancy (ASAP): protocol for a multicenter prospective cohort study in Burkina Faso, Kenya and Mozambique. *Reprod Health.* 2015;12:112.
 203. Manyando C, Njunju EM, Virtanen M, Hamed K, Gomes M, et al. Exposure to artemether-lumefantrine (Coartem®) in first trimester pregnancy in an observational study in Zambia. *Malar J.* 2015;14:77.
 204. Poespoprodjo JR, Fobia W, Kenangalem E, Lampah DA, Sugiarto P, et al. Dihydroartemisinin-piperaquine treatment of multidrug resistant falciparum and vivax malaria in pregnancy. *PLoS One.* 2014;9(1):e84976.
 205. Adam I, Idris HM, Elbashir MI. Quinine for chloroquine-resistant falciparum malaria in pregnant Sudanese women in the first trimester. *East Mediterr Health J.* 2004;10(4/5):560-5.
 206. Briggs GG, Freeman RK, Towers CV, Forinash AB. *Drugs in Pregnancy and Lactation.* 11th Edition ed: Wolters Kluwer Health; 2016.
 207. Kovacs SD, van Eijk AM, Sevene E, Dellicour S, Weiss NS, et al. The Safety of Artemisinin Derivatives for the Treatment of Malaria in the 2nd or 3rd Trimester of Pregnancy: A Systematic Review and Meta-Analysis. *PLoS One.* 2016;11(11):e0164963.
 208. McGready R, Stepniewska K, Lindegardh N, Ashley EA, La Y, et al. The pharmacokinetics of artemether and lumefantrine in pregnant women with uncomplicated falciparum malaria. *Eur J Clin Pharmacol.* 2006;62(12):1021-31.
 209. McGready R, Stepniewska K, Ward SA, Cho T, Gilveray G, et al. Pharmacokinetics of dihydroartemisinin following oral artesunate treatment of pregnant women with acute uncomplicated falciparum malaria. *Eur J Clin Pharmacol.* 2006;62(5):367-71.
 210. Klopogge F, McGready R, Phyto AP, Rijken MJ, Hanpithakpon W, et al. Opposite malaria and pregnancy effect on oral bioavailability of artesunate—a population pharmacokinetic evaluation. *Br J Clin Pharmacol.* 2015;80(4):642-53.
 211. McGready R, Phyto AP, Rijken MJ, Tarning J, Lindegardh N, et al. Artesunate/dihydroartemisinin pharmacokinetics in acute falciparum malaria in pregnancy: absorption, bioavailability, disposition and disease effects. *Br J Clin Pharmacol.* 2012;73(3):467-77.
 212. Morris CA, Onyamboko MA, Capparelli E, Koch MA, Atibu J, et al. Population pharmacokinetics of artesunate and dihydroartemisinin in pregnant and non-pregnant women with malaria. *Malar J.* 2011;10:114.
 213. Onyamboko MA, Meshnick SR, Fleckenstein L, Koch MA, Atibu J, et al. Pharmacokinetics and pharmacodynamics of artesunate and dihydroartemisinin following oral treatment in pregnant women with asymptomatic *Plasmodium falciparum* infections in Kinshasa DRC. *Malar J.* 2011;10:49.
 214. Rijken MJ, McGready R, Phyto AP, Lindegardh N, Tarning J, et al. Pharmacokinetics of dihydroartemisinin and piperaquine in pregnant and nonpregnant women with uncomplicated falciparum malaria. *Antimicrob Agents Chemother.* 2011;55(12):5500-6.
 215. Tarning J, Rijken MJ, McGready R, Phyto AP, Hanpithakpong W, et al. Population pharmacokinetics of dihydroartemisinin and piperaquine in pregnant and nonpregnant women with uncomplicated malaria.

- Antimicrob Agents Chemother.* 2012;56(4):1997-2007.
216. Rehman K, Lötsch F, Kremsner PG, Ramharter M. Haemolysis associated with the treatment of malaria with artemisinin derivatives: a systematic review of current evidence. *Int J Infect Dis.* 2014;29:268-73.
 217. Leonardi E, Gilvary G, White NJ, Nosten F. Severe allergic reactions to oral artesunate: a report of two cases. *Trans R Soc Trop Med Hyg.* 2001;95(2):182-3.
 218. McGready R, Cho T, Keo NK, Thwai KL, Villegas L, et al. Artemisinin antimalarials in pregnancy: a prospective treatment study of 539 episodes of multidrug-resistant *Plasmodium falciparum*. *Clin Infect Dis.* 2001;33(12):2009-16.
 219. McGready R, Tan SO, Ashley EA, Pimanpanarak M, Viladpai-Nguen J, et al. A randomised controlled trial of artemether-lumefantrine versus artesunate for uncomplicated *Plasmodium falciparum* treatment in pregnancy. *PLoS Med.* 2008;5(12):e253.
 220. Adam I, Elhassan EM, Omer EM, Abdulla MA, Mahgoub HM, et al. Safety of artemisinins during early pregnancy, assessed in 62 Sudanese women. *Ann Trop Med Parasitol.* 2009;103(3):205-10.
 221. Adam I, Elwasila E, Mohammed Ali DA, Elansari E, Elbashir MI. Artemether in the treatment of falciparum malaria during pregnancy in eastern Sudan. *Trans R Soc Trop Med Hyg.* 2004;98(9):509-13.
 222. Deen JL, von Seidlein L, Pinder M, Walraven GE, Greenwood BM. The safety of the combination artesunate and pyrimethamine-sulfadoxine given during pregnancy. *Trans R Soc Trop Med Hyg.* 2001;95(4):424-8.
 223. Willcox ML, Burton S, Oyweka R, Namyalo R, Challand S, et al. Evaluation and pharmacovigilance of projects promoting cultivation and local use of *Artemisia annua* for malaria. *Malar J.* 2011;10:84.
 224. Rouamba T, Kpoda H, Valea I, Mens PF, Gomes M, et al. Safety of antimalarial drug use during early pregnancy in Bobo Dioulasso: Examining low birth weight and congenital malformations as potential adverse outcomes. *Trop Med Int Health.* 2017;22 (Supplement 1):95.
 225. Manirakiza A, Soula G, Laganier R, Klement E, Djalle D, et al. Pattern of the Antimalarials Prescription during Pregnancy in Bangui, Central African Republic. *Malar Res Treat.* 2011;2011:414510.
 226. Sangaré LR, Weiss NS, Brentlinger PE, Richardson BA, Staedke SG, et al. Patterns of anti-malarial drug treatment among pregnant women in Uganda. *Malar J.* 2011;10:152.
 227. Kloprogge F, McGready R, Hanpithakpong W, Blessborn D, Day NPJ, et al. Lumefantrine and Desbutyl-Lumefantrine Population Pharmacokinetic-Pharmacodynamic Relationships in Pregnant Women with Uncomplicated *Plasmodium falciparum* Malaria on the Thailand-Myanmar Border. *Antimicrob Agents Chemother.* 2015;59(10):6375-84.
 228. Kloprogge F, Piola P, Dhorda M, Muwanga S, Turyakira E, et al. Population pharmacokinetics of lumefantrine in pregnant and nonpregnant women with uncomplicated *Plasmodium falciparum* malaria in Uganda. *CPT Pharmacometrics Syst Pharmacol.* 2013;2(11):e83.
 229. Tarning J, McGready R, Lindegardh N, Ashley EA, Pimanpanarak M, et al. Population pharmacokinetics of lumefantrine in pregnant women treated with artemether-lumefantrine for uncomplicated *Plasmodium falciparum* malaria. *Antimicrob Agents Chemother.* 2009;53(9):3837-46.
 230. Mutagonda RF, Kamuhabwa AAR, Minzi OMS, Massawe SN, Maganda BA, et al. Malaria prevalence, severity and treatment outcome in relation to day 7 lumefantrine plasma concentration in pregnant women. *Malar J.* 2016;15:278.

231. Mutagonda RF, Kamuhabwa AAR, Minzi OMS, Massawe SN, Asghar M, et al. Effect of pharmacogenetics on plasma lumefantrine pharmacokinetics and malaria treatment outcome in pregnant women. *Malar J.* 2017;16:267.
232. Mosha D, Guidi M, Mwingira F, Abdulla S, Mercier T, et al. Population pharmacokinetics and clinical response for artemether-lumefantrine in pregnant and nonpregnant women with uncomplicated *Plasmodium falciparum* malaria in Tanzania. *Antimicrob Agents Chemother.* 2014;58(8):4583-92.
233. Nyunt MM, Nguyen VK, Kajubi R, Huang L, Ssebuliba J, et al. Artemether-lumefantrine pharmacokinetics and clinical response are minimally altered in pregnant Ugandan women treated for uncomplicated falciparum malaria. *Antimicrob Agents Chemother.* 2016;60(3):1274-82.
234. Kloprogge F, Workman L, Borrmann S, Tekete M, Lefevre G, et al. Artemether-lumefantrine dosing for malaria treatment in young children and pregnant women: A pharmacokinetic-pharmacodynamic meta-analysis. *PLoS Med.* 2018;15(6):e1002579.
235. Onyamboko MA, Fanello CI, Turner G, Jackson N, Tarning J, et al. Comparison of two regimens of artemether-lumefantrine for the treatment of uncomplicated *Plasmodium falciparum* malaria in pregnant women in the Democratic Republic of Congo. *Am J Trop Med Hyg.* 2015;93(4 Supplement):6.
236. McGready R. Randomised Trial of 3 Artemisinin Combination Therapy for Malaria in Pregnancy (NCT01054248). 2010. [cited 30 April 2019]. Available from: <https://clinicaltrials.gov/ct2/show/NCT01054248>.
237. PREGACT Study Group, Pekyi D, Ampromfi AA, Tinto H, Traoré-Coulibaly M, et al. Four artemisinin-based treatments in African pregnant women with malaria. *N Engl J Med.* 2016;374(10):913-27.
238. Iribhogbe OI, Emmanuel I, Odianosen M. Comparative analysis of the safety and tolerability of fixed-dose artesunate/amodiaquine versus artemether/lumefantrine combinations for uncomplicated falciparum malaria in pregnancy: a randomized open label study. *Clin Pharmacol.* 2017;9:45-54.
239. Kaye DK, Nshemerirwe R, Mutyaba TS, Ndeezi G. A randomized clinical trial comparing safety, clinical and parasitological response to artemether-lumefantrine and chlorproguanil-dapsone in treatment of uncomplicated malaria in pregnancy in Mulago hospital, Uganda. *J Infect Dev Ctries.* 2008;2(2):135-9.
240. Rijken MJ, McGready R, Jullien V, Tarning J, Lindegardh N, et al. Pharmacokinetics of amodiaquine and desethylamodiaquine in pregnant and postpartum women with *Plasmodium vivax* malaria. *Antimicrob Agents Chemother.* 2011;55(9):4338-42.
241. Tarning J, Chotsiri P, Jullien V, Rijken MJ, Bergstrand M, et al. Population pharmacokinetic and pharmacodynamic modeling of amodiaquine and desethylamodiaquine in women with *Plasmodium vivax* malaria during and after pregnancy. *Antimicrob Agents Chemother.* 2012;56(11):5764-73.
242. Ukah M, Badejoko O, Ogunniyi S, Loto O, Aboderin O, et al. A randomized trial of artesunate-amodiaquine versus artemether-lumefantrine for the treatment of acute uncomplicated malaria in pregnancy. *Int J Gynaecol Obstet.* 2015;131(1):41-4.
243. Osarfo J, Tagbor H, Cairns M, Alifrangis M, Magnussen P. Dihydroartemisinin-piperaquine versus artesunate-amodiaquine for treatment of malaria infection in pregnancy in Ghana: an open-label, randomised, non-inferiority trial. *Trop Med Int Health.* 2017;22(8):1043-52.
244. Tagbor H, Bruce J, Browne E, Randal A, Greenwood B, et al. Efficacy, safety, and tolerability of amodiaquine plus sulphadoxine-pyrimethamine used alone or in combination for malaria treatment in

- pregnancy: a randomised trial. *Lancet*. 2006;368(9544):1349-56.
245. Clerk CA, Bruce J, Affipunguh PK, Mensah N, Hodgson A, et al. A randomized, controlled trial of intermittent preventive treatment with sulfadoxine-pyrimethamine, amodiaquine, or the combination in pregnant women in Ghana. *J Infect Dis*. 2008;198(8):1202-11.
 246. Ndiaye JL, Ndiaye A, Faye B, Ba M, Tine R, et al. Open-label in vivo drug study to evaluate the safety and efficacy of artesunate plus amodiaquine combination in pregnant women with uncomplicated *P. falciparum* malaria in Senegal. *Trop Med Int Health*. 2011;16(Supplement 1):140.
 247. Mutabingwa TK, Muze K, Ord R, Briceño M, Greenwood BM, et al. Randomized trial of artesunate+amodiaquine, sulfadoxine-pyrimethamine+amodiaquine, chlorproguanil-dapsone and SP for malaria in pregnancy in Tanzania. *PLoS One*. 2009;4(4):e5138.
 248. Iribhogbe OI, Igue EO, Odianosen M. Assessment of the safety of non-fixed-dose combination of artesunate and amodiaquine for uncomplicated falciparum malaria in pregnancy: a nonrandomized open-label study. *J Pharm Health Serv Res*. 2017;8(1):31-8.
 249. Burger RJ, Visser BJ, Grobusch MP, van Vugt M. The influence of pregnancy on the pharmacokinetic properties of artemisinin combination therapy (ACT): a systematic review. *Malar J*. 2016;15:99.
 250. Nosten F, Karbwang J, White NJ, Honeymoon, Na Bangchang K, et al. Mefloquine antimalarial prophylaxis in pregnancy: dose finding and pharmacokinetic study. *Br J Clin Pharmacol*. 1990;30(1):79-85.
 251. Na Bangchang K, Davis TME, Looareesuwan S, White NJJ, Bunnag D, et al. Mefloquine pharmacokinetics in pregnant women with acute falciparum malaria. *Trans R Soc Trop Med Hyg*. 1994;88(3):321-3.
 252. Valea I, Tinto H, Traore-Coulibaly M, Toe LC, Lindegardh N, et al. Pharmacokinetics of co-formulated mefloquine and artesunate in pregnant and non-pregnant women with uncomplicated *Plasmodium falciparum* infection in Burkina Faso. *J Antimicrob Chemother*. 2014;69(9):2499-507.
 253. McGready R, Cho T, Hkirijsaroen L, Simpson J, Chongsuphajaisiddhi T, et al. Quinine and mefloquine in the treatment of multidrug-resistant *Plasmodium falciparum* malaria in pregnancy. *Ann Trop Med Parasitol*. 1998;92(6):643-53.
 254. Adam I, Ali DA, Alwaseila A, Kheir MM, Elbashir MI, et al. Mefloquine in the treatment of falciparum malaria during pregnancy in Eastern Sudan. *Saudi Med J*. 2004;25(10):1400-2.
 255. Sowunmi A, Oduola AM, Ogundahunsi OA, Fehintola FA, Ilesanmi OA, et al. Randomised trial of artemether versus artemether and mefloquine for the treatment of chloroquine/sulfadoxine-pyrimethamine-resistant falciparum malaria during pregnancy. *J Obstet Gynaecol*. 1998;18(4):322-7.
 256. Carmona-Fonseca J, Agudelo-García OM, Arango-Flórez E. [Therapeutic efficacy and adverse events of treatments for vivax and falciparum malaria in pregnant women in the regions of Uraba and Alto San Jorge, Colombia, 2008-2011] (in Spanish). *Rev Colomb Obstet Ginecol*. 2013;64(1):27-37.
 257. Anvikar AR, Kuepfer I, Mishra V, Bruce J, Arya T, et al. Efficacy of two artemisinin-based combinations for the treatment of malaria in pregnancy in India: a randomized controlled trial. *Malar J*. 2018;17:246.
 258. Nosten F, ter Kuile F, Maelankiri L, Chongsuphajaisiddhi T, Nopdonrattakoon L, et al. Mefloquine prophylaxis prevents malaria during pregnancy: a double-blind, placebo-controlled study. *J Infect Dis*. 1994;169(3):595-603.
 259. Smoak BL, Writer JV, Keep LW, Cowan J, Chantelois JL. The effects of inadvertent exposure of

- mefloquine chemoprophylaxis on pregnancy outcomes and infants of US Army servicewomen. *J Infect Dis.* 1997;176(3):831-3.
260. Phillips-Howard PA, Steffen R, Kerr L, Vanhauwere B, Schildknecht J, et al. Safety of mefloquine and other antimalarial agents in the first trimester of pregnancy. *J Travel Med.* 1998;5(3):121-6.
 261. Schlagenhauf P, Blumentals WA, Suter P, Regep L, Vital-Durand G, et al. Pregnancy and fetal outcomes after exposure to mefloquine in the pre- and periconception period and during pregnancy. *Clin Infect Dis.* 2012;54(11):e124-31.
 262. Vanhauwere B, Maradit H, Kerr L. Post-marketing surveillance of prophylactic mefloquine (Lariam) use in pregnancy. *Am J Trop Med Hyg.* 1998;58(1):17-21.
 263. Harinasuta T, Kietinun S, Somlaw SB, Somlaw SP, Bunnag D, et al. A clinical trial of mefloquine on multi-resistant falciparum malaria in pregnant women in Thailand. *Bulletin de la Societe Francaise de Parasitologie.* 1990:419.
 264. Sowunmi A, Ilesanmi AO, Oduola AMJ, Omitowoju GUO, Ojengbede OA, et al. Efficacy of mefloquine in uncomplicated chloroquine-resistant falciparum malaria during pregnancy. *J Obstet Gynaecol.* 1996;16(5):362-3.
 265. Hoglund RM, Adam I, Hanpithakpong W, Ashton M, Lindegardh N, et al. A population pharmacokinetic model of piperaquine in pregnant and non-pregnant women with uncomplicated *Plasmodium falciparum* malaria in Sudan. *Malar J.* 2012;11:398.
 266. Adam I, Tarning J, Lindegardh N, Mahgoub H, McGready R, et al. Pharmacokinetics of piperaquine in pregnant women in Sudan with uncomplicated *Plasmodium falciparum* malaria. *Am J Trop Med Hyg.* 2012;87(1):35-40.
 267. Benjamin JM, Moore BR, Salman S, Page-Sharp M, Tawat S, et al. Population pharmacokinetics, tolerability, and safety of dihydroartemisinin-piperaquine and sulfadoxine-pyrimethamine-piperaquine in pregnant and nonpregnant Papua New Guinean women. *Antimicrob Agents Chemother.* 2015;59(7):4260-71.
 268. Rijken MJ, McGready R, Boel ME, Barends M, Proux S, et al. Dihydroartemisinin-piperaquine rescue treatment of multidrug-resistant *Plasmodium falciparum* malaria in pregnancy: a preliminary report. *Am J Trop Med Hyg.* 2008;78(4):543-5.
 269. Desai M, Gutman J, L'Lanziva A, Otieno K, Juma E, et al. Intermittent screening and treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine versus intermittent preventive treatment with sulfadoxine-pyrimethamine for the control of malaria during pregnancy in western Kenya: an open-lab. *Lancet.* 2015;386(10012):2507-19.
 270. Kajubi R, Huang L, Jagannathan P, Chamankhah N, Were M, et al. Antiretroviral Therapy With Efavirenz Accentuates Pregnancy-Associated Reduction of Dihydroartemisinin-Piperaquine Exposure During Malaria Chemoprevention. *Clin Pharmacol Ther.* 2017;102(3):520-8.
 271. Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, et al. Dihydroartemisinin-Piperaquine for the Prevention of Malaria in Pregnancy. *N Engl J Med.* 2016;374(10):928-39.
 272. Wallender E, Vucicevic K, Jagannathan P, Huang L, Natureeba P, et al. Predicting Optimal Dihydroartemisinin-Piperaquine Regimens to Prevent Malaria during Pregnancy for Human Immunodeficiency Virus-Infected Women Receiving Efavirenz. *J Infect Dis.* 2018;217(6):964-72.
 273. Batty KT, Moore BR, Stirling V, Ilett KF, Page-Sharp M, et al. Investigation of reproductive toxicity of

- piperaquine in mice. *Reprod Toxicol*. 2010;29(2):206-13.
274. McGready R, Ashley E, Nosten F, Rijken M. The prevention of malaria in pregnancy [Royal College of Obstetricians and Gynaecologists Green-top Guideline No. 54a]. London: Royal College of Obstetricians and Gynaecologists; 2010 [cited 17 June 2019]. Available from: https://www.rcog.org.uk/globalassets/documents/guidelines/gtg_54a.pdf.
 275. McGready R, Stepniewska K, Edstein MD, Cho T, Gilveray G, et al. The pharmacokinetics of atovaquone and proguanil in pregnant women with acute falciparum malaria. *Eur J Clin Pharmacol*. 2003;59(7):545-52.
 276. Na-Bangchang K, Manyando C, Ruengweerayut R, Kioy D, Mulenga M, et al. The pharmacokinetics and pharmacodynamics of atovaquone and proguanil for the treatment of uncomplicated falciparum malaria in third-trimester pregnant women. *Eur J Clin Pharmacol*. 2005;61(8):573-82.
 277. Wangboonskul J, White NJ, Nosten F, Kuile F, Moody RR, et al. Single dose pharmacokinetics of proguanil and its metabolites in pregnancy. *Eur J Clin Pharmacol*. 1993;44(3):247-51.
 278. McGready R, Stepniewska K, Seaton E, Cho T, Cho D, et al. Pregnancy and use of oral contraceptives reduces the biotransformation of proguanil to cycloguanil. *Eur J Clin Pharmacol*. 2003;59(7):553-7.
 279. Goodman CD, Siregar JE, Mollard V, Vega-Rodriguez J, Syafruddin D, et al. Parasites resistant to the antimalarial atovaquone fail to transmit by mosquitoes. *Science*. 2016;352(6283):349-53.
 280. McGready R, Keo NK, Villegas L, White NJ, Looareesuwan S, et al. Artesunate-atovaquone-proguanil rescue treatment of multidrug-resistant *Plasmodium falciparum* malaria in pregnancy: a preliminary report. *Trans R Soc Trop Med Hyg*. 2003;97(5):592-4.
 281. GlaxoSmithKline. Malarone - prescribing information 2008 [cited 24 April 2018]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2008/021078s0161bl.pdf.
 282. Andrejko KL, Mayer RC, Kovacs S, Slutsker E, Bartlett E, et al. The safety of atovaquone-proguanil for the prevention and treatment of malaria in pregnancy: A systematic review. *Travel Med Infect Dis*. 2019;27:20-6.
 283. Björn P, Anders H. Atovaquone-proguanil use in early pregnancy and the risk of birth defects. *Arch Intern Med*. 2011;171(3):259-60.
 284. Reuvers N, Vial T, Schaefer C, Elefant E, De Santis M, et al. Pregnancy outcome after first trimester exposure to malarone (atovaquone-proguanil): A prospective case-series. *Birth Defects Res A Clin Mol Teratol*. 2012;94(5):329.
 285. Tan KR, Fairley JK, Wang M, Gutman JR. A survey on outcomes of accidental atovaquone-proguanil exposure in pregnancy. *Malar J*. 2018;17:198.
 286. Mayer RC, Tan KR, Gutman JR. Safety of atovaquone-proguanil during pregnancy. *J Travel Med*. 2018;26(4):tay138.
 287. WorldWide Antimalarial Resistance Network. SP Molecular Surveyor. 2018. [cited 02. July 2018]. Available from: <http://www.wwarn.org/dhfr-dhps-surveyor/>.
 288. Saito M, Gilder ME, Nosten F, Guérin PJ, McGready R. Methodology of assessment and reporting of safety in anti-malarial treatment efficacy studies of uncomplicated falciparum malaria in pregnancy: a systematic literature review. *Malar J*. 2017;16:491.
 289. Moore KA, Fowkes FJI, Wiladphaingern J, Wai NS, Paw MK, et al. Mediation of the effect of malaria in pregnancy on stillbirth and neonatal death in an area of low transmission: observational data analysis. *BMC*

- Med.* 2017;15:98.
290. Cottrell G, Moussiliou A, Luty AJ, Cot M, Fievet N, et al. Submicroscopic *Plasmodium falciparum* infections are associated with maternal anemia, premature births, and low birth weight. *Clin Infect Dis.* 2015;60(10):1481-8.
 291. Achan J, Talisuna AO, Erhart A, Yeka A, Tibenderana JK, et al. Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. *Malar J.* 2011;10:144.
 292. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med.* 2009;6(7):e1000100.
 293. McGready R, Nosten F. Which drug is effective and safe for acute malaria in pregnancy? Reviewing the evidence. *Drug Dev Res.* 2010;71(1):56-68.
 294. McGready R, White NJ, Nosten F. Parasitological efficacy of antimalarials in the treatment and prevention of falciparum malaria in pregnancy 1998 to 2009: a systematic review. *BJOG.* 2011;118(2):123-35.
 295. Manyando C, Kayentao K, D'Alessandro U, Okafor HU, Juma E, et al. A systematic review of the safety and efficacy of artemether-lumefantrine against uncomplicated *Plasmodium falciparum* malaria during pregnancy. *Malar J.* 2012;11(Suppl 1):141.
 296. Burger RJ, van Eijk AM, Bussink M, Hill J, Ter Kuile FO. Artemisinin-based combination therapy versus quinine or other combinations for treatment of uncomplicated *Plasmodium falciparum* malaria in the second and third trimester of pregnancy: a systematic review and meta-analysis. *Open Forum Infect Dis.* 2016;3(1):ofv170.
 297. Moore BR, Salman S, Davis TME. Treatment regimens for pregnant women with falciparum malaria. *Expert Rev Anti Infect Ther.* 2016;14(8):691-704.
 298. Brown LD, Cai TT, DasGupta A. Interval estimation for a binomial proportion. *Stat Sci.* 2001;16(2):101-17.
 299. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med.* 2002;21(11):1539-58.
 300. Hunter JP, Saratzis A, Sutton AJ, Boucher RH, Sayers RD, et al. In meta-analyses of proportion studies, funnel plots were found to be an inaccurate method of assessing publication bias. *J Clin Epidemiol.* 2014;67(8):897-903.
 301. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* 1997;315(7109):629-34.
 302. Naing T, Win H, Nwe YY. Falciparum malaria and pregnancy: relationship and treatment response. *Southeast Asian J Trop Med Public Health.* 1988;19(2):253-8.
 303. Kalilani L, Mofolo I, Chaponda M, Rogerson SJ, Alker AP, et al. A randomized controlled pilot trial of azithromycin or artesunate added to sulfadoxine-pyrimethamine as treatment for malaria in pregnant women. *PLoS One.* 2007;2(11):e1166.
 304. Nambozi M, Kabuya J-BB, Hachizovu S, Mwakazanga D, Mulenga J, et al. Artemisinin-based combination therapy in pregnant women in Zambia: efficacy, safety and risk of recurrent malaria. *Malar J.* 2017;16:199.
 305. Chico RM, Chandramohan D, Greenwood B, Mosha F. Cardio-Safety of Dihydroartemisinin-Piperaquine

- and Pharmacokinetics of Piperaquine Amongst Pregnant Women in Tanzania (NCT02909712). 2016. [cited 30 April 2019]. Available from: <https://clinicaltrials.gov/ct2/show/NCT02909712>.
306. Juma EA, Ogutu BR, Oloo F, Barwa T, Aman R. Pharmacokinetics of artemether-lumefantrine in pregnant and non-pregnant women with uncomplicated *Plasmodium falciparum* malaria in western Kenya. *Am J Trop Med Hyg*. 2014;91(5 Supplement1):587.
 307. Adegnika AA, Breitling LP, Agnandji ST, Chai SK, Schütte D, et al. Effectiveness of quinine monotherapy for the treatment of *Plasmodium falciparum* infection in pregnant women in Lambaréné, Gabon. *Am J Trop Med Hyg*. 2005;73(2):263-6.
 308. Adam I, Ali DM, Abdalla MA. Artesunate plus sulfadoxine-pyrimethamine in the treatment of uncomplicated *Plasmodium falciparum* malaria during pregnancy in eastern Sudan. *Trans R Soc Trop Med Hyg*. 2006;100(7):632-5.
 309. McGready R, Cho T, Cho JJ, Simpson JA, Luxemburger C, et al. Artemisinin derivatives in the treatment of falciparum malaria in pregnancy. *Trans R Soc Trop Med Hyg*. 1998;92(4):430-3.
 310. Laochan N, Zaloumis SG, Imwong M, Lek-Uthai U, Brockman A, et al. Intervals to *Plasmodium falciparum* recurrence after anti-malarial treatment in pregnancy: a longitudinal prospective cohort. *Malar J*. 2015;14:221.
 311. Villegas L, Hernandez N, Vasquez C, Veliz F, Guevara M-E, et al. Treatment of multi drug-resistant falciparum malaria during pregnancy with mefloquine-artesunate in Venezuela: Preliminary results. *Am J Trop Med Hyg*. 2005;73(6 Supplement):225.
 312. Kalilani-Phiri L, Thesing PC, Nyirenda OM, Mawindo P, Madanitsa M, et al. Timing of malaria infection during pregnancy has characteristic maternal, infant and placental outcomes. *PLoS One*. 2013;8(9):e74643.
 313. Cohee LM, Kalilani-Phiri L, Mawindo P, Joshi S, Adams M, et al. Parasite dynamics in the peripheral blood and the placenta during pregnancy-associated malaria infection. *Malar J*. 2016;15:483.
 314. Das JL, Rulisa S, de Vries PJ, Mens PF, Kaligirwa N, et al. Population pharmacokinetics of artemether, dihydroartemisinin and lumefantrine in Rwandese pregnant women treated for uncomplicated *Plasmodium falciparum* malaria. *Antimicrob Agents Chemother*. 2018;62(10):e00518.
 315. Ismail MR, Ordi J, Menendez C, Ventura PJ, Aponte JJ, et al. Placental pathology in malaria: a histological, immunohistochemical, and quantitative study. *Hum Pathol*. 2000;31(1):85-93.
 316. Muehlenbachs A, Fried M, McGready R, Harrington WE, Mutabingwa TK, et al. A novel histological grading scheme for placental malaria applied in areas of high and low malaria transmission. *J Infect Dis*. 2010;202(10):1608-16.
 317. Bulmer JN, Rasheed FN, Francis N, Morrison L, Greenwood BM. Placental malaria. I. Pathological classification. *Histopathology*. 1993;22(3):211-8.
 318. Rogerson SJ, Mkundika P, Kanjala MK. Diagnosis of *Plasmodium falciparum* malaria at delivery: comparison of blood film preparation methods and of blood films with histology. *J Clin Microbiol*. 2003;41(4):1370-4.
 319. Hutagalung R, Paiphun L, Ashley EA, McGready R, Brockman A, et al. A randomized trial of artemether-lumefantrine versus mefloquine-artesunate for the treatment of uncomplicated multi-drug resistant *Plasmodium falciparum* on the western border of Thailand. *Malar J*. 2005;4:46.
 320. Vugt MV, Wilairatana P, Gemperli B, Gathmann I, Phaipun L, et al. Efficacy of six doses of artemether-

- lumefantrine (benflumetol) in multidrug-resistant *Plasmodium falciparum* malaria. *Am J Trop Med Hyg.* 1999;60(6):936-42.
321. Woodrow CJ, White NJ. The clinical impact of artemisinin resistance in Southeast Asia and the potential for future spread. *FEMS Microbiol Rev.* 2017;41(1):34-48.
 322. Stepniewska K, Taylor WR, Mayxay M, Price R, Smithuis F, et al. In vivo assessment of drug efficacy against *Plasmodium falciparum* malaria: duration of follow-up. *Antimicrob Agents Chemother.* 2004;48(11):4271-80.
 323. Mayor A, Serra-Casas E, Bardají A, Sanz S, Puyol L, et al. Sub-microscopic infections and long-term recrudescence of *Plasmodium falciparum* in Mozambican pregnant women. *Malar J.* 2009;8:9.
 324. Stepniewska K, White NJ. Some considerations in the design and interpretation of antimalarial drug trials in uncomplicated falciparum malaria. *Malar J.* 2006;5:127.
 325. Moore KA, Simpson JA, Thomas KH, Rijken MJ, White LJ, et al. Estimating gestational age in late presenters to antenatal care in a resource-limited setting on the Thai-Myanmar border. *PLoS One.* 2015;10(6):e0131025.
 326. McGready R, Davison BB, Stepniewska K, Cho T, Shee H, et al. The effects of *Plasmodium falciparum* and *P. vivax* infections on placental histopathology in an area of low malaria transmission. *Am J Trop Med Hyg.* 2004;70(4):398-407.
 327. WorldWide Antimalarial Resistance Network (WWARN). Malaria Case Record Form. 2017. [cited 17 June 2019]. Available from: <http://www.wwarn.org/tools-resources/procedures/malaria-case-record-form-crf>.
 328. Gutman J, Kovacs S, Dorsey G, Stergachis A, Ter Kuile FO. Safety, tolerability, and efficacy of repeated doses of dihydroartemisinin-piperaquine for prevention and treatment of malaria: a systematic review and meta-analysis. *Lancet Infect Dis.* 2017;17(2):184-93.
 329. Amiel-Tison C, Stewart A. Follow up studies during the first five years of life: a pervasive assessment of neurological function. *Arch Dis Child.* 1989;64:496-502.
 330. Resch B, Gedermann A, Maurer U, Ritschl E, Muller W. Neurodevelopmental outcome of hydrocephalus following intra-/periventricular hemorrhage in preterm infants: short- and long-term results. *Childs Nerv Syst.* 1996;12(1):27-33.
 331. Frankenburg WK, Dodds JB. The Denver Developmental Screening Test. *J Pediatr.* 1967;71(2):181-91.
 332. Haataja L, McGready R, Arunjerdja R, Simpson JA, Mercuri E, et al. A new approach for neurological evaluation of infants in resource-poor settings. *Ann Trop Paediatr.* 2002;22(4):355-68.
 333. McGready R, Simpson J, Panyavudhikrai S, Loo S, Mercuri E, et al. Neonatal neurological testing in resource-poor settings. *Ann Trop Paediatr.* 2000;20(4):323-36.
 334. WHO multicentre growth reference study group. Windows of achievement for six gross motor development milestones. *Acta Paediatr.* 2006;Supplement 450:86-95.
 335. Rijken MJ, Rijken JA, Papageorgiou AT, Kennedy SH, Visser GH, et al. Malaria in pregnancy: the difficulties in measuring birthweight. *BJOG.* 2011;118(6):671-8.
 336. McGready R, Prakash JAJ, Benjamin SJ, Wathanaworawit W, Anantatat T, et al. Pregnancy Outcome in Relation to Treatment of Murine Typhus and Scrub Typhus Infection: A Fever Cohort and a Case Series Analysis. *PLoS Negl Trop Dis.* 2014;8(11):e3327.
 337. Briand V, Saal J, Ghafari C, Huynh BT, Fievet N, et al. Fetal Growth Restriction Is Associated With

- Malaria in Pregnancy: A Prospective Longitudinal Study in Benin. *J Infect Dis*. 2016;214(3):417-25.
338. Rijken MJ, Papageorgiou AT, Thiptharakun S, Kiricharoen S, Dwell SLM, et al. Ultrasound Evidence of Early Fetal Growth Restriction after Maternal Malaria Infection. *PLoS One*. 2012;7(2):e31411.
 339. Unger H, Thriemer K, Ley B, Tinto H, Traore M, et al. The assessment of gestational age: a comparison of different methods from a malaria pregnancy cohort in sub-Saharan Africa. *BMC Pregnancy Childbirth*. 2019;19(1):12.
 340. Dubowitz LM, Dubowitz V, Goldberg C, Keith I. Rapid assessment of gestational age at birth. *Arch Dis Child*. 1976;51(12):986-7.
 341. Abbassi-Ghanavati M, Greer LG, Cunningham FG. Pregnancy and laboratory studies: a reference table for clinicians. *Obstet Gynecol*. 2009;114(6):1326-31.
 342. Centers for Disease Control and Prevention. Recommendations to prevent and control iron deficiency in the United States. *MMWR Recomm Rep*. 1998;47(RR-3):1-29.
 343. Shi T, McAllister DA, O'Brien KL, Simoes EAF, Madhi SA, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet*. 2017;390(10098):946-58.
 344. Desai M, Dellicour S. Effects of malaria and its treatment in early pregnancy. *Lancet Infect Dis*. 2012;12(5):359-60.
 345. Turner C, Carrara V, Thien NA, Paw NM, Rijken M, et al. Changes in the body weight of term infants, born in the tropics, during the first seven days of life. *BMC Pediatr*. 2013;13:93.
 346. Wright CM, Parkinson KN. Postnatal weight loss in term infants: what is normal and do growth charts allow for it? *Arch Dis Child Fetal Neonatal Ed*. 2004;89(3):F254-7.
 347. Rijken MJ, De Livera AM, Lee SJ, Boel ME, Rungwilaekhiri S, et al. Quantifying Low Birth Weight, Preterm Birth and Small-for-Gestational-Age Effects of Malaria in Pregnancy: A Population Cohort Study. *PLoS One*. 2014;9(7):e100247.
 348. Obonyo CO, Juma EA. Clindamycin plus quinine for treating uncomplicated falciparum malaria: a systematic review and meta-analysis. *Malar J*. 2012;11:2.
 349. Landis SH, Lokomba V, Ananth CV, Atibu J, Ryder RW, et al. Impact of maternal malaria and under-nutrition on intrauterine growth restriction: a prospective ultrasound study in Democratic Republic of Congo. *Epidemiol Infect*. 2009;137(2):294-304.
 350. White NJ. The parasite clearance curve. *Malar J*. 2011;10:278.
 351. Abdulla S, Ashley EA, Bassat Q, Bethell D, Bjorkman A, et al. Baseline data of parasite clearance in patients with falciparum malaria treated with an artemisinin derivative: an individual patient data meta-analysis. *Malar J*. 2015;14:359.
 352. Feghali M, Venkataramanan R, Caritis S. Pharmacokinetics of drugs in pregnancy. *Semin Perinatol*. 2015;39(7):512-9.
 353. Chotivanich K, Sattabongkot J, Udomsangpetch R, Looareesuwan S, Day NP, et al. Transmission-blocking activities of quinine, primaquine, and artesunate. *Antimicrob Agents Chemother*. 2006;50(6):1927-30.
 354. WWARN Gametocyte Study Group. Gametocyte carriage in uncomplicated *Plasmodium falciparum* malaria following treatment with artemisinin combination therapy: a systematic review and meta-analysis of

- individual patient data. *BMC Med.* 2016;14:79.
355. Price R, Nosten F, Simpson JA, Luxemburger C, Phaipun L, et al. Risk factors for gametocyte carriage in uncomplicated falciparum malaria. *Am J Trop Med Hyg.* 1999;60(6):1019-23.
 356. Lamprey H, Ofori MF, Kusi KA, Adu B, Owusu-Yeboah E, et al. The prevalence of submicroscopic *Plasmodium falciparum* gametocyte carriage and multiplicity of infection in children, pregnant women and adults in a low malaria transmission area in Southern Ghana. *Malar J.* 2018;17:331.
 357. Boudová S, Cohee LM, Kalilani-Phiri L, Thesing PC, Kamiza S, et al. Pregnant women are a reservoir of malaria transmission in Blantyre, Malawi. *Malar J.* 2014;13:506.
 358. Drakeley C, Sutherland C, Targett G, Doherty T, Walraven G, et al. Artesunate Reduces but Does Not Prevent Posttreatment Transmission of *Plasmodium falciparum* to *Anopheles gambiae*. *J Infect Dis.* 2001;183(8):1254-9.
 359. Tadesse FG, Meerstein-Kessel L, Goncalves BP, Drakeley C, Ranford-Cartwright L, et al. Gametocyte Sex Ratio: The Key to Understanding *Plasmodium falciparum* Transmission? *Trends Parasitol.* 2019;35(3):226-38.
 360. Sowunmi A, Fateye BA. Gametocyte sex ratios in children with asymptomatic, recrudescence, pyrimethamine-sulfadoxine-resistant, *Plasmodium falciparum* malaria. *Ann Trop Med Parasitol.* 2003;97(7):671-82.
 361. Wang J, Xu C, Liao FL, Jiang T, Krishna S, et al. A Temporizing Solution to “Artemisinin Resistance”. *N Engl J Med.* 2019;380:2087-9.
 362. Brockman A, Paul RE, Anderson TJ, Hackford I, Phaipun L, et al. Application of genetic markers to the identification of recrudescence *Plasmodium falciparum* infections on the northwestern border of Thailand. *Am J Trop Med Hyg.* 1999;60(1):14-21.
 363. Collett D. 6 Accelerated failure time and other parametric models. In: *Modelling survival data in medical research* Third edition. New York: CRC Press; 2015. p. 221-73.
 364. Collett D. 7 Model checking in parametric models. In: *Modelling survival data in medical research* Third edition. New York: CRC Press; 2015. p. 275-93.
 365. McLernon DJ, Donnan PT, Sullivan FM, Roderick P, Rosenberg WM, et al. Prediction of liver disease in patients whose liver function tests have been checked in primary care: model development and validation using population-based observational cohorts. *BMJ Open.* 2014;4:e004837.
 366. Klein JP, Moeschberger ML. *Survival analysis. Techniques for censored and truncated data.* Second Edition. New York: Springer; 2003.
 367. Royston P, Lambert PC. *Flexible parametric survival analysis using Stata: beyond the Cox model.* College Station, Texas: Stata Press; 2011.
 368. Douglas NM, Nosten F, Ashley EA, Phaipun L, van Vugt M, et al. *Plasmodium vivax* recurrence following falciparum and mixed species malaria: risk factors and effect of antimalarial kinetics. *Clin Infect Dis.* 2011;52(5):612-20.
 369. Worldwide Antimalarial Resistance Network (WWARN) AL Dose Impact Study Group. The effect of dose on the antimalarial efficacy of artemether-lumefantrine: a systematic review and pooled analysis of individual patient data. *Lancet Infect Dis.* 2015;15(6):692-702.
 370. Borrmann S, Peto T, Snow RW, Gutteridge W, White NJ. Revisiting the design of phase III clinical trials

- of antimalarial drugs for uncomplicated *Plasmodium falciparum* malaria. *PLoS Med.* 2008;5(11):e227.
371. White NJ, Pongtavornpinyo W, Maude RJ, Saralamba S, Aguas R, et al. Hyperparasitaemia and low dosing are an important source of anti-malarial drug resistance. *Malar J.* 2009;8:253.
 372. Price R, Luxemburger C, van Vugt M, Nosten F, Kham A, et al. Artesunate and mefloquine in the treatment of uncomplicated multidrug-resistant hyperparasitaemic falciparum malaria. *Trans R Soc Trop Med Hyg.* 1998;92(2):207-11.
 373. Ashley EA, White NJ. The duration of *Plasmodium falciparum* infections. *Malar J.* 2014;13:500.
 374. Nosten F, Luxemburger C, ter Kuile FO, Woodrow C, Eh JP, et al. Treatment of multidrug-resistant *Plasmodium falciparum* malaria with 3-day artesunate-mefloquine combination. *J Infect Dis.* 1994;170(4):971-7.
 375. Simpson JA, Watkins ER, Price RN, Aarons L, Kyle DE, et al. Mefloquine pharmacokinetic-pharmacodynamic models: implications for dosing and resistance. *Antimicrob Agents Chemother.* 2000;44(12):3414-24.
 376. Orbe J, Ferreira E, Nunez-Anton V. Comparing proportional hazards and accelerated failure time models for survival analysis. *Stat Med.* 2002;21(22):3493-510.
 377. Keene ON. Alternatives to the hazard ratio in summarizing efficacy in time-to-event studies: an example from influenza trials. *Stat Med.* 2002;21(23):3687-700.
 378. Patel K, Kay R, Rowell L. Comparing proportional hazards and accelerated failure time models: an application in influenza. *Pharm Stat.* 2006;5(3):213-24.
 379. Reid N. A Conversation with Sir David Cox. *Stat Sci.* 1994;9(3):439-55.
 380. Assefa SA, Preston MD, Campino S, Ocholla H, Sutherland CJ, et al. estMOI: estimating multiplicity of infection using parasite deep sequencing data. *Bioinformatics.* 2014;30(9):1292-4.
 381. Ocholla H, Preston MD, Mipando M, Jensen AT, Campino S, et al. Whole-genome scans provide evidence of adaptive evolution in Malawian *Plasmodium falciparum* isolates. *J Infect Dis.* 2014;210(12):1991-2000.
 382. Omer SA, Idress HE, Adam I, Abdelrahim M, Noureldein AN, et al. Placental malaria and its effect on pregnancy outcomes in Sudanese women from Blue Nile State. *Malar J.* 2017;16:374.
 383. Shulman CE, Marshall T, Dorman EK, Bulmer JN, Cutts F, et al. Malaria in pregnancy: adverse effects on haemoglobin levels and birthweight in primigravidae and multigravidae. *Trop Med Int Health.* 2001;6(10):770-8.
 384. Menendez C, Ordi J, Ismail MR, Ventura PJ, Aponte JJ, et al. The impact of placental malaria on gestational age and birth weight. *J Infect Dis.* 2000;181(5):1740-5.
 385. Van Buuren S. 5 Analysis of imputed data. In: Flexible Imputation of Missing Data. Second ed. Florida, US: CRC Press; 2018. p. 139-59.
 386. Wood AM, White IR, Royston P. How should variable selection be performed with multiply imputed data? *Stat Med.* 2008;27(17):3227-46.
 387. Plewes K, Lee T, Kajeewewa L, Thwin MM, Lee SJ, et al. Low seroprevalence of HIV and syphilis in pregnant women in refugee camps on the Thai-Burma border. *Int J STD AIDS.* 2008;19(12):833-7.
 388. Mishra N, Kaitholia K, Srivastava B, Shah NK, Narayan JP, et al. Declining efficacy of artesunate plus sulphadoxine-pyrimethamine in northeastern India. *Malar J.* 2014;13:284.

389. Flenady V, Koopmans L, Middleton P, Frøen JF, Smith GC, et al. Major risk factors for stillbirth in high-income countries: a systematic review and meta-analysis. *Lancet*. 2011;377(9774):1331-40.
390. Patel A, Prakash AA, Das PK, Gupta S, Pusdekar YV, et al. Maternal anemia and underweight as determinants of pregnancy outcomes: cohort study in eastern rural Maharashtra, India. *BMJ Open*. 2018;8(8):e021623.
391. Aune D, Saugstad OD, Henriksen T, Tonstad S. Maternal body mass index and the risk of fetal death, stillbirth, and infant death: a systematic review and meta-analysis. *JAMA*. 2014;311(15):1536-46.
392. Lee AC, Kozuki N, Cousens S, Stevens GA, Blencowe H, et al. Estimates of burden and consequences of infants born small for gestational age in low and middle income countries with INTERGROWTH-21(st) standard: analysis of CHERG datasets. *BMJ*. 2017;358:j3677.
393. Chawanpaiboon S, Vogel JP, Moller A-B, Lumbiganon P, Petzold M, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Glob Health*. 2019;7(1):e37-e46.
394. Cottrell G, Mary JY, Barro D, Cot M. The importance of the period of malarial infection during pregnancy on birth weight in tropical Africa. *Am J Trop Med Hyg*. 2007;76(5):849-54.
395. Griffin JB, Lokomba V, Landis SH, Thorp JM, Jr., Herring AH, et al. *Plasmodium falciparum* parasitaemia in the first half of pregnancy, uterine and umbilical artery blood flow, and foetal growth: a longitudinal Doppler ultrasound study. *Malar J*. 2012;11:319.
396. Umbers AJ, Stanicic DI, Ome M, Wangnapi R, Hanieh S, et al. Does malaria affect placental development? Evidence from in vitro models. *PLoS One*. 2013;8(1):e55269.
397. Malacova E, Regan A, Nassar N, Raynes-Greenow C, Leonard H, et al. Risk of stillbirth, preterm delivery, and fetal growth restriction following exposure in a previous birth: systematic review and meta-analysis. *BJOG*. 2018;125(2):183-92.
398. Shah PS. Parity and low birth weight and preterm birth: a systematic review and meta-analyses. *Acta Obstet Gynecol Scand*. 2010;89(7):862-75.
399. Kozuki N, Lee AC, Silveira MF, Sania A, Vogel JP, et al. The associations of parity and maternal age with small-for-gestational-age, preterm, and neonatal and infant mortality: a meta-analysis. *BMC Public Health*. 2013;13(3):S2.
400. Fuchs F, Monet B, Ducruet T, Chaillet N, Audibert F. Effect of maternal age on the risk of preterm birth: A large cohort study. *PLoS One*. 2018;13(1):e0191002.
401. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. *Bull World Health Organ*. 1987;65(5):663-737.
402. Kozuki N, Katz J, Lee AC, Vogel JP, Silveira MF, et al. Short Maternal Stature Increases Risk of Small-for-Gestational-Age and Preterm Births in Low- and Middle-Income Countries: Individual Participant Data Meta-Analysis and Population Attributable Fraction. *J Nutr*. 2015;145(11):2542-50.
403. Han Z, Lutsiv O, Mulla S, McDonald SD. Maternal height and the risk of preterm birth and low birth weight: a systematic review and meta-analyses. *J Obstet Gynaecol Can*. 2012;34(8):721-46.
404. Han Z, Group obotKS, Mulla S, Group obotKS, Beyene J, et al. Maternal underweight and the risk of preterm birth and low birth weight: a systematic review and meta-analyses. *Int J Epidemiol*. 2010;40(1):65-101.

405. De Beaudrap P, Turyakira E, White LJ, Nabasumba C, Tumwebaze B, et al. Impact of malaria during pregnancy on pregnancy outcomes in a Ugandan prospective cohort with intensive malaria screening and prompt treatment. *Malar J*. 2013;12:139.
406. Guyatt HL, Snow RW. Impact of malaria during pregnancy on low birth weight in sub-Saharan Africa. *Clin Microbiol Rev*. 2004;17(4):760-9.
407. Briggs J, Ategeka J, Kajubi R, Ochieng T, Kakuru A, et al. Impact of microscopic and submicroscopic parasitemia during pregnancy on placental malaria in a high-transmission setting in Uganda. *J Infect Dis*. 2019;doi: 10.1093/infdis/jiz130.
408. Fried M, Muga RO, Misore AO, Duffy PE. Malaria elicits type 1 cytokines in the human placenta: IFN-gamma and TNF-alpha associated with pregnancy outcomes. *J Immunol*. 1998;160(5):2523-30.
409. Leke RF, Bioga JD, Zhou J, Fouda GG, Leke RJ, et al. Longitudinal studies of *Plasmodium falciparum* malaria in pregnant women living in a rural Cameroonian village with high perennial transmission. *Am J Trop Med Hyg*. 2010;83(5):996-1004.
410. McGready R, Brockman A, Cho T, Levesque MA, Tkachuk AN, et al. Haemozoin as a marker of placental parasitization. *Trans R Soc Trop Med Hyg*. 2002;96(6):644-6.
411. Kominiarek MA, Peaceman AM. Gestational weight gain. *Am J Obstet Gynecol*. 2017;217(6):642-51.
412. Michalski ST, Porter J, Pauli RM. Costs and consequences of comprehensive stillbirth assessment. *Am J Obstet Gynecol*. 2002;186(5):1027-34.
413. Wojcieszek AM, Shepherd E, Middleton P, Gardener G, Ellwood DA, et al. Interventions for investigating and identifying the causes of stillbirth. *Cochrane Database Syst Rev*. 2018(4):CD012504.
414. Schmiegelow C, Matondo S, Minja DTR, Resende M, Pehrson C, et al. *Plasmodium falciparum* Infection Early in Pregnancy has Profound Consequences for Fetal Growth. *J Infect Dis*. 2017;216(12):1601-10.
415. White NJ. Cardiotoxicity of antimalarial drugs. *Lancet Infect Dis*. 2007;7(8):549-58.
416. White NJ, Looareesuwan S, Warrell DA, Chongsuphajaisiddhi T, Bunnag D, et al. Quinidine in falciparum malaria. *Lancet*. 1981;2(8255):1069-71.
417. van Aghtmael M, Bouchaud O, Malvy D, Delmont J, Danis M, et al. The comparative efficacy and tolerability of CGP 56697 (artemether + lumefantrine) versus halofantrine in the treatment of uncomplicated falciparum malaria in travellers returning from the Tropics to The Netherlands and France. *Int J Antimicrob Agents*. 1999;12(2):159-69.
418. Bouchaud O, Imbert P, Touze JE, Doodoo AN, Danis M, et al. Fatal cardiotoxicity related to halofantrine: a review based on a worldwide safety data base. *Malar J*. 2009;8:289.
419. Nosten F, ter Kuile FO, Luxemburger C, Woodrow C, Chongsuphajaisiddhi T, et al. Cardiac effects of antimalarial treatment with halofantrine. *Lancet*. 1993;341(8852):1054-6.
420. Haeusler IL, Chan XHS, Guerin PJ, White NJ. The arrhythmogenic cardiotoxicity of the quinoline and structurally related antimalarial drugs: a systematic review. *BMC Med*. 2018;16(1):200.
421. WHO Evidence Review Group Meeting. The cardiotoxicity of antimalarials. 2017. [cited 17 June 2019]. Available from: <http://www.who.int/malaria/mpac/mpac-mar2017-erg-cardiotoxicity-report-session2.pdf>.
422. Odening KE, Koren G, Kirk M. Normalization of QT interval duration in a long QT syndrome patient during pregnancy and the postpartum period due to sex hormone effects on cardiac repolarization. *HeartRhythm Case Rep*. 2016;2(3):223-7.

423. Drici MD, Clement N. Is gender a risk factor for adverse drug reactions? The example of drug-induced long QT syndrome. *Drug Saf.* 2001;24(8):575-85.
424. Abarientos C, Sperber K, Shapiro DL, Aronow WS, Chao CP, et al. Hydroxychloroquine in systemic lupus erythematosus and rheumatoid arthritis and its safety in pregnancy. *Expert Opin Drug Saf.* 2011;10(5):705-14.
425. Costedoat-Chalumeau N, Amoura Z, Huong DL, Lechat P, Piette JC. Safety of hydroxychloroquine in pregnant patients with connective tissue diseases. Review of the literature. *Autoimmun Rev.* 2005;4(2):111-5.
426. Natureeba P, Kakuru A, Muhindo M, Ochieng T, Ategeka J, et al. Intermittent Preventive Treatment With Dihydroartemisinin-Piperaquine for the Prevention of Malaria Among HIV-Infected Pregnant Women. *J Infect Dis.* 2017;216(1):29-35.
427. Postema PG, Wilde AA. The measurement of the QT interval. *Curr Cardiol Rev.* 2014;10(3):287-94.
428. Isbister GK, Page CB. Drug induced QT prolongation: the measurement and assessment of the QT interval in clinical practice. *Br J Clin Pharmacol.* 2013;76(1):48-57.
429. Woosley R, Heise C, Romero K. QT drugs List. AZCERT; 2018. [cited 30 July 2018]. Available from: www.Crediblemeds.org.
430. Vandenberg B, Vandael E, Robyns T, Vandenberghe J, Garweg C, et al. Which QT Correction Formulae to Use for QT Monitoring? *J Am Heart Assoc.* 2016;5(6):e003264.
431. Trinkley KE, Page RL, 2nd, Lien H, Yamanouye K, Tisdale JE. QT interval prolongation and the risk of torsades de pointes: essentials for clinicians. *Curr Med Res Opin.* 2013;29(12):1719-26.
432. Campbell RWF, Day CP, James OFW, Butler TJ. QT prolongation and sudden cardiac death in patients with alcoholic liver disease. *Lancet.* 1993;341(8858):1423-8.
433. Twisk J, Bosman L, Hoekstra T, Rijnhart J, Welten M, et al. Different ways to estimate treatment effects in randomised controlled trials. *Contemp Clin Trials Commun.* 2018;10:80-5.
434. Collett D. 3.6 Strategy for model selection. In: *Modelling survival data in medical research* Third edition. New York: CRC Press; 2015. p. 83-90.
435. Maldonado G, Greenland S. Simulation study of confounder-selection strategies. *Am J Epidemiol.* 1993;138(11):923-36.
436. Royston P. sg3.5: Comment on sg3.4 and an improved D'Agostino test. *Stata Technical Bulletin.* 1991;3:23-4.
437. Chan XHS, Win YN, Mawer LJ, Tan JY, Brugada J, et al. Risk of sudden unexplained death after use of dihydroartemisinin-piperaquine for malaria: a systematic review and Bayesian meta-analysis. *Lancet Infect Dis.* 2018;18(8):913-23.
438. Kajubi R, Ochieng T, Kakuru A, Jagannathan P, Nakalembe M, et al. Monthly sulfadoxine-pyrimethamine versus dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in pregnancy: a double-blind, randomised, controlled, superiority trial. *Lancet.* 2019;393:1428-39.
439. Ahmed R, Poespoprodjo JR, Syafruddin D, Khairallah C, Pace C, et al. Efficacy and safety of intermittent preventive treatment and intermittent screening and treatment versus single screening and treatment with dihydroartemisinin-piperaquine for the control of malaria in pregnancy in Indonesia: a cluster-randomised, open-label, superiority trial. *Lancet Infect Dis.* 2019;19(9):973-87.
440. Pukrittayakamee S, Tarning J, Jittamala P, Charunwatthana P, Lawpoolsri S, et al. Pharmacokinetic

- interactions between primaquine and chloroquine. *Antimicrob Agents Chemother*. 2014;58(6):3354-9.
441. Hanboonkunupakarn B, Ashley EA, Jittamala P, Tarning J, Pukrittayakamee S, et al. Open-label crossover study of primaquine and dihydroartemisinin-piperaquine pharmacokinetics in healthy adult thai subjects. *Antimicrob Agents Chemother*. 2014;58(12):7340-6.
 442. van Vugt M, Ezzet F, Nosten F, Gathmann I, Wilairatana P, et al. No evidence of cardiotoxicity during antimalarial treatment with artemether-lumefantrine. *Am J Trop Med Hyg*. 1999;61(6):964-7.
 443. Magnano AR, Holleran S, Ramakrishnan R, Reiffel JA, Bloomfield DM. Autonomic nervous system influences on QT interval in normal subjects. *J Am Coll Cardiol*. 2002;39(11):1820-6.
 444. Rabkin SW, Cheng X-BJ, Thompson DJS. Detailed analysis of the impact of age on the QT interval. *J Geriatr Cardiol*. 2016;13(9):740-8.
 445. Porta-Sánchez A, Gilbert C, Spears D, Amir E, Chan J, et al. Incidence, Diagnosis, and Management of QT Prolongation Induced by Cancer Therapies: A Systematic Review. *J Am Heart Assoc*. 2017;6(12):e007724.
 446. Sedlak T, Shufelt C, Iribarren C, Merz CNB. Sex Hormones and the QT Interval: A Review. *J Womens Health*. 2012;21(9):933-41.
 447. Abehsira G, Bachelot A, Badilini F, Koehl L, Lebot M, et al. Complex Influence of Gonadotropins and Sex Steroid Hormones on QT Interval Duration. *J Clin Endocrinol Metab*. 2016;101(7):2776-84.
 448. Seth R, Moss AJ, McNitt S, Zareba W, Andrews ML, et al. Long QT syndrome and pregnancy. *J Am Coll Cardiol*. 2007;49(10):1092-8.
 449. von Seidlein L, Jaffar S, Greenwood B. Prolongation of the QTc interval in African children treated for falciparum malaria. *Am J Trop Med Hyg*. 1997;56(5):494-7.
 450. Molnar J, Zhang F, Weiss J, Ehlert FA, Rosenthal JE. Diurnal pattern of QTc interval: How long is prolonged? *J Am Coll Cardiol*. 1996;27(1):76-83.
 451. Bexton RS, Vallin HO, Camm AJ. Diurnal variation of the QT interval--influence of the autonomic nervous system. *Br Heart J*. 1986;55(3):253-8.
 452. Smetana P, Batchvarov V, Hnatkova K, Camm AJ, Malik M. Circadian rhythm of the corrected QT interval: impact of different heart rate correction models. *Pacing Clin Electrophysiol*. 2003;26(1 Pt 2):383-6.
 453. Price RN, Nosten F, White NJ. Comment on 'Prolongation of the QTc interval in African children treated for falciparum malaria'. *Am J Trop Med Hyg*. 1998;59(4):503.
 454. Kasamaki Y, Ozawa Y, Ohta M, Sezai A, Yamaki T, et al. Automated versus manual measurement of the QT interval and corrected QT interval. *Ann Noninvasive Electrocardiol*. 2011;16(2):156-64.
 455. World Health Organization. Severe malaria. *Trop Med Int Health*. 2014;19 Suppl 1:7-131.
 456. Dondorp A, Nosten F, Stepniewska K, Day N, White N, et al. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *Lancet*. 2005;366(9487):717-25.
 457. Dondorp AM, Fanello CI, Hendriksen IC, Gomes E, Seni A, et al. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. *Lancet*. 2010;376(9753):1647-57.
 458. Newton PN, Angus BJ, Chierakul W, Dondorp A, Ruangveerayuth R, et al. Randomized comparison of artesunate and quinine in the treatment of severe falciparum malaria. *Clin Infect Dis*. 2003;37(1):7-16.
 459. Kovacs SD, Rijken MJ, Stergachis A. Treating severe malaria in pregnancy: a review of the evidence.

- Drug Saf.* 2015;38(2):165-81.
460. Luxemburger C, Ricci F, Nosten F, Raimond D, Bathet S, et al. The epidemiology of severe malaria in an area of low transmission in Thailand. *Trans R Soc Trop Med Hyg.* 1997;91(3):256-62.
 461. Sholapurkar SL, Gupta AN, Mahajan RC. Clinical course of malaria in pregnancy--a prospective controlled study from India. *Trans R Soc Trop Med Hyg.* 1988;82(3):376-9.
 462. Mishra SK, Panigrahi P, Mishra R, Mohanty S. Prediction of outcome in adults with severe falciparum malaria: a new scoring system. *Malar J.* 2007;6:24.
 463. Kochar DK, Thanvi I, Joshi A, Subhakaran, Aseri S, et al. Falciparum malaria and pregnancy. *Indian J Malariol.* 1998;35(3):123-30.
 464. Kochar DK, Shubhakaran, Kumawat BL, Kochar SK, Halwai M, et al. Cerebral malaria in Indian adults: a prospective study of 441 patients from Bikaner, north-west India. *J Assoc Physicians India.* 2002;50:234-41.
 465. Dondorp AM, Lee SJ, Faiz MA, Mishra S, Price R, et al. The relationship between age and the manifestations of and mortality associated with severe malaria. *Clin Infect Dis.* 2008;47(2):151-7.
 466. Marsh K, Forster D, Waruiru C, Mwangi I, Winstanley M, et al. Indicators of life-threatening malaria in African children. *N Engl J Med.* 1995;332(21):1399-404.
 467. Brand NR, Opoka RO, Hamre KE, John CC. Differing Causes of Lactic Acidosis and Deep Breathing in Cerebral Malaria and Severe Malarial Anemia May Explain Differences in Acidosis-Related Mortality. *PLoS One.* 2016;11(9):e0163728.
 468. Gbotosho GO, Okuboyejo TM, Happi CT, Sowunmi A. *Plasmodium falciparum* hyperparasitaemia in Nigerian children: epidemiology, clinical characteristics, and therapeutic responses to oral artemisinin-based combination treatments. *Asian Pac J Trop Dis.* 2011;1(2):85-93.
 469. Hanson J, Lee SJ, Mohanty S, Faiz MA, Anstey NM, et al. A Simple Score to Predict the Outcome of Severe Malaria in Adults. *Clin Infect Dis.* 2010;50(5):679-85.
 470. McGready R, Kang J, Watts I, Tyrosvoutis MEG, Torchinsky MB, et al. Audit of antenatal screening for syphilis and HIV in migrant and refugee women on the Thai-Myanmar border: a descriptive study. *F1000Res.* 2014;3:123.
 471. Sweeting MJ, Sutton AJ, Lambert PC. What to add to nothing? Use and avoidance of continuity corrections in meta-analysis of sparse data. *Stat Med.* 2004;23(9):1351-75.
 472. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* 1986;7(3):177-88.
 473. Steyerberg EW, Vickers AJ, Cook NR, Gerds T, Gonen M, et al. Assessing the performance of prediction models: a framework for traditional and novel measures. *Epidemiology.* 2010;21(1):128-38.
 474. Myint HY, Tipmanee P, Nosten F, Day NP, Pukrittayakamee S, et al. A systematic overview of published antimalarial drug trials. *Trans R Soc Trop Med Hyg.* 2004;98(2):73-81.
 475. WorldWide Antimalarial Resistance Network. WWARN clinical trials publication library. 2017. [cited 20 March 2019]. Available from: <https://www.wwarn.org/tools-resources/literature-reviews/wwarn-clinical-trials-publication-library>.
 476. Otienoburu SD, Suay I, Garcia S, Thomas NV, Srisutham S, et al. An online mapping database of molecular markers of drug resistance in *Plasmodium falciparum*: the ACT Partner Drug Molecular Surveyor. *Malar J.* 2019;18:12.

477. Daru J, Zamora J, Fernández-Félix BM, Vogel J, Oladapo OT, et al. Risk of maternal mortality in women with severe anaemia during pregnancy and post partum: a multilevel analysis. *Lancet Glob Health*. 2018;6(5):e548-e554.
478. Haider BA, Olofin I, Wang M, Spiegelman D, Ezzati M, et al. Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: systematic review and meta-analysis. *BMJ*. 2013;346:f3443.
479. Gilder ME, Simpson JA, Bancone G, McFarlane L, Shah N, et al. Evaluation of a treatment protocol for anaemia in pregnancy nested in routine antenatal care in a limited-resource setting. *Glob Health Action*. 2019;12(1):1621589.
480. Bardaji A, Martinez-Espinosa FE, Arevalo-Herrera M, Padilla N, Kochar S, et al. Burden and impact of *Plasmodium vivax* in pregnancy: A multi-centre prospective observational study. *PLoS Negl Trop Dis*. 2017;11(6):e0005606.

Acknowledgement for copyright

Figure 1.1: Reprinted from World Malaria Report 2018, World Health Organization, Page 2, Copyright (2018).

Figure 1.4: Reprinted from The Lancet, Vol. 383 (9918), White NJ, *et al.*, Malaria, 723-35, Copyright (2014), with permission from Elsevier.

Box 2-3: Modified and reprinted from Methods for surveillance of antimalarial drug efficacy, World Health Organization, Page 7, Copyright (2009).

Box 8-1: Reprinted from Guidelines for the treatment of malaria. Third edition, World Health Organization, Page 73-4, Copyright (2015).

Appendices

Appendix 2-1. Standardized variable list for WWARN IPD meta-analysis on malaria in pregnancy

Study information (meta-data)

- Unique study ID
- Geographical location(s) of the study
- Study year(s)
- Study design
- Longitudinal (repeated malaria episodes) or not
- Obstetric outcomes are available or not
- Newborn information is available or not

Baseline patient data

Core variables

- Unique patient identifier
- Unique episode identifier
- Clinic site identifier if multi-centre study
- Date of enrolment
- Age at enrolment or date of birth of mother
- Estimated gestational age (EGA) at enrolment (calculated backwards)
- Estimation method(s) of gestational week used for patient
 - If ultrasound was used, EGA at time of examination and measurement used
 - If postnatal examination was used, hours after birth (or date).
- Estimated gestational age on day 0 of each episode
- Estimated date of delivery
- Date of first day of last menstrual period
- Parity (total number of babies born ≥ 28 weeks)
- Gravidity (total number of previous pregnancies plus 1 for current pregnancy)
- Body weight on day 0 of each episode (kg)

Optional variables

- Date of (or EGA at) first antenatal clinic visit
- Number of antenatal clinic visits during pregnancy
- First body weight measured and date (preferably in the first trimester)
- Height (cm)
- Mid upper arm circumference (cm)
- Number of previous miscarriages
- Number of previous stillbirths
- Previous history of neonatal death
- Previous history of having low-birth infants (<2,500 g)
- Previous history of pre-term delivery (<37 weeks day 0)
- Previous history of caesarean section
- Baseline haematocrit or haemoglobin at enrolment if participants are enrolled before malaria
- History of haematinics (iron and/or folic acid) use during pregnancy
 - Purpose (prophylaxis or treatment doses)
 - Dose (mg/day) if available
- Smoking during pregnancy
- Alcohol during pregnancy
- Marital status at time of enrolment
- Literacy
- Highest education
- Comorbidity/complication by end of pregnancy (Yes/No/Information not available)
 - Diabetes
 - Hypertension
 - Preeclampsia
 - Eclampsia
 - Epilepsy
 - Thyroid diseases

- Syphilis
- HIV sero-status
 - If assessed, ask date of test
 - If yes, ask CD4 count, viral load, antiretroviral treatment, sulphamethoxazole-trimethoprim
- History of non-malarial febrile illness during the pregnancy
 - If yes, ask date
- History (date) of malaria within 28 days before study enrolment
- History of antimalarial intake including IPTp within 28 days before study enrolment
- Bed net use

Clinical assessment including symptoms and examination

Core variables

- Symptomatic or asymptomatic
- Body temperature
- Fever history in the last 24 hours
- Days of fever
- Severity (signs of severe malaria by WHO definition)
- Clinical outcomes on day 28, 42, 63, at delivery and after delivery

Optional variables

- Fetal heart rate
- Fundal height
- Pulse rate
- Respiratory rate
- Blood pressure (systolic / diastolic)
- Hepatomegaly
- Splenomegaly
- Other symptoms
- Thermometer type used

Treatment

Core variables

- Date or day(s) after the initiation of the treatment
- Purpose of drug administration (treatment/IPT/rescue/other infection)
- Drug(s) received
- Dose
- Supervision of drug administration (meta-data)
- Source of treatment information (meta-data)

Optional variables

- Post treatment vomiting
- Concomitant medication
- Fat intake (meta-data)

Laboratory data

Core variables

- Diagnostics used for malaria
- Species
- Asexual *P. falciparum* parasitaemia count (and date)
- Haematology (haematocrit / haemoglobin, white blood cell) (and date)

Optional variables

- Assessment of co-infection (meta-data)
- Asexual *P. vivax* parasitaemia count (and date)
- Assessment of gametocytaemia (meta-data)
- Gametocytaemia count (and date)
- Biochemistry
- Placental malaria
 - "Diagnosis of placental malaria 1: Placental histopathology (Bulmer's classification)"
 - Inflammation score (I/II/III) (if available)
 - Pigment score (I/II/III) (if available)
 - Fixative (formalin/Streck/alcohol)
 - "Diagnosis of placental malaria 2: Placental blood/crush/stamp smear"

- "Diagnosis of placental malaria 3: Placental blood by rapid diagnostic test"
- "Diagnosis of placental malaria 4: Placental blood/tissue by PCR"
- "Diagnosis of placental malaria 5: Cord blood smear"
- "Diagnosis of placental malaria 6: Cord blood by rapid diagnostic test"
- "Diagnosis of placental malaria 7: Cord blood by PCR"
- Newborn peripheral blood smear / rapid diagnostic test
- Newborn haematocrit / haemoglobin

PCR genotyping result

Core variables

- PCR result

Optional variables

- Duration of follow-up period in which PCR is used for recurrence (meta-data)
- Marker(s) used for PCR (meta-data)

Ultrasound

Optional variables (details below are optional if EGA at examination is provided)

- Date of examination
- Crown rump length (CRL)
 - Length (cm/mm)
 - Estimated gestational age
 - Estimated date of delivery
- Biparietal diameter (BP)
 - Length (cm/mm)
 - Estimated gestational age
 - Estimated date of delivery
- Head circumference (HC)
 - Length (cm/mm)
 - Estimated gestational age
 - Estimated date of delivery
- Femur length (FL)

- Length (cm/mm)
- Estimated gestational age
- Estimated date of delivery
- Abdominal Circumference (AC)
 - Length (cm/mm)
 - Estimated gestational age
 - Estimated date of delivery

Obstetric outcomes

Core variables

- Date of birth (date of delivery or date of termination, whichever comes first)
- Estimated gestational age at delivery (or termination of pregnancy, whichever comes first)
- Last date of follow up
- Life status of mother at end of follow-up (alive / dead/ lost)
- Birth outcome
- Number of fetuses (singleton / twins or more)
- Life status of newborn, if delivered
- Birthweight (g)
- Date (or timing) of anthropometric measurement
- Gender of newborn
- External congenital abnormality of neonates
 - If yes -> number of major and number of minor, then free text description (optional)

Optional variables

- Place of delivery
- Onset of labour (induced/spontaneous)
- Prolonged preterm premature rupture of membranes (PPROM) (Yes/No)
- Delivery method
- Minimum precision of body weight scale
- Length of newborn at birth (cm)

- Head circumference of newborn at birth (cm)
- Apgar score at 1 minute
- Apgar score at 5 minutes
- Weight of placenta (g)
- Last date of follow-up of newborn (date of death)
- Life status of newborn at end of follow-up

Appendix 2-2. Waiver of ethics approval for secondary data use at IDDO/WWARN by

OxTREC

Oxford Tropical Research Ethics Committee

University of Oxford
Research Services, University Offices
Wellington Square, Oxford OX1 2JD
Tel. +44 (0)1865 (2)82106
E mail: oxtrece@admin.ox.ac.uk



Laura Merson
Centre for Tropical Medicine and Global Health
Nuffield Department of Medicine Research Building
University of Oxford
Old Road Campus
Roosevelt Drive
Headington
Oxford OX3 7FZ

09 November 2016

Dear Laura

Infectious Diseases Data Observatory (IDDO) data platforms and analyses

Thank you for your letter of 3 November 2016.

I can confirm that, on the understanding that the data platforms hosted by the IDDO (a) receive and hold only anonymised data that cannot be traced back to individuals, and (b) that those data have been obtained with the full knowledge and consent of the individuals concerned in accordance with the laws and ethical review processes of the countries of origin, these data platforms are exempt from ethical review by the Oxford Tropical Research Ethics Committee (OxTREC).

I can also confirm that analysis by IDDO scientists of the data held in the IDDO data platforms is exempt from ethical review by OxTREC, since this comprises secondary analysis of anonymised data that cannot be traced back to individuals.

Thus no further action is required from you regarding ethical approval for the next five years of the project. I would advise making contact with OxTREC again if the project extends beyond five years, so that we can confirm that the position with regard to ethical approval of such data platforms remains the same.

Yours sincerely

A handwritten signature in black ink, appearing to read 'Rebecca Bryant'.

Dr Rebecca Bryant
Research Ethics Manager, OxTREC

Appendix 2-3. Ethics approval for secondary clinical data collected at SMRU by

OxTREC

Oxford Tropical Research Ethics Committee

University of Oxford
Research Services, University Offices
Wellington Square, Oxford OX1 2JD
Tel. +44 (0)1865 (2)82106
E mail: oxtrece@admin.ox.ac.uk



Professor Francois Nosten
Shoklo Malaria Research Unit
68/30 Bantung Road, PO Box 46
Mae Sot, Tak 63110 Thailand

09 March 2018

Dear Professor Nosten

Full Title of Study: Retrospective analysis of hospital records and stored samples in Shoklo Malaria Research Unit field based hospital from 1986-2008
OXTREC Reference: 28-09

Thank you for your email of the 9th March 2018, in which you have included the annual report. The study continues to have OxTREC approval.

We will update our database accordingly.

Yours sincerely

A handwritten signature in black ink, appearing to read 'Rebecca Bryant'.

Dr Rebecca Bryant
Research Ethics Manager, OxTREC

Direct Line Tel: +44 (0)1865 (2)82106 Email: oxtrece@admin.ox.ac.uk
Web: <https://researchsupport.admin.ox.ac.uk/governance/ethics>

**Appendix 2-4. Ethics approval for secondary clinical data collected at SMRU by Tak
Province Community Ethics Advisory Board (T-CAB)**

Tak Province Community Ethics Advisory Board (T-CAB)
Mae Sot, Tak Province
Thailand



29th April 2015

Dr Rose McGready
Shoklo Malaria Research Unit
68/30 Bann Tung Road
Maesot, Tak Province
Thailand

Dear Dr McGready

Full Title of Study: "SMRU Antenatal and birth records and reporting observational outcomes.

Meeting held on 29th April 2015"

T-CAB reference: TCAB-4/1/2015

The T-CAB reviewed the above study at the meeting held on 29th April 2015.

The committee confirms its favorable opinion of the study.

Yours sincerely

A handwritten signature in blue ink, appearing to read 'Yar Zar Hein', written over a horizontal line.

Yar Zar Hein
Meeting Chair

Appendix 3-1. Summary of the study design and reported efficacy outcomes

Study Country Study year	Study design Treatment: N	Trimester	Efficacy endpoint reported	Intermittent preventive treatment	Dosing of treatment
Naing, 1988 Myanmar 1985-1986	RCT Q: 23 AQ: 19	2,3	Fever clearance time Parasite clearance time	Not applicable (not Q: quinine dihydrochloride 10mg salt/kg x 3 times/day for Sub-Saharan Africa)	7 days orally or intravenously depending on the clinical condition
Harinasuta, 1990 Thailand INA (conference abstract)	RCT Q: 82 MFQ: 85	1,2,3	Parasite clearance on day 7	Not applicable (not Q: quinine sulfate 600mg x 3times/day for 7 days Sub-Saharan Africa)	
Nosten, 1993 Thailand 1989-1990	RCT Q5: 16 Q5 + spiramycin: 16	2,3	Recurrence rate within 28 days Parasite clearance time	Not applicable (not Q: quinine sulphate 30mg salt/kg/day for 5 days Sub-Saharan Africa)	spiramycin: 1.2M IU x 3 times/day for 5 days
Sowunmi, 1998 Nigeria 1994-1997	RCT AM: 23 AMMQ: 22	2,3	Cure rate on day 14 /28 Fever clearance time Parasite clearance time Peripheral parasitaemia of newborns at birth	Information not available	AM: 3.2 mg/kg given intramuscularly on day 0, followed by 1.6 mg/kg/day for 4 days AMMQ: single dose of 3.2 mg/kg AM intramuscularly on day 0, followed by 7.5mg/kg/day MQ for 2 days
Bounyasong, 2001 Thailand 1995-1998	RCT ASMQ: 29 Q: 28	2,3	Recrudescence within 28 days Fever clearance time Parasite clearance time	Not applicable (not AS: Oral artesunate 2 mg/kg for the first dose, 1 mg/kg Sub-Saharan Africa)	every 12 hours orally for 5 days minimally (The treatment might be extended until the parasites were absent and the patients had clinical improvement) MQ: 25mg/kg (split on day 6: 15 mg/kg and 6 hours later 10 mg/kg) Q: Quinine sulfate 10 mg/kg x 3 times/day for at least 7 days until the patients had clinically recovered

Appendix 3-1 continued.

Study Country Study year	Study design Treatment: N	Trimester	Efficacy endpoint reported	Intermittent preventive treatment	Dosing of treatment
McGready, 2000 Thailand 1995-1997	RCT Q: 42 ASMQ: 66	2,3	Cure rates on day 63 Fever clearance by 48h Parasite clearance by 48h	Not applicable (not Sub-Saharan Africa)	Q: 10 mg salt/kg x 3 times/day for 7 days AS (Guilin): 4mg/kg/day for 3 days MQ (Lariam): 25 mg base/kg given as 15 mg/kg on day 1 and 10 mg/kg on day 2
McGready, 2001a Thailand 1997-2000	RCT QC: 65 AS: 64	2,3	Cumulative cure rate on day 42 Fever clearance by 48h Parasite clearance by 48h Gametocyte carriage	Not applicable (not Sub-Saharan Africa)	Q: 10 mg salt/kg x 3 times/day for 7 days Clindamycin: 5 mg/kg x 3 times/day for 7 days AS (Guilin): 2 mg/kg daily on days 0, 1, 2, 3 and 4 and 1 mg/kg daily on days 5 and 6 (total 10mg/kg)
McGready, 2005 Thailand 2001-2003	RCT AAP: 39 Q: 42	2,3	Cumulative cure rate at delivery or on day 63 Fever clearance on day 2, 3 & 4 Parasite clearance time Gametocyte carriage	Not applicable (not Sub-Saharan Africa)	AS (Guilin): 4mg/kg/day for 3 days (50mg tablet) AP (Malarone): (atovaquone 20 mg/kg/day and 8 mg/kg/d) for 3 days (250+100mg tablet) With 200mL of chocolate milk (8% fat) Q: 10 mg salt/kg x 3 times/day for 7 days
Adam, 2004a Sudan 2002-2003	RCT Q (low dose): 18 Q (standard dose): 24	2,3	Failure rate within 28 days Fever clearance time Parasite clearance time	Information not available	Low dose group: quinine 10mg/kg x 2 times/day for 7 days Standard dose group: quinine 10mg/kg x 3 times/day for 7 days Quinine was administered intravenously until patients could tolerate
Kalilani, 2007 Malawi 2003-2004	RCT ASSP: 47 SP: 47 SP + azithromycin: 47	2	Recrudescence rate within 28 days Fever clearance on day 3 Parasite clearance on day 2 & 3 Parasitaemia at delivery Prevalence of placental malaria	The second dose of the same drug was administered at least 4 weeks after the first dose instead of IPTp	AS: 200mg/day for 3 days SP: 1500+75mg as a single dose
McGready, 2008 Thailand 2004-2006	RCT AL: 125 AS: 128	2,3	Cumulative cure rate at delivery or on day 42 Fever clearance time Parasite clearance time Placental / cord blood malaria	Not applicable (not Sub-Saharan Africa)	AL (Coartem): (80+480mg) x 2 times/day for 3 days (at 0, 8, 24, 36, 48 and 60 h) With 250mL of chocolate milk containing 7g of fat AS (Guilin): 2mg/kg x 1 time/day for 7 days (50mg tablet)

Appendix 3-1 continued.

Study Country Study year	Study design Treatment: N	Trimester	Efficacy endpoint reported	Intermittent preventive treatment	Dosing of treatment
Mutabingwa, 2009 Tanzania 2004-2006	RCT ASAQ: 83 AQSP: 80 CD: 81 SP: 28	2,3	Recurrence rate within 28 days Gametocyte carriage	Information not available	AQ: 10 mg/kg/day for 3 days AS: 4 mg/kg/day for 3 days
Kaye, 2008 Uganda 2006	RCT AL: 57 CD: 57	2,3	Cure rate on day 28 Fever clearance Parasite clearance	Information not available	AL (Coartem): (80+400mg) x 2 times/day for 3 days *Assumed to be 80+480mg
Piola, 2010 Uganda 2006-2009	RCT AL: 152 Q: 152	2,3	Cure rate and cumulative recurrence by day 42 Cure rate and cumulative recurrence by delivery Fever clearance on day 0, 1, 2 & 3 Parasite clearance on day 2 & 3 Gametocyte clearance within 7 days Gametocyte carriage Placental malaria	IPTp was interrupted while study period	AL: (80+480mg) x 2 times/day for 3 days (at 0, 8, 24, 36, 48 and 60 h) With 200mL milk Q: quinine hydrochloride 10 mg base/kg x 3 times/day for 7 days (300 mg of the base per tablet)
Carmona- Fonseca, 2013 Colombia 2008-2011	RCT AL: 15 ASMQ: 15	2,3	Cure rate on day 28	Not applicable (not Sub-Saharan Africa)	AL (80mg+480mg) x 2times/day for 3 days AS 4 mg/kg x 1 time/day for 3 days (50mg tablet) MQ 25 mg base/kg, divided into 2 or 3 days (250mg tablet)
D'Alessandro, 2016 Burkina Faso, Ghana, Malawi and Zambia 2010-2013	RCT AL: 880 ASAQ: 842 ASMQ: 848 DP: 853	2,3	Cure rate on day 63 Parasite clearance on day 1 & 2 Prevalence of placental malaria Gametocytaemia	Some patients received IPTp-SP before the trial IPTp-SP was restarted after 63 days	AL (Coartem): (80+480mg) x 2times/day for 3 days With recommendation to take high-fat food or drinks together ASAQ (Winthrop): (200+540mg) x 1 time/day for 3 days ASMQ (Farmanguinhos): (200+440mg/day) x 1 time/day for 3 days DP (Eurartesim): (120+960mg) x 1time/day for 3 days

Appendix 3-1 continued.

Study Country Study year	Study design Treatment: N	Trimester	Efficacy endpoint reported	Intermittent preventive treatment	Dosing of treatment
Osarfo, 2017 Ghana 2011-2012	RCT ASAQ: 205 DP: 212	2,3	Cure rate on day 42 Cure rate on day 28 Placental malaria	IPTp-SP was not given during the study period (42 days)	ASAQ (Arsuamoon) AS 4mg/kg and AQ 10mg/kg in twelve hourly doses over 3 days DP (P-alaxin) Total dosing of 6.75mg/kg DHA and 55mg/kg PPQ over 3 days rounded to the nearest half tablet
Onyamboko, 2015 DRC 2013-2014 (conference abstract)	RCT AL: 48 AL5: 48	2,3	Cure rate on day 42 Placental malaria	Information not available	AL for 3 days (6 doses) AL for 5 days (10 doses) With milk
Ukah, 2015 Nigeria 2013	RCT AL: 75 ASAQ: 75	2,3	Cure rate on day 28 Symptom clearance time Parasite clearance by day 3	IPTp-SP x 2	AL (GloatemForte): (80+480mg) x 2 times/day for 3 days ASAQ (Winthrop): (100+270mg) x 2 times/day for 3 days
Iribhogbe, 2017a Nigeria 2013-2014	RCT AL: 40 ASAQ: 40	2,3	Recurrence within 28 days Fever clearance on day 3 Parasite clearance on day 3	Information not available	AL: (80+480mg) x 2 times/day for 3 days ASAQ: (300/810mg) x once a day for 3 days
Anvikar, 2018 India 2010-2013	RCT ASMQ (123) ASSP (125)	2,3	Cure rate on day 63 Recurrence by day 63	Not applicable (not Sub-Saharan Africa)	AS: 200mg/day for 3 days MQ: 440mg/day for 3 days AS: 200mg/day for 3 days SP: 1500+75mg single dose on day1

Appendix 3-1 continued.

Study Country Study year	Study design Treatment: N	Trimester	Efficacy endpoint reported	Intermittent preventive treatment	Dosing of treatment
NCT01054248 Thailand 2010-2016	RCT AL4 (335) ASMQ (335) DP (335) *Planned number of participants including vivax malaria patients	2,3	Cure rate on day 63 or until delivery Gametocyte carriage Placental malaria	Not applicable (not AL (Coartem): (100+600mg) x 2 times/day for 4 days (at 0, Sub-Saharan Africa)	AS (Guillain Factory no.1, 50mg/tablet): 4mg/kg/day for 3 days (rounded to the nearest quarter tablet) MQ (Mequin, 250mg/tablet): 8mg/kg/day for 3 days (rounded to the nearest quarter tablet) ASMQ (Farmanguinhos: 100+220mg/tablet): 2 tablets/day for 3 days (if this fixed combination is available) DP (Holley-Cotec: 40+320mg/tablet): (2.4 mg/kg DHA and 20 mg/kg PPQ) x 1 time/day for 3 days (rounded to the nearest half tablet)
NCT02909712 Tanzania 2016-	RCT DP (50) SP (50) * Planned	2,3	Cure rate on day 28	Information not available	DP (Eurartesim): (120+960mg) x 1 time/day for 3 days
McGready, 2003a Thailand 2000-2001	PK AAP: 24	2,3	Cure rate on day 28 Fever clearance by 48h Parasite clearance time	Not applicable (not AS (Guilin): 4mg/kg/day for 3 days (50mg tablet) Sub-Saharan Africa)	AP (Malarone): (atovaquone 20 mg/kg/day and 8 mg/kg/d) for 3 days (250+100mg tablet) With 200mL of chocolate milk (8% fat)
Adam, 2012 Sudan 2007-2008	PK study DP: 12 (control: 12 matched non- pregnant women)	2,3	Cure rate on day 63 Fever clearance within 2 days Parasite clearance within 2 days	Information not available	DHA/PQ-phosphate (DuoCotecxin): 2.4 mg of DHA/kg/day and 20 mg of PQ (as phosphate)/kg/day rounded to the nearest half tablet for 3 days

Appendix 3-1 continued.

Study Country Study year	Study design Treatment: N	Trimester	Efficacy endpoint reported	Intermittent preventive treatment	Dosing of treatment
Onyamboko, 2011 DRC 2007-2008	PK ASSP: 24 (control: 14 non-pregnant women)	2,3	Recurrence within 28 days Parasite clearance by 12 h	Information not available	AS (Guilin): 200mg once SP: 1725mg 24 hours after AS administration
McGready, 2012 Thailand 2008-2009	PK AS: 20	2,3	Cumulative cure rate on day 63 Fever clearance time Parasite clearance time	Not applicable (not Sub-Saharan Africa)	Group 1: intravenous AS (Guilin) 4mg/kg on day 0 followed by oral AS 4mg/kg for 6 days Group 2: oral AS (Guilin) 4mg/kg on day 0 followed by intravenous AS 4mg/kg on day 1 followed by oral artesunate 4mg/kg daily for 5 days
Rijken, 2011 Thailand 2008	PK DP: 24 (control: 24 matched non- pregnant women)	2,3	Cumulative cure rate on day 63 Fever clearance Parasite clearance Placental malaria	Not applicable (not Sub-Saharan Africa)	DP (Holley Pharm) Total dosing of 6.5 mg/kg DHA and 51.2mg/kg PPQ over 3 days The tablets (40+320mg) were divided to the nearest quarter
Valea, 2014 Burkina Faso 2008-2009	PK ASMQ: 24 (control: 24 non-pregnant women)	2,3	Cure rate on day 63 Parasite clearance on day 3	IPTp-SP x 2	ASMQ (Farmanguinhos) AS: 3.6mg/kg x 1 time/day for 3 days MQ: 8mg/kg x 1 time/day for 3 days (<50kg: 150+330mg/day 50-60kg: 200+440mg/day >60kg: 250+550mg/day)
Juma, 2014 Kenia 2012 (conference abstract)	PK AL: 45 (control: 25 non-pregnant women)	2,3	Treatment response on day 28	Information not available	AL: (80+480mg) x 2 times/day for 3 days (at hours 0, 8, 24, 36, 48 and 60)

Appendix 3-1 continued.

Study Country Study year	Study design Treatment: N	Trimester	Efficacy endpoint reported	Intermittent preventive treatment	Dosing of treatment
Mosha, 2014 Tanzania 2012	PK AL: 33	2,3	Cure rate / recurrence rate on day 42 Number of recrudescence	Information not available	AL (Coartem): (80+480mg) x 2 times/day for 3 days (at 0, 8, 24, 36, 48 and 60 h) With 200mL of milk containing 4.5g of fat
Nyunt, 2016 Uganda 2013-2014	PK AL: 30 (control: 30 non pregnant adults)	2,3	Cure rate / recurrence rate on day 42 Parasite clearance on day 2	SP x 2 (16-24w, 28-36 weeks)	AL (Coartem): (80+480mg) x 2 times/day for 3 days With 200mL milk
Mutagonda, 2017 Tanzania 2014-2015	PK AL: 92	2,3	Cure rate / recurrence rate on day 28 (Cumulative cure rate on day 28 was reported in sub-cohort [66])	Information not available	AL (Coartem): (80+480mg) x 2 times/day for 3 days (at 0, 8, 24, 36, 48 and 60 h) With milk containing 4.5g of fat
Adam, 2004b Sudan 2000-2002	Single-arm Q: 26	1	Cure rate on day 28 Fever clearance within 3 days Parasite clearance within 3 days	Information not available	Q: 30 mg salt/kg/day for 7 days Quinine was administered intravenously until patients could tolerate
Adam, 2004c Sudan 1997-2001	Single-arm AM im: 28	1,2,3	Number of recurrence Parasite clearance on day 7	Information not available	AM(Kunming): given intramuscularly, 80mg initially then 80mg after 12 h, then 80mg daily (total 480 mg)
Adegnika, 2005 Gabon 2003-2004	Single-arm Q: 50	2,3	Cure rate on day 28 Cure rate on day 56 Prevalence of placental malaria	Information not available	Q:10 mg salt/kg x 3 times/day for 7 days rounded to the nearest half tablet of 300mg quinine

Appendix 3-1 continued.

Study Country Study year	Study design Treatment: N	Trimester	Efficacy endpoint reported	Intermittent preventive treatment	Dosing of treatment
Adam, 2006 Sudan 2004-2005	Single-arm ASSP: 32	2,3	Cure rate on day 28 Fever clearance within 2 days Parasite clearance within 2 days	Information not available	AS: 200mg/day for 3 days on days 0—2 SP: 1500+75mg given as a single dose on day 0
Ndiaye, 2011 Senegal 2009-2011 (conference abstract)	Single-arm ASAQ: 28	2,3	Recurrence rate within 42 days Parasite clearance Fever clearance Placental malaria Congenital malaria	Information not available	ASAQ (Coarsucam) AS: 200mg/day for 3 days AQ: 540mg/day for 3 days
Iribhogbe, 2017b Nigeria 2013-2014	Single-arm ASAQ: 50	2,3	Recurrence within 28 days	INA	AS: 4mg/kg/day for 3 days AQ: 10mg/kg/day for 3 days
McGready, 1998a Thailand 1992-1996	Observational cohort AS: 61 AMMQ: 3 ASMQ: 26	1,2,3	Recurrence within 28 days Parasite clearance on day 7 Gametocyte carriage	Not applicable (not AS (Guilin) Sub-Saharan Africa)	4 mg/kg on day 0, 2 mg/kg on days 1 and 2, and 1 mg/kg on days 3-6 (total dose of 12 mg/kg) or 4 mg/kg/day for 3 days Mefloquine (Laham): 25 mg/kg, usually as a split regimen on days 5 and 6, was added when there had been no previous intake of mefloquine during the pregnancy AM (Kunming): a total dose of 12 mg/kg over 3 or 5 d. AM was given intramuscularly if the patient could not tolerate oral treatment

Appendix 3-1 continued.

Study Country Study year	Study design Treatment: N	Trimester	Efficacy endpoint reported	Intermittent preventive treatment	Dosing of treatment
McGready, 1998b Thailand 1991-1996	Observational cohort Q Primary treatment: 113 Retreatment: 91	1,2,3	Recurrence within 42 days Parasite clearance on day 3 Fever clearance at 48h Gametocyte carriage	Not applicable (not Q: 30 mg salt/kg/day for 7 days Sub-Saharan Africa)	
McGready, 2001b Thailand 1992-2000	Observational cohort artemisinins Primary treatment: 229 Retreatment: 310	1,2,3	Cumulative recurrence rate within 42 days Proportion of recurrence within 42 days Fever clearance time Parasite clearance time Gametocyte carriage	Not applicable (not AS (Guilin): 12-14mg/kg in total over 3 or 7 days Sub-Saharan Africa)	MQ (Lariam): 25 mg base/kg given over 2 days AP (Malarone): (atovaquone 20 mg/kg/day and 8 mg/kg/d) for 3 days
Laochan, 2015 Thailand 1994-2009	collation of an observational cohort and interventional studies† AS: 151, AAP: 11, AC: 143, AL: 43, ASMQ: 5, DP 29, Q: 476, QC: 29 MQ: 22	1,2,3	Description of recurrent episodes of malaria with PCR	Not applicable (not Q: 10 mg salt/kg x 3 times/day for 7 days Sub-Saharan Africa)	AS (Guilin): 2 mg/kg x 1 time/day for 7 days (total dose 10–16 mg/kg) AS (Guilin): 4mg/kg x 1 time/day for 3 days AL (Coartem): (80+480mg) x 2 times/day for 3 days MQ: 25 mg/kg stat dose or over two days DP (Duo-Cotexcin): DHA 3 mg/kg, PPQ 15–17 mg/kg x 1 time/day for 3 days AP (Malarone): (atovaquone 20 mg/kg/day and 8 mg/kg/d) for 3 days Clindamycin: 300mg x 3 times/day for 7 days
McGready, 2002 Thailand 1995-2000	observational Q novel: 25 retreatment: 209	1	Recurrence within 28 days Fever clearance Parasite clearance Gametocyte carriage	Not applicable (not Q: 30 mg salt/kg/day for 7 days Sub-Saharan Africa)	

Appendix 3-1 continued.

Study Country Study year	Study design Treatment: N	Trimester	Efficacy endpoint reported	Intermittent preventive treatment	Dosing of treatment
McGready, 2003b Thailand 1999-2001	Observational cohort AAP: 27	1,2,3	Recrudescence within day 42 Fever clearance by 48h Parasite clearance time Gametocyte carriage Placental malaria	Not applicable (not AS: 4mg/kg/day for 3 days or 2mg/kg/day for 7 days (with Sub-Saharan Africa)	a loading dose of 4 mg/kg in hyperparasitaemic patients with >4%) AP: (atovaquone 20 mg/kg/day and 8 mg/kg/d) for 3 days
Villegas, 2005 Venezuela 2002-2005 (conference abstract)	Observational cohort ASMQ: 27	INA	INA (efficacy)	Not applicable (not ASMQ for 2-3 days Sub-Saharan Africa)	
Rijken, 2008 Thailand 2006-2007	Observational cohort DP: 62	1,2,3	Cumulative cure rate by day 63 Fever clearance on day 2 Parasite clearance time	Not applicable (not DP (Holley Pharm) Sub-Saharan Africa)	Total dosing of 6.5 mg/kg DHA and 51.2mg/kg PPQ over 3 days (40+320mg tablet)
Rulisa, 2012 Rwanda 2007-2009	Observational cohort AL: (1072)†	1,2,3	Cure rate on day 56 Recurrence within 56 days	23.4% received IPTp-SP	Rwanda National Guideline 22 patients enrolled PK study: AL: 80+480mg at 0 hr, 8, 24, 36, 48 and 60 hr with a glass of milk or a small cake
Kalilani, 2013 and Cohee, 2016 Malawi 2009-2010	Observational cohort AL: 56	1,2	Recrudescence rate by delivery Placental malaria Temporal relationship between peripheral parasitaemia and placental malaria	IPTp-SP x 3	AL: (80+480mg) x 2 times/day for 3 days (at 0h, 8h, day2 AM, day2 PM, day3 AM, day3 PM)

AAP: artesunate-atovaquone-proguanil, AC: artesunate-clindamycin, AL: artemether-lumefantrine, AQ: amodiaquine, AS: artesunate, CD: chlorproguanil-Dapsone, CHQ: chloroquine, DP: dihydroartemisinin(DHA)-piperazine(PPQ), DRC: Democratic Republic of Congo, ECG: electrocardiography, INA: information not available, MQ: mefloquine, N: number of pregnant women included, PCR: polymerase chain reaction, PK: pharmacokinetic study, Q: quinine, QC: quinine-clindamycin, RCT: randomized controlled trial, SP: sulfadoxine-pyrimethamine. *Planned number of participants in clinical trial registry. NCT01054248 includes *P. vivax* patients. † This number includes patients without parasitological confirmation, 22 were enrolled in a PK study with confirmed parasitaemia. The exact number of parasitologically confirmed patients is not known. ‡ This study included patients enrolled in other studies.

Appendix 3-2. Summary of the quality of studies

Study	Study design	Randomisation	Allocation concealment	Blinding	Loss to follow-up for efficacy assessment
Naing, 1988	RCT	Unclear: 'Patients were randomised [...] depending on clinical severity and parasite density.'	Unclear	Unclear	Unclear
Harinasuta, 1990*	RCT	INA	INA	INA	0/82 (Q) 0/85 (MQ)
Nosten, 1993	RCT	Yes, but the method is not specified.	Unclear	At least patients were blinded by using identical placebo.	11/43 were dropped out.
Sowunmi, 1998	RCT	Yes, but the method is not specified.	Unclear	Unclear	Unclear
Bounyasong, 2001	RCT	Yes, but the method is not specified.	Unclear	Unclear	1/30 (Q) 2/30 (ASSP)
McGready, 2000	RCT	Block randomisation	Unclear	Open-label	0/66 (ASMQ) 0/42 (Q)
McGready, 2001a	RCT	Block randomisation	Unclear	Open-label	19/65 (QC) 17/64 (AS)
McGready, 2005	RCT	Computer-generated block randomisation	Sealed envelope	Open-label	0/39 (AAP) 0/42 (Q)
Adam, 2004a	RCT	Yes, but the method is not specified.	Unclear	Unclear	7/25 (20mg/kg/day) 2/26 (30mg/kg/day)
Kalilani, 2007	RCT	Block randomisation	Sealed envelope	Open-label Lab technicians were blinded.	8/47 (ASSP) 7/47 (SP) 5/47 (SP + azithromycin)
McGready, 2008	RCT	Block randomisation	Sealed envelope	Open-label Lab technicians and patient examiners were blinded.	5/125 (AL) 3/125 (AS)
Mutabingwa, 2009	RCT	Block randomisation	Sealed envelope	Open-label Microscopists were blinded.	8/83 (ASAQ) 8/80 (AQSP) 4/81 (CD) 2/28 (SP)
Kaye, 2008	RCT	Computer-generated random number	Sealed envelope	Open-label	5/57 (AL) 5/57 (CD)
Piola, 2010	RCT	Computer-generated permuted block of eight	Sealed envelope	Open-label Microscopists and clinical investigators were blinded.	10/152 (Q) 6/152 (AL)
Carmona-Fonseca, 2013	RCT	Ballot system	Yes	Unclear	0/15 (AL) 0/15 (ASMQ)
D'Alessandro, 2016	RCT	Block randomisation	Sealed envelope	Open-label Lab technicians (interpretation of PCR) were blinded.	50/880 (AL) 51/842 (ASAQ) 94/853 (DP) 77/848 (ASMQ)
Osarfo, 2017	RCT	Computer-generated random number	Sealed envelope	Open-label. Microscopists were blinded.	24/206 (ASAQ) 18/212 (DP)
Onyamboko, 2015*	RCT	Yes, but the method is not specified.	INA	Open-label	INA
Ukah, 2015	RCT	Computer-generated	Only pharmacy had access to the allocation sequence.	Double blind	3/75 (AL) 7/75 (ASAQ)

Appendix 3-2 continued.

Study	Study design	Randomisation	Allocation concealment	Blinding	Loss to follow-up for efficacy assessment
Iribhogbe, 2017a	RCT	Balloting	Unclear	Open-label	2/40 (AL)
Anvikar, 2018	RCT	Permuted block of 12	Sealed envelope	Open-label	3/40 (ASAQ)
NCT01054248*	RCT	Computer generated permuted block of 15	sealed envelope	Open-label	4/123 (ASMQ)
				Microscopists were blinded.	2/125 (ASSP)
NCT02909712*	RCT	INA	INA	Assessors	INA
McGready, 2003a	PK study	Not applicable	Not applicable	Unclear	8/24
Adam, 2012	PK study	Not applicable	Not applicable	Unclear	0/12
Onyamboko, 2011	PK study	Not applicable	Not applicable	Unclear	0/26
McGready, 2012	PK study	No: assigned alternatively	Not applicable	Unclear	0/20
Rijken, 2011	PK study	Not applicable	Not applicable	Unclear	2/24 (delivery)
Valea, 2014	PK study	Not applicable	Not applicable	Unclear	1/24
Juma, 2014*	PK study	Not applicable	Not applicable	Open-label	INA
Mosha, 2014	PK study	Not applicable	Not applicable	Unclear	0/33
Nyunt, 2016	PK study	Not applicable	Not applicable	Open-label	0/30
Mutagonda, 2017	PK study	Not applicable	Not applicable	Unclear	10/92
Adam, 2004b	Single-arm	Not applicable	Not applicable	Unclear	0/26
Adam, 2004c	Single-arm	Not applicable	Not applicable	Unclear	0/28
Adegnika, 2005	Single-arm	Not applicable	Not applicable	Unclear	0/50 (day 28)
					9/50 (day 56)
Adam, 2006	Single-arm	Not applicable	Not applicable	Unclear	0/32
Ndiaye, 2011*	Single-arm	Not applicable	Not applicable	Open-label	0/27
Iribhogbe, 2017b	Single-arm	Not applicable	Not applicable	Open-label	8/50
McGready, 1998a	Observational cohort	Not applicable	Not applicable	Unclear	5/83 (all)
McGready, 1998b	Observational cohort	Not applicable	Not applicable	Unclear	50/372 to delivery (including Q and MQ)
McGready, 2001b	Observational cohort	Not applicable	Not applicable	Unclear	47/461 to delivery
Laochan, 2015	Collation of observational cohorts and RCTs [40-42, 45, 58, 61, 61, 80, 82	refer to each study	refer to each study	refer to each study	refer to each study
McGready, 2002	Observational cohort	Not applicable	Not applicable	Unclear	54/300 to delivery (including Q and CHQ)
McGready, 2003b	Observational cohort	Not applicable	Not applicable	Unclear	6/27
Villegas, 2005*	Observational cohort	Not applicable	Not applicable	INA	INA
Rijken, 2008	Observational cohort	Not applicable	Not applicable	Unclear	5/50 (delivery)
Rulisa, 2012	Observational cohort / PK study	Not applicable	Not applicable	Unclear	Unclear
Kalilani, 2013	Observational cohort	Not applicable	Not applicable	Unclear	120/450 (delivery) including those without malaria

AAP: artesunate-atovaquone-proguanil, AL: artemether-lumefantrine, AQ: amodiaquine, AS: artesunate, CHQ: chloroquine, CD: chlorproguanil-Dapsone, DP: dihydroartemisinin-piperaquine, INA: information not available (conference abstract or registered trial). MQ: mefloquine, PK: pharmacokinetic, Q: quinine, QC: quinine-clindamycin, RCT: randomized controlled trial, SP: sulfadoxine-pyrimethamine. * conference abstract or registered trial.

Appendix 3-3. Summary of the quality of observational cohort studies by Newcastle-Ottawa scale

Study	Total score	Selection				Comparability		Outcome		
		1	2	3	4	1	2	1	2	3
McGready, 2003a	8	b	-	a	a	Yes	Yes	b	a	b
Adam, 2012	7	a	-	a	a	Yes	No	b	a	a
Onyamboko, 2011	8	a	-	a	a	Yes	Yes	b	a	a
McGready, 2012	8	a	-	a	a	Yes	Yes	b	a	a
Rijken, 2011	8	a	-	a	a	Yes	Yes	b	a	b
Valea, 2014	8	a	-	a	a	Yes	Yes	b	a	b
Juma, 2014*	8	a	-	a	a	Yes	Yes	b	a	a
Mosha, 2014	7 (8) ^a	a	-	a	a	Yes	No ^a	b	a	a
Nyunt, 2016	7 (8) ^a	a	-	a	a	Yes	No ^a	b	a	a
Mutagonda, 2017	8	a	-	a	a	Yes	Yes	b	a	b
Adam, 2004b	8	b	-	a	a	Yes	Yes	b	a	a
Adam, 2004c	8	b	-	a	a	Yes	Yes	b	a	a
Adegnika, 2005	8	a	-	a	a	Yes	Yes	b	a	a
Adam, 2006	8	a	-	a	a	Yes	Yes	b	a	a
Ndiaye, 2011*	7 (8) ^a	a	-	a	a	Yes	No ^a	b	a	a
Iribhogbe, 2017b	8	a	-	a	a	Yes	Yes	b	a	b
McGready, 1998a	7	b	-	a	a	Yes	No	b	a	b
McGready, 1998b	8	b	-	a	a	Yes	Yes	b	a	b
McGready, 2001b	8	b	-	a	a	Yes	Yes	b	a	b
McGready, 2002	8	a	-	a	a	Yes	Yes	b	a	b
McGready, 2003b	7	b	-	a	a	Yes	Yes	b	a	c
Villegas, 2005*	INA									
Rijken, 2008	8	b	-	a	a	Yes	Yes	b	a	b
Rulisa, 2012	5	a	-	a/b	b	No	Yes	b	a	d
Kalilani, 2013	5 (7) ^a	a	-	a	a	No ^a	No ^a	b	a	c

INA: information not available (because the studies with * have not been fully published).

^aNot available in publication, but available in IPD.

Selection 2 is not relevant for all studies as these are not comparative studies. Total score is thus 0-8.

Comparability: baseline parasitaemia and parity (or gravidity) are regarded as the most important (1) and an additional factor (2) for the efficacy outcome.

Outcome 3: if the proportion of lost to follow-up was >20%, it was judged as (c).

Appendix 3-4. The number of study arms with PCR-corrected outcomes for each comparison of drugs

	Q	QC	AS	AM	AL	ASAQ	ASMQ	DP	AAP	AC	ASSP	AMMQ
Q	2 (8)				1		2		1			
QC			1									
AS			1 (1)		1							
AM				1								(1)
AL					7 (1)	1 (2)	2 [1]	1 [1]				
ASAQ						2 (1)	1	1 (1)				
ASMQ							1 (2)	1 [1]			1	
DP								3 [1]				
AAP									2			
AC												
ASSP											2 (1)	
AMMQ												(1)

The number of studies in which PCR-corrected outcomes were not available is shown in round brackets. The number of unpublished registered trials is shown in square brackets. The numbers of non-comparative studies were shown on the diagonal. Two observational studies which include more than five treatment groups (McGready, 2001b and Laochan, 2015) were excluded from this figure. AAP: artesunate + atovaquone-proguanil, AC: artesunate + clindamycin, AL: artemether-lumefantrine, AM: artemether, AS: artesunate, AQ: amodiaquine, MQ: mefloquine, SP: sulfadoxine-pyrimethamine, DP: dihydroartemisinin-piperaquine, Q: quinine, QC: quinine + clindamycin.

Appendix 3-5. Characteristics of women enrolled in the treatment efficacy studies

Study	Treatment group	N	Age (year)	Weight (kg)	Gestational age at malaria (week)	Gravidity	Parity	Parasitaemia (/μL)	Haemoglobin (g/dL)	Body temperature (°C)	Note
Naing, 1988	Q	23	26.7 ± 1.2	NA	2nd tri (50%) 3rd tri (50%)	NA	0 (41%) 1–3 (31%) ≥4 (29%)	Log mean 4.3 ± 0.3	NA	NA	Gestational age and parity includes patients treated with amodiaquine. Conference abstract
Harinasuta, 1990	Q	82	NA	NA	1st tri (9%) 2nd tri (59%) 3rd tri (33%)	NA	NA	NA	NA	NA	
Nosten, 1993	Q5	16	28 ± 6.8	48 ± 5.1	19.3 ± 6.5	NA	4*	631‡ (91–8256)	Hct 26 ± 4 %	NA	
	Q5+spiramycin	16	26 ± 6.2	49 ± 5.5	21.7 ± 9		2*	537‡ (53–5512)	Hct 28 ± 5 %		
Sowunmi, 1998	AM	23	29.9 ± 6.1 (21–41)	63.9 ± 12.1 (50–119)	NA	NA	0 (65%) 1 (13%) 2 (9%)	29719‡ (1674–250138)	Anaemia ^a 30%	38.1 ± 0.2 (37.5–39.0)	
	AM-MQ	22	28.9 ± 5.1 (20–40)	64.1 ± 11.9 (53–121)			≥3 (13%) 0 (64%) 1 (18%) 2 (5%) ≥3 (14%)	28044‡ (2001–199992)	Anaemia ^a 30%	38.1 ± 0.1 (36.7–39.3)	
Bounyasong, 2001	Q	29	27.2	58.7	27.4	NA	1.6	1313.35 /200WBC	Hct 35.2 %	NA	Body weight before pregnancy.
	ASMQ	28	26.1	57.1	26.7		1.4	1329.68 /200WBC	Hct 34.3 %		
McGready, 2000	ASMQ	66	24* (15–37)	48* (36–68)	24* (12–40)	NA	1 (27%) 1 (29%)	11651‡ (32–241127)	Hct 29.4* (17.5–39.0) %	Fever 15%	
	Q	42	23* (16–36)	50* (41–67)	24* (15–38)			19086‡ (79–149389)	Hct 28.7* (21.0–37.2) %	Fever 21%	
McGready, 2001a	QC	65	25* (15–41)	49* (40–64)	26* (16–40) 28* (16–40)	NA	1 (26%) 1 (27%)	11099‡ (16–93019)	Hct 29.2* (14.9–38.5) %	Fever 22%	
	AS	64	24* (15–41)	49* (39–74)				9822‡ (16–109724)	Hct 29.6* (20.0–38.9) %	Fever 14%	
McGready, 2005	Q	42	26 ± 7 (16–39)	50 ± 7 (30–68)	21 ± 4.5 (14.9–30.2)	1 (26%)	NA	2084‡ (33–109648)	Anaemia ^a 45.2%	Fever 74%	History of fever.
	AAP	39	26 ± 6 (16–37)	49 ± 7 (40–69)	21 ± 5.3 (10.1–36.2)	1 (31%)		2596‡ (33–123027)	Anaemia ^a 38.5%	Fever 59%	
Adam, 2004a	Q 20mg/kg/d	18	25.2 ± 6.0	59.3 ± 15.9	26.1 ± 9.9	NA	2.6 ± 1.9	5837 ± 8361	8.9 ± 0.6	38.1 ± 0.9	
	Q 30mg/kg/d	24	25.5 ± 1.9	52.2 ± 9.3	26.1 ± 8.9		2.6 ± 2.2	4207 ± 12325	8.6 ± 0.9	37.8 ± 1.0	

Appendix 3-5 continued.

Study	Treatment group	N	Age (year)	Weight (kg)	Gestational age at malaria (week)	Gravidity	Parity	Parasitaemia (/μL)	Haemoglobin (g/dL)	Body temperature (°C)	Note
Kalilani, 2007	ASSP	47	20* [17–24]	52.9 ± 5.9	22* [20–26]	1 (60%) 2 (9%) ≥3 (32%)	NA	686.6‡ (120–4260)	10.5 ± 1.6	Fever 4%	Summary of all 47 enrolled patients. 7 HIV-positive cases.
McGready, 2008	AL	125	26 ± 7 (14–42)	50 ± 6 (35–65)	23.7 ± 6.8 (13.1–39.2)	3* (1–13)	1* (0–9)	3548‡ (65–457088)	Hct 30.0 ± 4.4 %	36.8 ± 1.0	
	AS	128	27 ± 8 (14–44)	49 ± 7 (37–71)	24.8 ± 7.6 (13.1–39.4)	3* (1–11)	2* (0–9)	3162‡ (48–158489)	Hct 29.9 ± 5.0 %	37.0 ± 1.1	
Mutabingwa, 2009	AL	83	21* [19–26]	NA	6* [5–7] months	NA	1 (59%)	181* [62–628] /200WBC	9.3 ± 1.3	NA	All HIV negative.
Kaye, 2008	AL	57	23.5 ± 5.29	NA	2nd tri (25.5%) 3rd tri (74.5%)	1 (33%) 2 (26%) ≥3 (42%)	NA	24900 ± 3368.2	NA	36.9	Summary of all 57 enrolled patients.
Piola, 2010	Q	152	22.6 (17–38)	58 (IQR 10)	22.3 (9–38)	2* (1–7)	NA	1995‡ (IQR 9771)	10.9*	Fever 20%	
	AL	152	22.5 (15–38)	58 (IQR 10)	24.7 (10–39)	2* (1–8)		1418‡ (IQR 4727)	10.9*	Fever 23%	
Carmona-Fonseca, 2013	AL & ASMQ	30	22.0 ± 6.7	NA	2nd tri (50%) 3rd tri (50%)	NA	1 (42%) 2 (18%) ≥3 (40%)	NA	10.6 ± 1.2	NA	Age, parity and haemoglobin are the average of those of 60 P.vivax cases and 30 P.f cases.
D'Alessandro, AL 2016		880	22.6 ± 5.6	NA	2nd tri (71.8%) 3rd tri (28.2%)	1 (36.3%) 2 (23.1%) ≥3 (40.6%)	NA	800* [213–2880]	10.2* [9.2–11.0]	Fever 6.5%	Patients taking antiretroviral treatment were not enrolled.
	ASAQ	842	23.4 ± 5.9		2nd tri (75.0%) 3rd tri (24.9%)	1 (37.4%) 2 (22.2%) ≥3 (40.4%)		569* [165–2025]	10.1* [9.1–11.0]	Fever 6.8%	
	DP	853	22.3 ± 5.4		2nd tri (68.5%) 3rd tri (31.5%)	1 (40.0%) 2 (25.3%) ≥3 (34.7%)		680* [200–2760]	10.1* [9.1–11.0]	Fever 3.2%	
	ASMQ	848	23.5 ± 5.9		2nd tri (65.8%) 3rd tri (34.2%)	1 (32.7%) 2 (23.7%) ≥3 (43.6%)		840* [218–3040]	10.1* [9.1–10.9]	Fever 8.0%	

Appendix 3-5 continued.

Study	Treatment group	N	Age (year)	Weight (kg)	Gestational age at malaria (week)	Gravidity	Parity	Parasitaemia (/μL)	Haemoglobin (g/dL)	Body temperature (°C)	Note
Osarfo, 2017	ASAQ	205	15-19 (20%), NA 20-24 (27%), 25-29 (27%), ≥30 (26%)		22.3 (95% CI: 21.6-22.9)	NA	0 (26%), 1 (22%), ≥2 (51%)	238‡ (95%CI: 199-285)	10.1 (95%CI: 9.9-10.2)	Asymptomatic	Women with positive HIV status were excluded.
	DP	212	15-19 (14%), 20-24 (36%), 25-29 (32%), ≥30 (18%)		22.7 (95%CI: 22.1-23.4)		0 (35%), 1 (22%), ≥2 (43%)	236‡ (95%CI: 193-289)	10.0 (95%CI: 9.8-10.2)		
Onyamboko, 2015	AL3 AL5	48 48	NA	NA	NA	NA	NA	NA	NA	NA	Conference abstract
Ukah, 2015	ASAQ	75	29.2 ± 4.8 (20–42)	NA	28.5 ± 6.8 (14–40)	NA	1* (0–4)	13174 ± 12445	Hct 32.3 ± 2.4 %	Fever 60%	HIV-positive cases: 1/75 in ASAQ, 5/75 in AL..
	AL	75	29.4 ± 4.6 (18–41)		27.2 ± 7.3 (14–40)		0* (0–4)	12484 ± 11051	Hct 32.2 ± 2.3 %	Fever 53%	
Iribhogbe, 2017a	AL	40	31.0 ± 0.5	68.2 ± 0.3	27.9 ± 0.3	3.4 ± 0.7	4.3 ± 0.6	4884 ± 386.6	NA	38.5 ± 0.1	
	ASAQ	40	27.2 ± 0.5	69.0 ± 0.9	31.3 ± 0.0	2.9 ± 0.1	3.7 ± 0.1	3634 ± 537		38.1 ± 0.1	
Anvikar, 2018	ASSP	125	23.1 ± 3.9	NA	25.5 ± 6.2	1 (46%) 2 (30%) ≥3 (25%)	0 (51%) 1 (29%) ≥2 (20%)	16261 ± 30494 (32-165520)	8.8 ± 1.1	Overall 24.6% (61/248) were >37.5	
	ASMQ	123	23.4 ± 3.8		24.8 ± 5.7	1 (42%) 2 (31%) ≥3 (27%)	0 (50%) 1 (33%) ≥2 (18%)	17126 ± 30705 (32-153000)	9.1 ± 1.3		
McGready, 2003a	AAP	24	20* (16–37)	50* (39–64)	28.5* (19.1–35.9)	2* (1–7)	1* (0–4)	3467‡ (33–138038)	NA	37.6* (36.0–40.0)	
Adam, 2012	DP	12	26.2 ± 8.7	61.3 ± 10.1	32.0 (15.0–40.0)	NA	NA	12642‡ (1716–89700)	9.0 ± 1.0	NA	
Onyamboko, 2011	ASSP	13	21.7 ± 2.7	55.5 ± 8.3	22.4 ± 0.8	NA	0* (0–1)		Hct 30.0* [30.0–30.0] %	36.5* [36.1–36.7]	All HIV negative. Including 2 women with submicroscopic parasitaemia.
	EGA 22–26w EGA 32–36w	13 13	26.6 ± 4.4	63.0 ± 7.2	33.2 ± 1.4		1* (0–4)	528* [372–842]	Hct 32.0* [31.0–33.0] %	36.4* [36.2–36.5]	

Appendix 3-5 continued.

Study	Treatment group	N	Age (year)	Weight (kg)	Gestational age at malaria (week)	Gravidity	Parity	Parasitaemia (/μL)	Haemoglobin (g/dL)	Body temperature (°C)	Note
McGready, 2012	AS (iv on day0)	10	24* (17–40)	48* (40–59)	24.8* (17.1–24.2)	1* (1–6) 4* (1–8)	0* (0–5)	7933‡ (575–63096)	Hct 30* (25–46) %	37.8* (36.0–40.0)	
	AS (iv on day1)	10	32* (22–40)	50* (40–64)	25.5* (14.0–37.4)		2* (0–6)	2864 ‡ (129–51286)	Hct 30* (27–34) %	37.1* (36.1–39.0)	
Rijken, 2011	DP	24	28.1 ± 8.0	49 ± 6	24.6 ± 5.9	3* (1–9)	2* (0–8)	7349.06‡ (96–176594)	Hct 31.0 ± 4.5 %	37.7 ± 0.9	
Valea, 2014	ASMQ	24	23.6 ± 6.1	52.0* (46–69.5)	2nd tri (50%) 3rd tri (50%)	NA	1 (42%) 2 (33%) 3 (25%)	1029‡ (95%CI 455–2327)	9.0 ± 1.1	36.9 ± 0.4	
Juma, 2014	AL	45	NA	NA	NA	NA	NA	NA	NA	NA	Conference abstract and registry information
Mosha, 2014	AL	33	25* (18–41)	52* (40–80)	27* (14–37)	NA	NA	25280* (560–198080)	10.2* (7.1–13.3)	37.1* (36.0–39)	
Nyunt, 2016	AL	30	25* (18–39)	59.4* (44.5–81.1)	28* (14–34)	NA	NA	13227‡ (95%CI 7728–22639)	10.5* (7.6–13.1)	NA	All HIV negative.
Mutagonda, 2017	AL	92	23* (15–41)	NA	2nd tri (61%) 3rd tri (39%)	1 (46%) 2 (19%) ≥3 (36%)	NA	2700* (400–72500)	NA	NA	
Adam, 2004b	Q	26	26.2 ± 3.5	65.8 ± 4.6	8.5 ± 0.9	NA	2.8 ± 2.6	5856 ± 1652	9.2 ± 1.3	38.2 ± 0.6	
Adam, 2004c	AM	28	27.1 ± 5.8	65.8 ± 6.4	27 ± 7.3	NA	1* (0–8)	8468.54‡ (525–35000)	8.3 ± 0.9	38.6 ± 1.1	
Adegnika, 2005	Q Recurrence	20	20 ± 4	54 ± 6	25.5 ± 5.7	2.2 (1–5)	0.8 (0–3)	2104‡ (125–240000)	9.0 ± 1.2	36.8 ± 0.5	
	Q No recurrence	30	23 ± 6	61 ± 8	24.5 ± 6.3	3.2 (1–13)	1.6 (0–9)	695‡ (12–19800)	9.5 ± 1.1	36.8 ± 0.4	
Adam, 2006	ASSP	32	29.4 ± 4.3	68.7 ± 12.7	29.7 ± 8.4	NA	3.2 ± 1.8	7762‡ (3800–15250)	9.8 ± 1.4	38.1 ± 0.6	
Ndiaye, 2011	ASAQ	28	NA	NA	NA	NA	NA	9.91 ± 1.24‡	NA	NA	Conference abstract. Parasitaemia is shown in log scale.

Appendix 3-5 continued.

Study	Treatment group	N	Age (year)	Weight (kg)	Gestational age at malaria (week)	Gravidity	Parity	Parasitaemia (/μL)	Haemoglobin (g/dL)	Body temperature (°C)	Note
Iribhogbe, 2017b	ASAQ	50	NA	NA	NA	NA	NA	5721.3 ± 287.0	NA	100%	Demographic information was shown together with non-malaria pregnant women.
McGready, 1998a	Artemisinins Retreatment	61	25 (17–38)	NA	1st (28%)	NA	NA	814*	Anaemia 72%	Fever 16%	Gestational age was reported for combined group of retreatment and hyperparasitaemia. Percentage of anaemia was reported but without definition.
		13	22 (16–32)		2nd–3rd (82%)			(16–130385)	Anaemia 50%	Fever 23%	
	Hyperparasitaemia Inadvertent use	16	20 (15–40)		1st (88%)			115484* (960–312924)	Anaemia 29%	Fever 67%	
McGready, 1998b	Q Primary treatment	113	24.1 ± 5.8	47 ± 5.9	14.7 ± 7.3	1 (37%)	NA	852‡	Hct	36.7 ± 1	Including multiple episodes from a single patient.
		91	24.5 ± 6	49.6 ± 7	22.6 ± 8	2–3 (31%)		(16–2000332)	29.5 ± 5.3%	36.6 ± 0.82	
	Retreatment					>3 (32%)		1078‡	Hct		
McGready, 2001b	Artemisinins Primary treatment	229	24.8 ± 6.4	48.8 ± 6.7	24.6 ± 9.3 (3–40)	1 (35%)		1023‡	Hct		Including 55 cases of severe or hyperparasitaemia. Parasitaemia of uncomplicated cases are shown.
		310	(14–48)	(32–78)	3* (1–17)	2–3 (35%)	1* (0–14)	(16–131825)	28.9 ± 5.1 %	Fever 18%	
	Retreatment					>3 (31%)		1412‡	Hct		
Laochan, 2015	AS	151	24.5*	47*	21* (4–37)	NA	NA	3216*	Hct	36.8*	Summary of all 700 pregnant women included in the analysis except gestational age.
	AAP	11	(14–45)	(30–78)	23* (18–32)			(16.4–4768)	31* (12–48) %	(35.0–40.4)	
	AC	143			23* (1–39)						
	AL	43			23* (13–36)						
	ASMQ	5			28* (-0.4–39)						
	DP	29			20* (8–34)						
	Q	476			17* (3–36)						
	QC	29			10* (4–24)						
	MQ	22			26* (12–38)						
McGready, 2002	Q	204	24 ± 7 (14–42)	45.4 ± 5.9 (35–66)	7.9 ± 2.8 (1.0–12.8)	2* (1–13)	1* (0–10)	1230‡ (15.8–138038)	Anaemia ^a 29%	Fever 18%	

Appendix 3-5 continued.

Study	Treatment group	N	Age (year)	Weight (kg)	Gestational age at malaria (week)	Gravidity	Parity	Parasitaemia (/μL)	Haemoglobin (g/dL)	Body temperature (°C)	Note
McGready, 2003b	AAP	27	25* (16–38)	NA	28.2* (-1.6–37.3)	3* (1–7)	1* (0–6)	2206‡ (16–309030)	Hct 29.3 ± 5.1 %	Fever 7%	
Villegas, 2005	ASMQ	27	NA	NA	NA	NA	NA	NA	NA	NA	Conference abstract
Rijken, 2008	DP	62	24* (16–39)	48 ± 6 (36–60)	23.7 ± 8.2 (9.2–39.1)	2* (1–12)	1* (0–9)	1951‡ (12–85114)	Hct 29.9 ± 4.3 % (18–37)	36.6 ± 1.0 (35.1–40.0)	62 episodes in 50 patients.
Rulisa, 2012	AL	(107 2)	26.0* (17–48)	59.0 (40.0–65.0) (n=22)	25.8* (95%CI 25.3–26.4)	2* (1–12)	1* (0–10)	34800 (11700–96000) (n=22)	NA	36.5 (35.1–38.6) (n=22)	Including patients with clinical diagnosis. The number of patients with confirmed parasitaemia is not specified. A subset (n=22) were enrolled in a PK study.
Kalilani, 2013	AL	56	NA	NA	NA	NA	NA	NA	NA	NA	Only summary of the whole cohort including women without malaria was reported.

AAP: artesunate-atovaquone-proguanil, AC: artesunate-clindamycin, AL: artemether-lumefantrine, AQ: amodiaquine, AS: artesunate, CHQ: chloroquine, CI: confidence interval, DP: dihydroartemisinin-piperaquine, EGA: estimated gestational age, Hct: haematocrit, IQR: interquartile range. MQ: mefloquine, N: number of pregnant women who were assessed, NA: data not available, Q: quinine, QC: quinine-clindamycin, SP: sulfadoxine-pyrimethamine, tri: trimester.

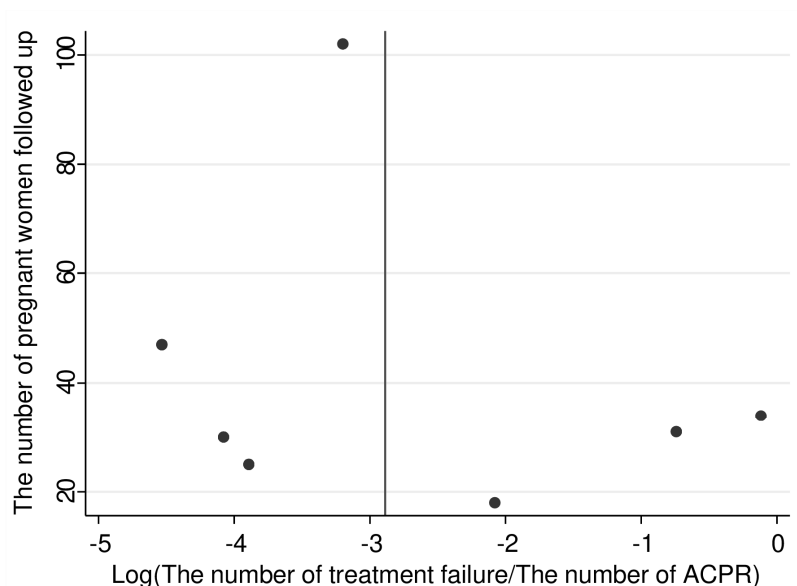
Figures are shown in mean (± standard deviation) unless indicated otherwise. *: median. ‡: geometric mean. Ranges are shown in () and inter-quartile ranges are shown in [].

Gestational age at malaria, parasitaemia and haemoglobin were shown in week, /μL and g/dL, respectively, unless indicated otherwise. Gestational age, gravidity and parity are shown as category (%) depending on the reporting.

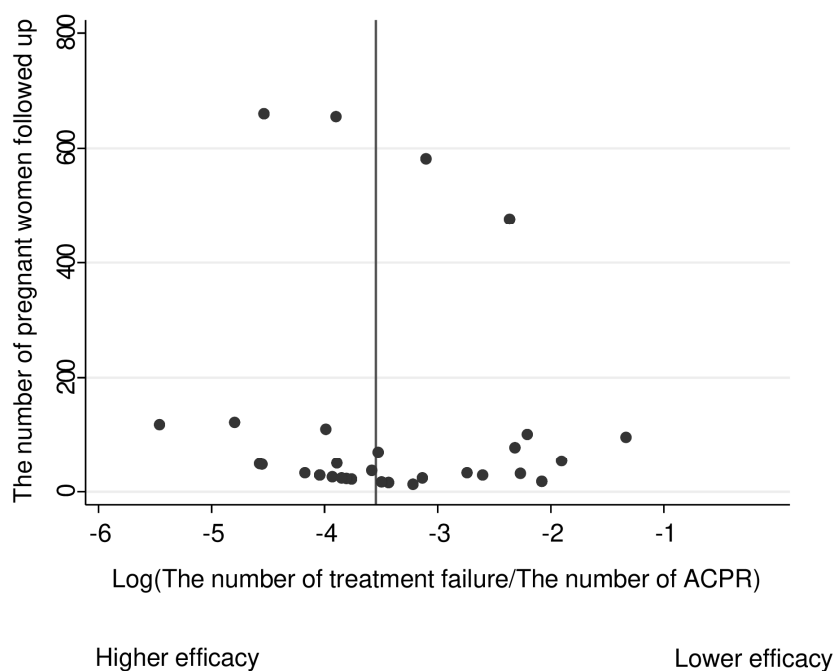
^a: anaemia was defined as haematocrit <30%.

Appendix 3-6.

Quinine-based treatment



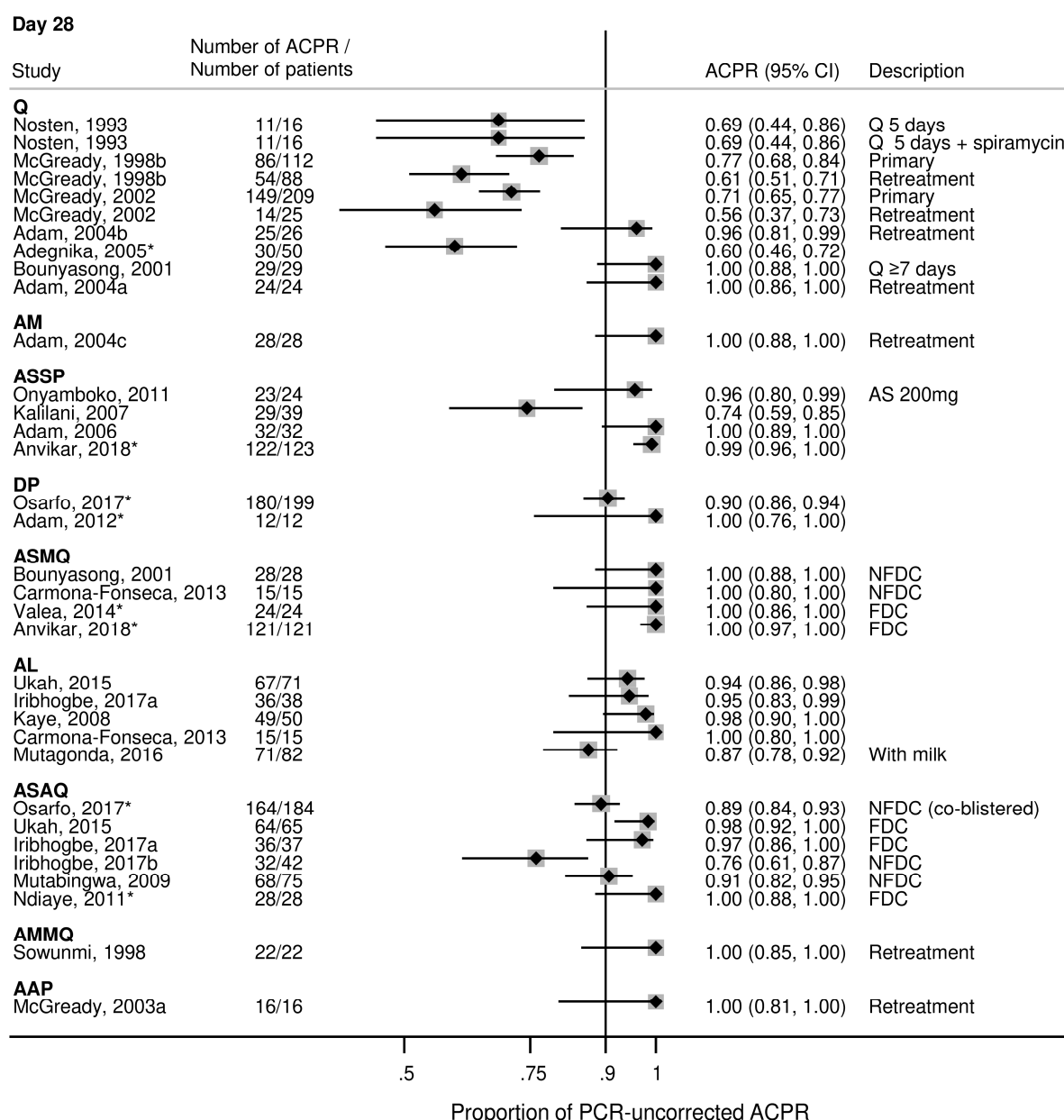
Artemisinin-based treatment



Appendix Figure 3-6. Funnel plots for quinine-based and artemisinin-based treatments.

Log odds (the number of PCR-confirmed recrudescence / the number of PCR-corrected ACPR) was plotted on the x-axis against the number of pregnant women followed-up on the y-axis. Each study arm was plotted once using the treatment outcome of the longest follow-up. Continuity correction was made for the studies without treatment failure by adding 0.5. The vertical line shows the average of the log odds weighted by the study size.

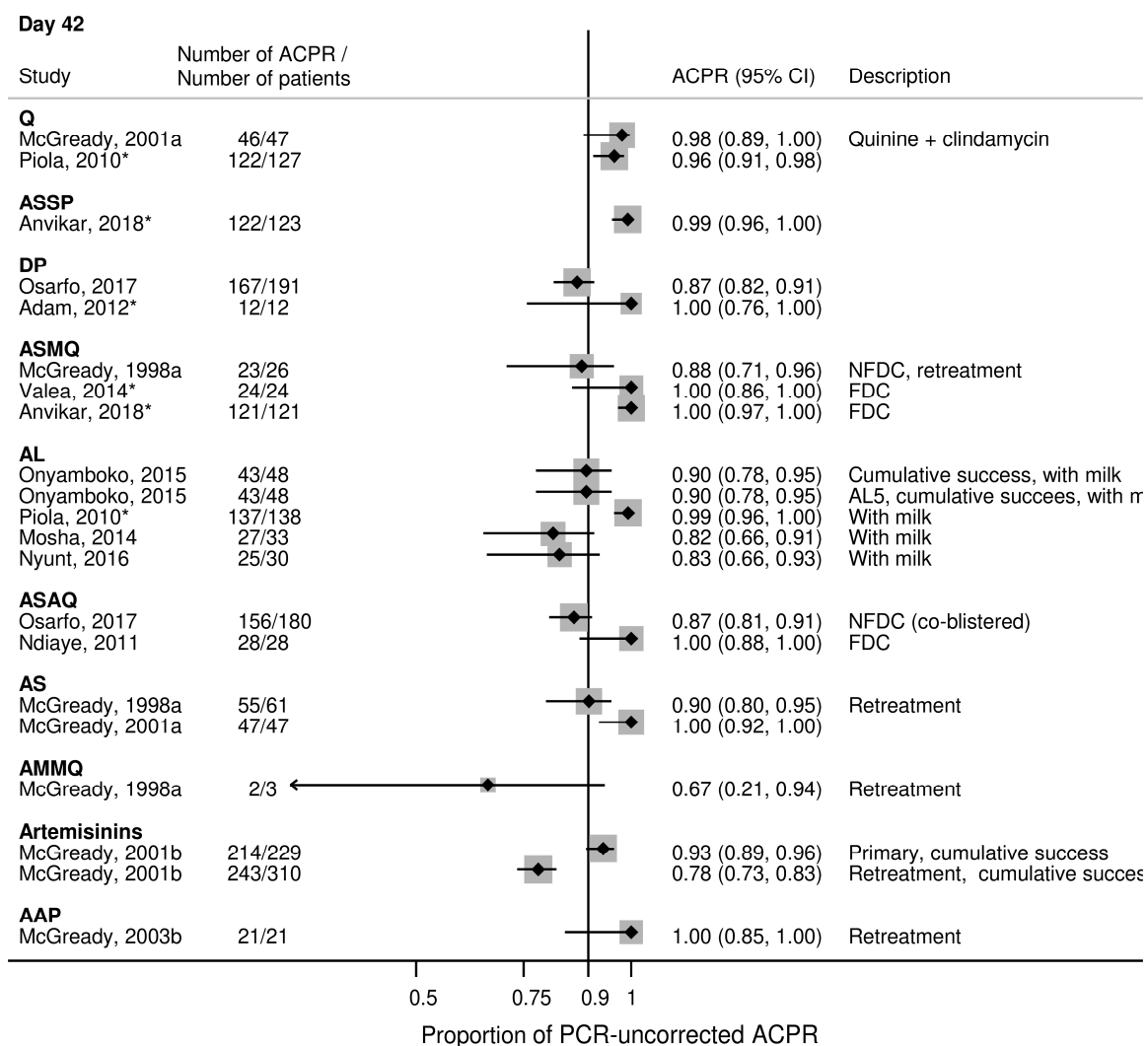
Appendix 3-7.



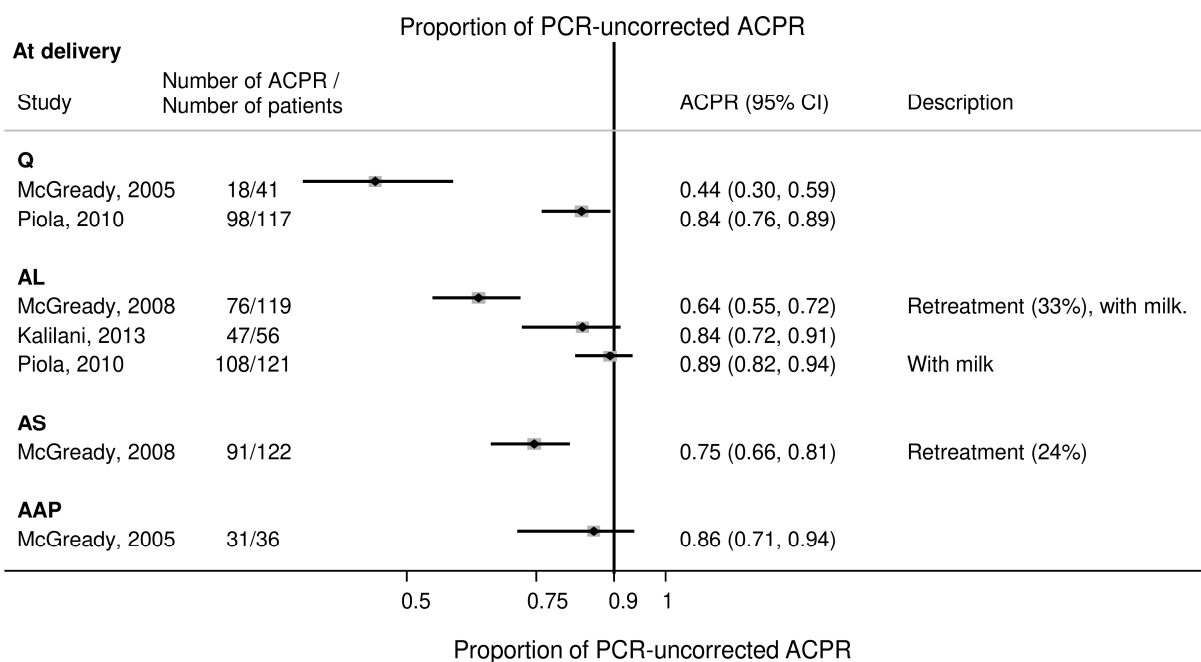
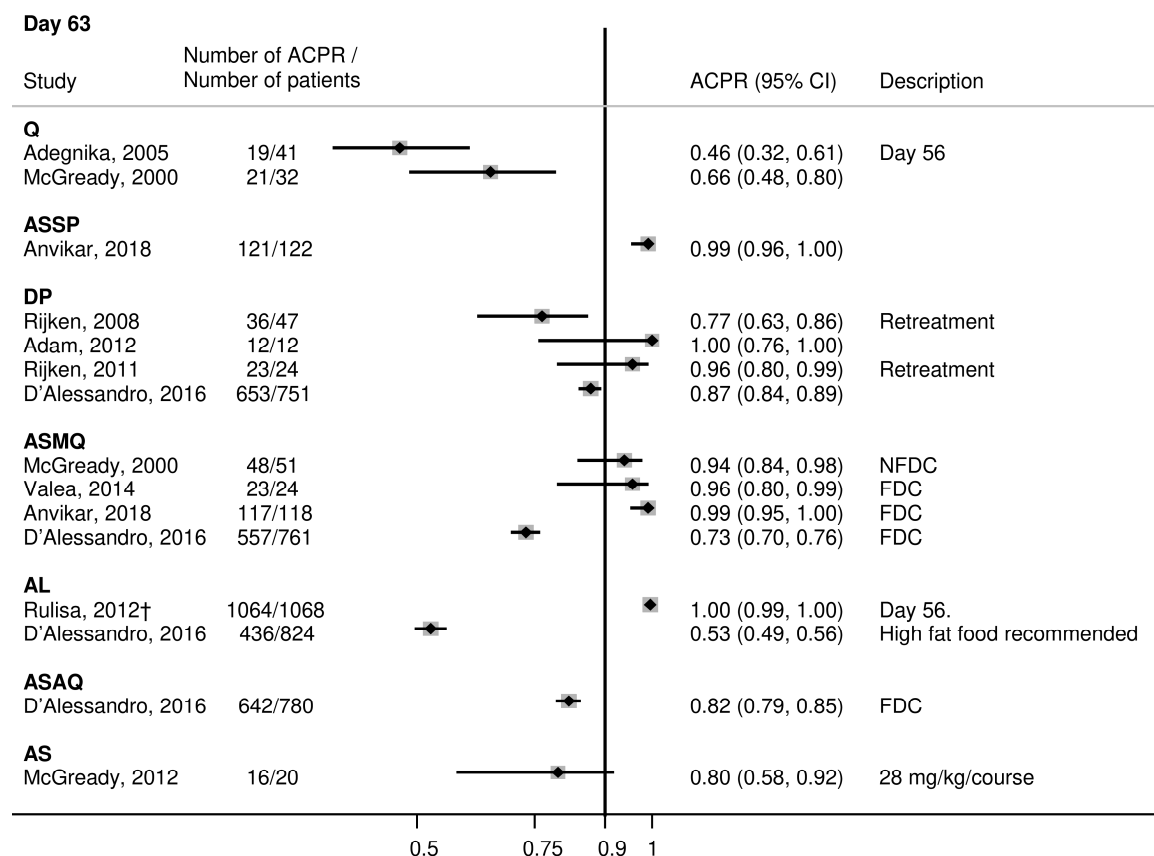
Appendix Figure 3-7. The PCR-uncorrected proportional appropriate adequate clinical and parasitological response (ACPR) for each study

ACPR are shown by treatment group and the duration of follow-up (i.e. day 28, 42, 63 and at delivery) with 95% confidence intervals. *: ACPR at later follow-up day is available. † Parasitaemia was not confirmed in all women. AAP: artesunate + atovaquone-proguanil, AC: artesunate with clindamycin, AL: artemether-lumefantrine, AM: artemether, AS: artesunate, AQ: amodiaquine, DP: dihydroartemisinin-piperaquine, FDC: fixed dose combination, MQ: mefloquine, NFDC: non-fixed dose combination, Q: quinine, QC: quinine with clindamycin, SP: sulfadoxine-pyrimethamine.

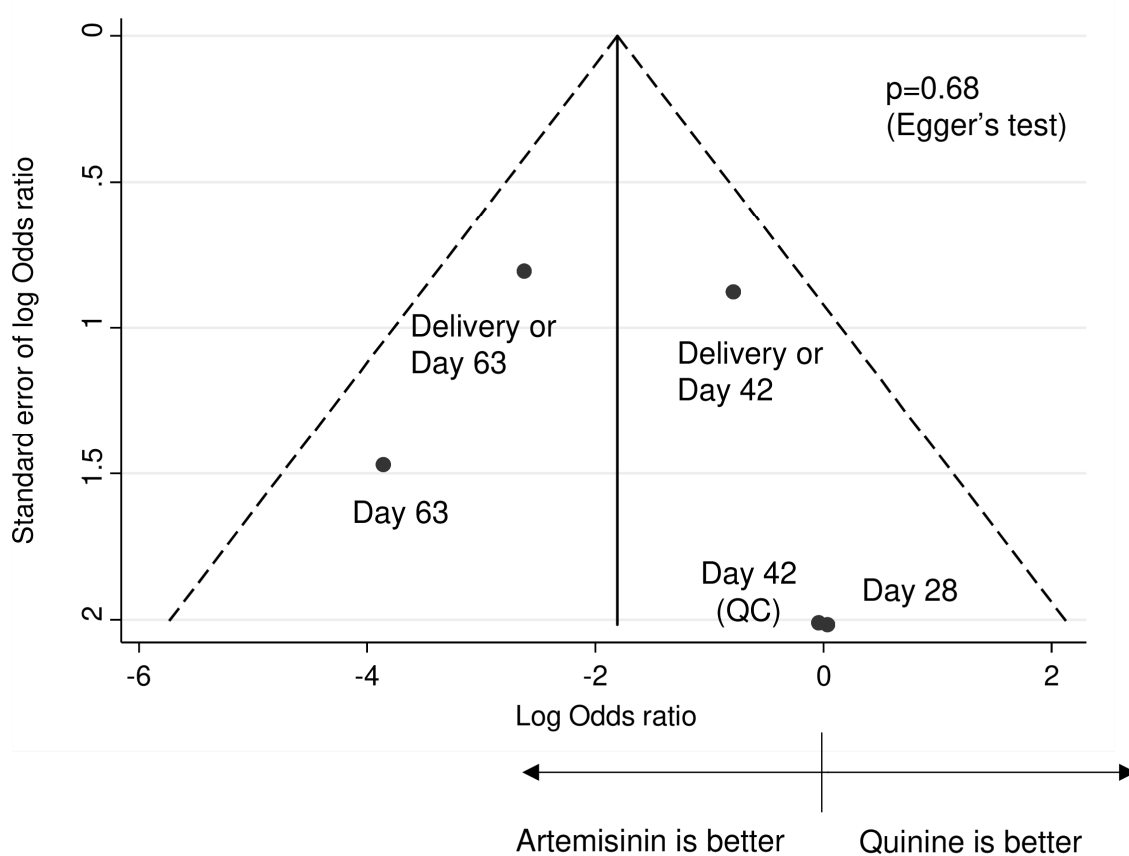
Appendix 3-7 continued.



Appendix 3-7 continued.



Appendix 3-8.



Appendix Figure 3-8. Funnel plot of odds ratio of PCR-corrected treatment failure comparing quinine-based and artemisinin-based treatments

Continuity correction was made for the studies without treatment failure by adding 0.5. QC: quinine with clindamycin.

Appendix 3-9. The methodology of assessing placental malaria

Study	Method of evaluating placental malaria
Bounyasong, 2001	Calcification of placenta
Kalilani, 2007	Pathological examination of placenta
McGready, 2008	Placental blood and cord blood thick smears
	Placental histopathology (Rogerson method ³¹⁸)
	Placental blood (smear and PCR)
	Placental histopathology (Muehlenbachs method ³¹⁶)
	Cord blood (smear and PCR)
Mutabingwa, 2009*	Placental blood smear
	Cord blood smear
Piola, 2010	Placental histopathology (Muehlenbachs method ³¹⁶)
	Placental blood (smear and PCR)
	Placental crush smear
	Cord blood (smear and PCR)
D'Alessandro, 2016	Placental biopsy Giemsa stain.
	Placental histopathology (Ismail method ³¹⁵)
Osarfo, 2017	Placental parasitaemia
	Cord blood parasitaemia
Onyamboko, 2015	Yes (details not available)
Anvikar, 2018	Placental biopsy smear
	Placental blood (smear, RDT)
NCT01054248	Cord blood (smear and PCR)
	Placenta (smear and PCR)
	Placenta histopathology
Rijken, 2011	Placental smear
Adegnika, 2005	Placental thick blood smear
Ndiaye, 2011	Placental blood
	Cord blood
McGready, 2003b	Placental smear
Kalilani, 2013	Placental histopathology (Bulmer method ³¹⁷)
	Placental blood quantitative PCR if histopathology is positive.

PCR: polymerase chain reaction.

* Planned in the protocol but not reported.

Appendix 3-10. Summary of the study design and reported safety outcomes

Study Country Study year	Study design Treatment: N	Trimester	Estimation of gestational age	Parity or gravidity reported	Follow up until delivery	Length of follow-up after delivery	ECG	Blood test	Pregnancy outcomes reported
Naing, 1988 Myanmar 1985-1986	RCT Q: 23 AQ: 19	2,3	Not specified	Parity	Weekly	Not followed	Not reported	Routine blood test was carried out, but the results were not reported. Blood glucose	Stillbirth/miscarriage Preterm birth Neonatal mortality
Harinasuta, 1990 ^a Thailand INA	RCT Q: 82 MFQ: 85	1,2,3	Not specified	INA	Yes	6 months	INA		Stillbirth/miscarriage Preterm birth Low birth weight (%) Congenital abnormality Child development (6 months)
Nosten, 1993 Thailand 1989-1990	RCT Q5: 16 Q5 + spiramycin: 16	2,3	Not specified	Parity	Not followed	Not followed	Not reported	WBC count at baseline	Stillbirth/miscarriage during the treatment
Sowunmi, 1998 Nigeria 1994-1997	RCT AM: 23 AMMQ: 22	2,3	Ultrasound	Parity	3-6 weekly	3 years	Not reported	Total WBC count, platelet count, ALT, AST, bilirubin, sodium, potassium, chloride, bicarbonate, uric acid, creatinine and blood glucose at baseline and on day 7	Stillbirth/miscarriage Preterm birth Congenital abnormality Birth weight Child development
Bounyasong, 2001 Thailand 1995-1998	RCT ASMQ: 29 Q: 28	2,3	Fundal height + ultrasound	Parity	Weekly	2 years	Not reported	CBC at baseline and daily during the study period, but not reported. Blood glucose.	Calcification of placenta Intra-uterine growth restriction Estimated gestational age at delivery Birth weight Congenital abnormality Child development (up to 2 years)

Appendix 3-10 continued.

Study Country Study year	Study design Treatment: N	Trimester	Estimation of gestational age	Parity or gravidity reported	Follow up until delivery	Length of follow-up after ECG delivery		Blood test	Pregnancy outcomes reported
McGready, 2000 Thailand 1995-1997	RCT Q: 42 ASMQ: 66	2,3	Fundal height + Dubowitz	Parity	Weekly	1 year	Not reported	Not reported	Stillbirth/miscarriage Estimated gestational age at delivery Birth weight / low birth weight (%) Placental weight Congenital abnormality Child development Neonatal mortality / Infantile mortality Maternal mortality
McGready, 2001a Thailand 1997-2000	RCT QC: 65 AS: 64	2,3	Fundal height + Dubowitz	Parity	Weekly	1 year	Not reported	Not reported	Stillbirth/miscarriage Estimated gestational age at delivery Birth weight / low birth weight (%) Congenital abnormality Placental weight Neonatal mortality / infantile mortality Child development
McGready, 2005 Thailand 2001-2003	RCT AAP: 39 Q: 42	2,3	Ultrasound, Dubowitz or Fundal height	Gravidity	Weekly	1 year	Not reported	CBC at baseline	Stillbirth/miscarriage Estimated gestational age at delivery Birth weight / low birth weight (%) Height, head circumference, arm circumference Congenital abnormality Infantile mortality Child development Maternal mortality
Adam, 2004a Sudan 2002-2003	RCT Q (low dose): 18 Q (standard dose): 24	2,3	Not specified	Parity	Yes	Not followed	Not reported	Blood glucose	Preterm birth Perinatal mortality

Appendix 3-10 continued.

Study Country Study year	Study design Treatment: N	Trimester	Estimation of gestational age	Parity or gravidity reported	Follow up until delivery	Length of follow-up after delivery	ECG	Blood test	Pregnancy outcomes reported
Kalilani, 2007 Malawi 2003-2004	RCT ASSP: 47 SP: 47 SP + azithromycin: 47	2	Ballard score	Gravidity	Yes	Not followed	Not reported	Not reported	Anaemia at delivery Stillbirth/miscarriage Preterm birth Birth weight and low birth weight Congenital abnormality Neonatal death
McGready, 2008 Thailand 2004-2006	RCT AL: 125 AS: 128	2,3	Ultrasound or Dubowitz	Both	Weekly	1 year	Yes (subset)	CBC and biochemistry at baseline and on day 14 (subset of patients)	Stillbirth/miscarriage Estimated gestational age at delivery Birth weight / low birth weight (%) Height, head circumference, arm circumference Congenital abnormality Neonatal neurological test Neonatal / Infantile mortality Child development Maternal mortality
Mutabingwa, 2009 Tanzania 2004-2006	RCT ASAP: 83 AQSP: 80 CD: 81 SP: 28	2,3	Dubowitz	Parity	At delivery and 42d after delivery	6 weeks	Yes	Total WBC count with differential, platelet count, creatinine, total bilirubin, ALT and albumin at baseline, on day 3 and 7	Stillbirth/miscarriage Preterm birth Birth weight Congenital abnormality Placenta weight Maternal mortality
Kaye, 2008 Uganda 2006	RCT AL: 57 CD: 57	2,3	Not specified	Gravidity	Not followed	Not followed	Not reported	Not reported	None

Appendix 3-10 continued.

Study Country Study year	Study design Treatment: N	Trimester	Estimation of gestational age	Parity or gravidity reported	Follow up until delivery	Length of follow-up after delivery	ECG	Blood test	Pregnancy outcomes reported
Piola, 2010 Uganda 2006-2009	RCT AL: 152 Q: 152	2,3	Ultrasound or LMP	Gravidity	Weekly	1 year	Yes	Creatinine, ALT and bilirubin at baseline, on day 7, 14 and 42. CBC at baseline, on day 14 and 42	Stillbirth/miscarriage Preterm birth / gestational age at delivery Birth weight / low birth weight (%) Congenital abnormality Early neonatal mortality Maternal mortality
Carmona-Fonseca, 2013 Colombia 2008-2011	RCT AL: 15 ASMQ: 15	2,3	Not specified	Gravidity	Not followed	Not followed	Not reported	Creatinine, AST, ALT, direct and indirect bilirubin, and platelet 24 hours after the treatment	Maternal mortality
D'Alessandro, 2016 Burkina Faso Ghana Malawi Zambia 2010-2013	RCT AL: 880 ASAQ: 842 ASMQ: 848 DP: 853	2,3	Ultrasound, Fundal height + New Ballard score	Gravidity	Monthly	1 year	Not reported	Total WBC count with differential at baseline, on day 7, 14, 28 and 63. Total bilirubin, ALT and creatinine at baseline, on day 7 and 14.	Stillbirth/miscarriage Preterm birth Birth weight Congenital abnormality Maternal mortality
Osarfo, 2017 Ghana 2011-2012	RCT ASAQ: 205 DP: 212	2,3	ultrasound (or Ballard if not done)	Parity	Yes	6 weeks	Not reported	CBC at baseline, on day 14, 28 and 42	Stillbirth/miscarriage Preterm birth Birth weight / low birth weight Congenital abnormality Neonatal mortality and morbidity Maternal mortality
Onyamboko, 2015 ^a DRC 2013-2014	RCT AL: 48 AL5: 48	2,3	Ultrasound	INA	Yes	1 year	INA	Biochemistry	Stillbirth/miscarriage Congenital abnormality Neonatal mortality Child development

Appendix 3-10 continued.

Study Country Study year	Study design Treatment: N	Trimester	Estimation of gestational age	Parity or gravidity reported	Follow up until delivery	Length of follow-up after delivery	ECG	Blood test	Pregnancy outcomes reported
Ukah, 2015 Nigeria 2013	RCT AL: 75 ASAQ: 75	2,3	Not specified	Parity	Telephone confirmation after birth	Not followed	Not reported	Not reported	Stillbirth/miscarriage
Iribhogbe, 2017a Nigeria INA	RCT AL: 40 ASAQ: 40	2,3	Not specified	Both	No	No	Not reported	Differential WBC count, total and conjugated bilirubin, ALT, AST, ALP, cholesterol) on day 4. Biochemistry	None
Anvikar, 2018 India 2010-2013	RCT ASMQ (123) ASSP (125)	2,3	Ultrasound	Both	Yes	42 days	No		Stillbirth/miscarriage Preterm birth Congenital abnormality Birth weight / low birth weight (%)
NCT01054248 ^b Thailand 2010-2016	RCT AL4 (335) ASMQ (335) DP (335)	2,3	Ultrasound (Dubowitz, LMP or fundal height)	Both	Yes	1 year	INA	Full biochemistry and CBC at baseline, on day 0, 14 and 28 (subset of the patients)	Stillbirth/miscarriage Preterm birth Congenital abnormality Birth weight / low birth weight (%) Neonatal and infant mortality Child development INA (Not mentioned)
NCT02909712 ^b Tanzania 2016-	RCT DP (50) SP (50)	2, 3	Ultrasound	INA	INA	INA	Yes	INA	
McGready, 2003a Thailand 2000-2001	PK AAP: 24	2,3	Fundal height + Dubowitz	Both	Weekly	Not followed	Yes	CBC and blood glucose at baseline. Biochemistry at baseline and 72 h after treatment	Stillbirth/miscarriage Estimated gestational age at delivery / preterm birth Birth weight / low birth weight (%) Congenital abnormality Maternal mortality

Appendix 3-10 continued.

Study Country Study year	Study design Treatment: N	Trimester	Estimation of gestational age	Parity or gravidity reported	Follow up until delivery	Length of follow-up after ECG delivery		Blood test	Pregnancy outcomes reported
Adam, 2012 Sudan 2007-2008	PK study DP: 12 (control: 12 matched non- pregnant women)	2,3	LMP+ ultrasound	Parity	Weekly	1 year	Not reported	Urea, AST and ALT at baseline and on day 14	Stillbirth/miscarriage Preterm birth Birth weight Congenital abnormality Child development (up to 1 year)
Onyamboko, 2011 DRC 2007-2008	PK ASSP: 26 (control: 25 non-pregnant women)	2,3	Ultrasound	Parity	Yes	1 year	Not reported	Creatinine, albumin, ALT, AST and alpha-1- acid glycoprotein at baseline and after the first dose of artesunate	Stillbirth/miscarriage Birth weight / low birth weight (%) Congenital abnormality Child development (1 year)
McGready, 2012 Thailand 2008-2009	PK AS: 20	2,3	Ultrasound	Both	Weekly	1 year	Not reported	CBC and biochemistry at baseline	Stillbirth/miscarriage Estimated gestational age at delivery / preterm birth Birth weight / low birth weight (%) Congenital abnormality Child development
Rijken, 2011 Thailand 2008	PK DP: 24 (control: 24 matched non- pregnant women)	2,3	Ultrasound	Both	Weekly	1 months	Not reported	CBC and biochemistry at baseline and on day 14	Stillbirth/miscarriage Congenital abnormality Neonatal mortality
Valea, 2014 Burkina Faso 2008-2009	PK ASMQ: 24 (control: 24 non-pregnant women)	2,3	LMP or fundal height	Parity	Passive follow-up	Not followed	Not reported	Total WBC count, total bilirubin, ALT, AST, creatinine at baseline and on day 14	Stillbirth/miscarriage Birth weight / low birth weight (%) Congenital abnormality

Appendix 3-10 continued.

Study Country Study year	Study design Treatment: N	Trimester	Estimation of gestational age	Parity or gravidity reported	Follow up until delivery	Length of follow-up after ECG delivery	Blood test	Pregnancy outcomes reported	
Juma, 2014 ^a Kenia 2012	PK AL: 45 (control: 25 non-pregnant women)	2,3	Not specified	INA	Not followed	Not followed	INA Not reported	Not planned	
Mosha, 2014 Tanzania 2012	PK AL: 33	2,3	LMP + fundal height	No	Not followed	Not followed	Not reported	WBC count with differential, platelet count, creatinine, ALT and AST at baseline, on day 14 and 28 Not reported	Stillbirth/miscarriage within 42 days
Nyunt, 2016 Uganda 2013-2014	PK AL: 30 (control: 30 non pregnant adults)	2,3	LMP + ultrasound	No	Not followed	Not followed	Not reported	Not reported	None
Mutagonda, 2017 Tanzania 2014-2015	PK AL: 60	2,3	Not specified	Gravidity	Not followed	Not followed	Not reported	Urea, creatinine, albumin and bilirubin at baseline	None
Adam, 2004b Sudan 2000-2002	Single-arm Q: 26	1	Ultrasound	Parity	Every 2 weeks	1 year	Not reported	Urea, creatinine, albumin and bilirubin at baseline	Stillbirth/miscarriage Preterm birth Birth weight Congenital abnormality Child development (up to 1 year) Infantile mortality

Appendix 3-10 continued.

Study Country Study year	Study design Treatment: N	Trimester	Estimation of gestational age	Parity or gravidity reported	Follow up until delivery	Length of follow-up after delivery	ECG	Blood test	Pregnancy outcomes reported
Adam, 2004c Sudan 1997-2001	Single-arm AM im: 28	1,2,3	Ultrasound	Parity	Every 2 weeks	1 year	Not reported	Urea, creatinine, albumin and bilirubin at baseline	Stillbirth/miscarriage Preterm birth Birth weight Head circumference Congenital abnormality Child development (up to 1 year) Perinatal mortality Maternal mortality None
Adegnika, 2005 Gabon 2003-2004	Single-arm Q: 50	2,3	Not specified	Both	Monthly	Not followed	Not reported	Not reported	
Adam, 2006 Sudan 2004-2005	Single-arm ASSP: 32	2,3	Ultrasound	Parity	Every 2 weeks	1 month	Not reported	Not reported	Stillbirth/miscarriage Preterm birth Birth weight Head circumference Congenital abnormality Child development (up to 1 month) Perinatal mortality Maternal mortality
Ndiaye, 2011 ^a Senegal 2009-2011	Single-arm ASAQ: 28	2,3	Fundal height + Dubowitz	INA	At delivery	9 months	Not reported	WBC count with differential, creatinine, ALT, and bilirubin at baseline, on day 7, 14, 28, 42 and at delivery. Blood glucose at baseline.	Stillbirth/miscarriage Gestational age at delivery Birth weight / low birth weight Height Congenital abnormality Child development (up to 9 months) Perinatal mortality

Appendix 3-10 continued.

Study Country Study year	Study design Treatment: N	Trimester	Estimation of gestational age	Parity or gravidity reported	Follow up until delivery	Length of follow-up after ECG delivery		Blood test	Pregnancy outcomes reported
Iribhogbe, 2017b Nigeria INA	Single-arm ASAQ: 42	2,3	Not specified	Both	No	No	Not reported	WBC count with differential, total and conjugated bilirubin, ALT, AST, ALP, cholesterol) on day 4	None
McGready, 1998a Thailand 1992-1996	Observational cohort AS: 55 AMMQ: 2 ASMQ: 23	1,2,3	LMP + fundal height + Dubowitz	No	Weekly	1-2 year	Not reported	Not reported	Stillbirth/miscarriage Gestational age at delivery Congenital abnormality Child development
McGready, 1998b Thailand 1991-1996	Observational cohort Q: 204	1,2,3	Dubowitz or LMP	Gravidity	Weekly	Not followed	Not reported	Total WBC count and platelet count at baseline	Stillbirth/miscarriage Gestational age at delivery Birth weight Congenital abnormality
McGready, 2001b Thailand 1992-2000	Observational cohort artemisinins: 539	1,2,3	Fundal height + Dubowitz or LMP	Both	Weekly	Not followed	Not reported	Not reported	Stillbirth/miscarriage Estimated gestational age at delivery Birth weight / low birth weight (%) Congenital abnormality Placental weight Maternal mortality

Appendix 3-10 continued.

Study Country Study year	Study design Treatment: N	Trimester	Estimation of gestational age	Parity or gravidity reported	Follow up until delivery	Length of follow-up after ECG delivery		Blood test	Pregnancy outcomes reported
Laochan, 2015 Thailand 1994-2009	collation of an observational cohort and interventional studies‡ AS: 151, AAP: 11, AC: 143, AL: 43, ASMQ: 5, DP 29, Q: 476, QC: 29 MQ: 22	1,2,3	Ultrasound, Dubowitz or Fundal height	Parity	Weekly	Not followed	Not reported	Not reported	None
McGready, 2002 Thailand 1995-2000	observational Q:204	1	Fundal height + Dubowitz	Both	Weekly	Not followed	Not reported	Not reported	Stillbirth/miscarriage Estimated gestational age at delivery Birth weight / low birth weight (%) Congenital abnormality
McGready, 2003b Thailand 1999-2001	Observational cohort AAP: 27	1,2,3	Not specified	Both	Weekly	Not followed	Not reported	Not reported	Stillbirth/miscarriage Estimated gestational age at delivery Birth weight / low birth weight (%) Congenital abnormality Neonatal mortality
Villegas, 2005 ^a Venezuela 2002-2005	Observational cohort ASMQ: 27	INA	INA	INA	INA	INA	INA	INA	NA

Appendix 3-10 continued.

Study Country Study year	Study design Treatment: N	Trimester	Estimation of gestational age	Parity or gravidity reported	Follow up until delivery	Length of follow-up after delivery	ECG	Blood test	Pregnancy outcomes reported
Rijken, 2008 Thailand 2006-2007	Observational cohort DP: 62	1,2,3	Not specified	Both	Weekly	1 month	Not reported	Not reported	Stillbirth/miscarriage Preterm birth Birth weight / low birth weight (%) Congenital abnormality Neonatal mortality
Rulisa, 2012 Rwanda 2007-2009	Observational cohort AL: (1072) †	1,2,3	LMP + fundal height + ultrasound(so me)	Both	Monthly	Not specified	Not reported	Not reported	Stillbirth/miscarriage Preterm birth Birth weight Length, head circumference Congenital abnormality Neonatal mortality Maternal mortality
Kalilani, 2013 Malawi 2009-2010	Observational cohort AL: 56	1,2	LMP + fundal height + Ballard score	Gravidity	Monthly	14weeks	Not reported	Not reported	Anaemia at delivery Stillbirth/miscarriage Estimated gestational age at delivery / preterm birth (%) Birth weight / low birth weight (%) / small for gestational age (%) Neonatal death

AAP: artesunate-atovaquone-proguanil, AC: artesunate-clindamycin, AL: artemether-lumefantrine, ALT: alanine aminotransferase, AQ: amodiaquine, AS: artesunate, AST: aspartate aminotransferase, CBC: complete blood count, CD: chlorproguanil-Dapsone, CHQ: chloroquine, DP: dihydroartemisinin(DHA)-piperaquine(PPQ), DRC: Democratic Republic of Congo, ECG: electrocardiography, LMP: last menstrual period, MQ: mefloquine, N: number of pregnant women who completed the follow-up, NA: data not available, PCR: polymerase chain reaction, PK: pharmacokinetic study, Q: quinine, QC: quinine-clindamycin, RCT: randomized controlled trial, SP: sulfadoxine-pyrimethamine. WBC: white blood cell.

† This number includes patients without parasitological confirmation. The exact number of parasitologically confirmed patients is not known. ‡ This study included patients enrolled in other studies. ^a: Conference abstract. ^b: Registered clinical trial. Planned number of participants is shown. NCT01054248 included *P. vivax* cases.

Appendix 3-11. Definition and reporting of fetal and neonatal outcomes

Study	Miscarriage		Stillbirth		EGA	Prematurity (preterm)		Perinatal death		Neonatal death	
	Definition	Report	Definition	Report	Report	Definition	Report	Definition	Report	Definition	Report
Naing, 1988	NR	Yes	NR	NR	NR	NR	Yes	NR	NR	NR	Yes
Harinasuta, 1990	INA	Yes (0)*	INA	Yes (0)*	NR	INA	Yes	INA	INA	INA	INA
Sowunmi, 1998	NR	Yes (0)*	NR	Yes (0)*	NR	NR	Yes (0)	NR	NR	NR	NR
Bounyasong, 2001	NR	Yes (0)*	NR	Yes (0)*	Yes	NR	NR	NR	NR	NR	NR
McGready, 2000	NR	Yes	NR	Yes (0)	Yes	<37w	NR	NR	NR	NR	Yes‡
McGready, 2001a	NR	Yes (0)	NR	Yes	Yes	<37w	NR	NR	NR	NR	Yes‡
McGready, 2005	<28w	Yes (0)	≥28w	Yes	Yes	<37w	Yes	NR	NR	NR	Yes‡
Adam, 2004a	NR	NR	NR	NR	NR	<37w	Yes	NR	NR	NR	Yes
Kalilani, 2007	<28w	Yes (0)	≥28w	Yes	Yes	<37w	NR	Yes‡	Yes	≤27 days of life	Yes
McGready, 2008	NR	Yes	NR	Yes	Yes	<37w	Yes	NR	NR	0-32 days	Yes‡
Mutabingwa, 2009	NR	NR	NR	Yes	NR	NR	NR	NR	NR	NR	Yes
Piola, 2010	<20w (spontaneous abortions) >20w (intrauterine fetal deaths)	Yes	>21w (protocol)	Yes	Yes	Prematurity (<37w) Severe prematurity (<28w)	Yes	NR	NR	early neonatal death (1 week after birth)	Yes
D'Alessandro, 2016	NR	Yes	NR	Yes	NR	<37w	Yes	NR	NR	NR	NR
Osarfo, 2017	<28w	Yes	≥28w	Yes	NR	<37w	Yes	NR	NR	NR	Yes
Onyamboko, 2015	INA	INA	INA	Yes	NR	INA	INA	INA	INA	INA	Yes
Ukah, 2015	NR	Yes	NR	Yes	NR	NR	NR	NR	NR	NR	NR
Anvikar, 2018	<28w	Yes	≥28w	Yes	No	NR	Yes	NR	NR	NR	NR
NCT01054248	INA	Planned	INA	Planned	INA	INA	Planned	INA	INA	INA	Planned

Appendix 3-11 continued.

Study	Miscarriage		Stillbirth		EGA	Prematurity (preterm)		Perinatal death		Neonatal death	
	Definition	Report	Definition	Report	Report	Definition	Report	Definition	Report	Definition	Report
McGready, 2003a	NR	Yes (0)*	NR	Yes (0)*	Yes	<37w	Yes (0)	NR	NR	NR	NR
Adam, 2012	<28w	Yes (0)	NR	NR	NR	28-37w	Yes	NR	NR	NR	NR
Onyamboko, 2011	NR	Yes (0)*	NR	Yes (0)*	NR	NR	NR	NR	NR	NR	NR
McGready, 2012	NR	Yes (0)*	NR	Yes (0)*	Yes	NR	Yes	NR	NR	NR	Yes (0)
Rijken, 2011	NR	Yes (0)*	NR	Yes (0)*	NR	NR	NR	NR	NR	1 month	Yes (0)
Valea, 2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Adam, 2004b	<28w	Yes	NR	NR	NR	28-37w	NR	from 28 weeks until 1 week after delivery	NR	NR	Yes‡
Adam, 2004c	<28w	Yes (0)	NR	Yes (0)	NR	NR	Yes	NR	NR	NR	Yes
Adegnika, 2005	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Adam, 2006	NR	Yes (0)	NR	Yes (0)	NR	NR	Yes (0)	NR	NR	NR	Yes
Ndiaye, 2011	NR	Yes (0)*	NR	Yes (0)*	Yes	NR	Yes	NR	NR	NR	Yes
McGready, 1998a	<28w	Yes	≥28w	Yes	Yes	NR	NR	NR	NR	NR	NR
McGready, 1998b	<28w	Yes	NR	Yes	Yes	NR	NR	NR	NR	NR	Yes (0)
McGready, 2001b	<28w	Yes	≥28w	Yes	Yes	<37w	NR	NR	NR	NR	NR
Laochan, 2015	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
McGready, 2002	<28w	Yes	≥28w	Yes	Yes	<37w	NR	NR	NR	NR	NR
McGready, 2003b	NR	Yes (0)*	NR	Yes (0)*	Yes	NR	Yes	NR	NR	NR	Yes‡
Rijken, 2008	NR	Yes (0)*	NR	Yes (0)*	NR	<37w	Yes	NR	NR	1 month	Yes
Rulisa, 2012	NR	Yes	NR	Yes	NR	NR	Yes	NR	NR	≤ 7 days	Yes
Kalilani, 2013	NR	NR	NR	Yes	Yes	<37w	Yes	NR	NR	NR	Yes

EGA: estimated gestational age at delivery. INA: information not available (conference abstract or registered trial), NR: not reported.

* No fetal losses were reported. ‡ include deaths after a month. †Total of abortion, stillbirth and neonatal deaths.

Appendix 3-12. Anthropometric and developmental assessment of the newborns

Study	Birth-weight	Timing	Adjustment for			Minimal precision of scale	LBW		Height	Child follow-up	Developmental test
			Sex	Gestational age	Twins		Definition	Reported			
Naing, 1988	No	NoBW	NoBW	NoBW	NR	NoBW	NR	NR	NR	Not followed	-
Harinasuta, 1990	Yes	NR	No	No	INA	INA	<2500g	Yes	INA	6 months	Not specified
Sowunmi, 1998	Yes	NR	No	No	NR	NR	NR	NR	NR	3 years	Yes ^{329, 330}
Bounyasong, 2001	Yes	24h	No	No	NR	NR	NR	NR	NR	2 years	Denver developmental screening test ³³¹
McGready, 2000	Yes	NR	No	No	Body weight and ega were reported in singletons only.	50g	NR	Yes	Assessed, but not reported.	1 year	Basic developmental milestones
McGready, 2001a	Yes	NR	No	No	Body weight were reported in singletons only.	50g	NR	Yes	Assessed, but not reported	1 year	Developmental milestones
McGready, 2005	Yes	24h	Assessed, No but not reported	No	Body weight and ega were reported in singletons only.	NR	<2500g	Yes	Yes	1 year	Yes ³³²
Adam, 2004a	No	NoBW	NoBW	NoBW	NR	NoBW	NR	NR	NR	NR	-
Kalilani, 2007	Yes	24h	No	No	Women with multiple gestations were excluded from the study.	To the nearest gram	<2500g	Yes	NR	Not followed	-
McGready, 2008	Yes	72h	No	Term low birthweight	Body weight and ega were reported in singletons only.	NR	<2500g	Yes	Yes	1 year	Shoklo developmental score ³³²
Mutabingwa, 2009	Yes	NR	No	No	Women with multiple gestations were excluded from the study.	NR	NR	NR	NR	6 weeks	Not specified
Piola, 2010	Yes	NR	No	Term low birthweight	Proportion of multiple births was planned to be reported but not.	NR	<2500g	Yes	Assessed, but not reported	1 year	Yes ³³³
D'Alessandro, 2016	Yes	72h	No	No	Analysis of body weight was restricted to singleton births.	Digital baby scale	<2500g	Yes	NR	1 year	Not specified

Appendix 3-12 continued.

Study	Birth-weight	Timing	Adjustment for			Minimal precision of scale	LBW		Height	Child follow-up	Developmental test
			Sex	Gestational age	Twins		Definition	Reported			
Osarfo, 2017	Yes	NR	No	No	Women with multiple gestations were excluded from the study.	NR	<2500g	Yes	Assessed, but not reported	6 weeks	Not specified
Onyamboko, 2015	INA	INA	INA	INA	Women with multiple gestations were excluded from the study.	INA	INA	INA	INA	1 year	Not specified
Ukah, 2015	No	NoBW	NoBW	NoBW	NR	NoBW	NR	NR	NR	Not followed	-
Anvikar, 2018	Yes	7 days	Sex ratio was reported	Term LBW / preterm LBW	NR	NR	<2500g	Yes	NR	42 days	Not specified
NCT01054248	Yes	As soon as possible	Planned	No	INA	INA	INA	Planned	Planned	1 year	Yes ³³²
McGready, 2003a	Yes	<5 days	Assessed, but not reported	No	All delivered singleton.	NR	<2500g	Yes	NR	Not followed	-
Adam, 2012	Yes	NR	Assessed, but not reported	No	NR	NR	<2500g	Yes	NR	1 year	Neurologic developmental assessment
Onyamboko, 2011	Yes	NR	No	No	All delivered singleton.	NR	≤2500g	Yes	NR	1 year	Not specified
McGready, 2012	Yes	72h	Assessed, but not reported	No	All delivered singleton.	10g	<2500g	Yes	NR	1 year	Yes ³³²
Rijken, 2011	No	NoBW	NoBW	NoBW	All delivered singleton. Birthweight was not reported.	± 10g	NR	NR	Assessed, but not reported	1 months	Not specified
Valea, 2014	Yes	NR	No	No	NR	10g	<2500g	NR	NR	Not followed	-
Adam, 2004b	Yes	NR	No	No	NR	NR	NR	NR	NR	1 year	Not specified

Appendix 3-12 continued.

Study	Birth-weight	Timing	Adjustment for			Minimal precision of scale	LBW		Height	Child follow-up	Developmental test
			Sex	Gestational age	Twins		Definition	Reported			
Adam, 2004c	Yes	24h	No	No	NR	NR	NR	NR	NR	1 year	Not specified
Adegnika, 2005	No	NoBW	NoBW	NoBW	NR	NoBW	NR	NR	NR	Not followed	-
Adam, 2006	Yes	24h	No	No	NR	NR	NR	NR	NR	1 month	Not specified
Ndiaye, 2011	Yes	48h	No	No	NR	NR	NR	NR	Yes	9 months	Not specified
McGready, 1998a	Yes, in methods but no results	NoBW	NoBW	NoBW	Twins were included. Birthweight was not reported.	NoBW	<2500g	NR	NR	1-2 year	Not specified
McGready, 1998b	Yes	NR	No	No	NR	NR	NR	NR	NR	Not followed	-
McGready, 2001b	Yes	NR	No	No	Body weight and ega were reported in singletons only.	NR	<2500g	Yes	NR	Not followed	-
Laochan, 2015	No	NoBW	NoBW	NoBW	NR	NoBW	NR	NR	NR	Not followed	-
McGready, 2002	Yes	NR	No	No	Body weight and ega were reported in singletons only.	NR	<2500g	Yes	NR	Not followed	-
McGready, 2003b	Yes	NR	No	No	All delivered singleton.	NR	<2500g	Yes	NR	Not followed	-
Rijken, 2008	Yes	3d	No	No	Body weight was reported in singletons only.	NR	<2500g	Yes	NR	1 month	Not specified
Rulisa, 2012	Yes	NR	No	Term low birthweight	Number of twins was reported, but it is unclear whether they were included in birthweight.	NR	NR	NR	Yes	Not specified	WHO motor development milestones ³³⁴
Kalilani, 2013	Yes	NR	Yes*	Weight for gestational age*	Placental malaria was reported in singletons only. It is unclear for other assessment (e.g. body weight).	NR	<2500g	Yes	NR	14weeks	Not specified

ega: estimated gestational age. INA: information not available (conference abstract or registered clinical trial). LBW: low birth weight. NoBW: birth weight was not reported. NR: Not reported (information was not reported in the publication). -: not applicable because newborns were not followed. *: WHO standard growth curve was used.

Appendix 3-13. Assessment and reporting of risk factors for adverse pregnancy outcomes

Study	Smoking	Marital status	Educational level	Height	Nutrition status	Chronic diseases
Naing, 1988	NR	NR	NR	NR	NR	NR
Harinasuta, 1990	INA	INA	INA	INA	INA	INA
Nosten, 1993	NR‡	NR‡	NR‡	NR‡	NR‡	NR‡
Sowunmi, 1998	NR	NR	NR	NR	NR	NR
Bounyasong, 2001	NR	NR	NR	Yes	NR	NR
McGready, 2000	NR	NR	NR	NR	NR	Women with a history of mental disorder excluded.
McGready, 2001a	NR	NR	NR	NR	NR	Women with major liver or kidney disease were excluded.
McGready, 2005	NR	NR	NR	NR	NR	Women with chronic diseases were excluded from the study.
Adam, 2004a	NR	NR	NR	NR	NR	NR
Kalilani, 2007	NR	Yes	Yes	NR	NR	Women with a history of chronic disease or pregnancy complications were excluded from the study. HIV status was assessed.
McGready, 2008	Yes	NR	NR	NR	BMI	Women with chronic diseases were excluded from the study.
Mutabingwa, 2009	NR	NR	Yes	NR	NR	HIV status was assessed. Women with concomitant disease masking assessment of the response to treatment were excluded.
Kaye, 2008	NR‡	NR‡	Yes	NR‡	NR‡	NR‡
Piola, 2010	Recorded but NR.	NR	NR	NR	NR	HIV patient was enrolled.
Carmona-Fonseca, 2013	NR‡	NR‡	NR‡	NR‡	NR‡	NR‡
D'Alessandro, 2016	NR	NR	NR	NR	NR	Women with chronic diseases were excluded. HIV positive patients were enrolled only if she received neither antiretroviral treatment nor cotrimoxazole.
Osarfo, 2017	NR	NR	Yes	Yes, but not reported	NR	Women with chronic diseases (diabetes, hypertension, renal/cardiac) or HIV were excluded from the study.
Onyamboko, 2015	INA	INA	INA	INA	INA	HIV positive women and women with medical conditions requiring concomitant drug treatment were excluded from the study.
Ukah, 2015	NR	NR	NR	NR	NR	HIV was assessed and reported.
Iribhogbe, 2017a	NR‡	NR‡	NR‡	Yes	BMI	Women with known severe underlying diseases were excluded from the study.‡
Anvikar, 2018	NR	NR	NR	NR	NR	Women with known chronic diseases (cardiac, haemoglobinopathy), with abnormal liver or renal function tests, with a history of convulsion, psychiatric disorders or seizures were excluded from the study.
NCT01054248	Yes	INA	Yes (literacy)	Yes	INA	Women with a history or known liver diseases or other chronic diseases were excluded.
NCT02909712	INA‡	INA‡	INA‡	INA‡	INA‡	Women with known heart disease or known cardiac ailment were excluded. HIV was screened and HIV-positive women were excluded.
McGready, 2003a	NR	NR	NR	NR	NR	Women with chronic diseases were excluded from the study.

Appendix 3-13 continued.

Study	Smoking	Marital status	Educational level	Height	Nutrition status	Chronic diseases
Adam, 2012	NR	NR	NR	Yes	NR	Women with chronic diseases were excluded.
Onyamboko, 2011	NR	NR	Yes	Yes	BMI	Women with chronic diseases (diabetes, hypertension, etc.) or HIV were excluded.
McGready, 2012	Yes	NR	NR	NR	BMI	NR
Rijken, 2011	Yes	NR	NR	Yes	NR	NR
Valea, 2014	NR	Yes	Yes	Yes	BMI	Patients with chronic medical conditions requiring special care beyond what the study could provide were excluded.
Juma, 2014	INA	INA	INA	INA	INA	Patients with a history of heart disease or arrhythmia were excluded.
Mosha, 2014	No history of smoking.	NR‡	NR‡	Yes	BMI	No history of chronic disease.
Nyunt, 2016	NR‡	NR‡	NR‡	NR	BMI	Women with significant other illness including HIV were excluded from the study. ‡
Mutagonda, 2016	NR‡	NR‡	NR‡	NR‡	NR‡	NR‡
Adam, 2004b	NR	NR	NR	NR	NR	NR
Adam, 2004c	NR	NR	NR	NR	NR	NR
Adegnika, 2005	NR	NR	NR	NR	NR	NR
Adam, 2006	NR	NR	NR	NR	NR	NR
Ndiaye, 2011	NR	NR	NR	NR	NR	Patients with underlying hepatic or renal illness were excluded.
Iribhogbe, 2017b	NR‡	NR‡	NR‡	Yes	BMI	Women with known severe underlying diseases were excluded from the study. ‡
McGready, 1998a	NR	NR	NR	NR	NR	NR
McGready, 1998b	NR	NR	NR	Yes	NR	NR
McGready, 2001b	NR	NR	NR	NR	NR	NR
Laochan, 2015	NR‡	NR‡	NR‡	NR‡	NR‡	NR‡
McGready, 2002	NR	NR	NR	NR	NR	NR
McGready, 2003b	NR	NR	NR	NR	NR	NR
Villegas, 2005	INA	INA	INA	INA	INA	INA
Rijken, 2008	NR	NR	NR	NR	NR	NR
Rulisa, 2012	NR	NR	NR	NR	NR*	Diabetes was reported
Kalilani, 2013	NR	Yes	Yes	NR	NR	Patients with major illness and HIV-positive patients receiving cotrimoxazole were excluded from the study.

BMI: body mass index. INA: information not available (conference abstract or registered clinical trial). NR: not reported. ‡: pregnancy outcomes were not reported. * BMI was reported in a subset who were enrolled in a PK study, which did not report pregnancy outcomes.

Appendix Table 4-1. Summary of the designs of studies included and not included in this pooled analysis

Study	Study design	Randomisation	Allocation concealment	Blinding	Loss to follow-up for efficacy assessment	NOS	Reason for exclusion
Studies those IPD were shared							
McGready, 2000	RCT	Block randomisation	Unclear	Open-label	0/66 (ASMQ) 0/42 (Q)	-	
McGready, 2001a	RCT	Block randomisation	Unclear	Open-label	19/65 (QC) 17/64 (AS)	-	
McGready, 2005	RCT	Computer-generated block randomisation	Sealed envelope	Open-label	0/39 (AAP) 0/42 (Q)	-	
Kalilani, 2007	RCT	Block randomisation	Sealed envelope	Open-label Lab technicians were blinded.	8/47 (ASSP) 7/47 (SP) 5/47 (SP + azithromycin)	-	
McGready, 2008	RCT	Block randomisation	Sealed envelope	Open-label Lab technicians and patient examiners were blinded.	5/125 (AL) 3/125 (AS)	-	
Piola, 2010	RCT	Computer-generated permuted block of eight	Sealed envelope	Open-label Microscopists and clinical investigators were blinded.	10/152 (Q) 6/152 (AL)	-	
D'Alessandro, 2016	RCT	Block randomisation	Sealed envelope	Open-label Lab technicians (interpretation of PCR) were blinded.	50/880 (AL) 51/842 (ASAQ) 94/853 (DP) 77/848 (ASMQ)	-	
Anvikar, 2018	RCT	Permuted block of 12	Sealed envelope	Open-label	4/123 (ASMQ) 2/125 (ASSP)	-	
McGready, 2003a	PK study	Not applicable	Not applicable	Unclear	8/24	8/8	
McGready, 2012	PK study	No: assigned alternatively	Not applicable	Unclear	0/20	8/8	
Rijken, 2011	PK study	Not applicable	Not applicable	Unclear	2/24 (delivery)	8/8	

Appendix Table 4-1 continued.

Study	Study design	Randomisation	Allocation concealment	Blinding	Loss to follow-up for efficacy assessment	NOS	Reason for exclusion
Valea, 2014	PK study	Not applicable	Not applicable		1/24	8/8	
Juma, 2014	PK study	Not applicable	Not applicable		INA	8/8	
Mosha, 2014	PK study	Not applicable	Not applicable	Unclear	0/33	8/8	
Nyunt, 2016	PK study	Not applicable	Not applicable	Open-label	0/30	8/8	
Ndiaye, 2011	Single-arm	Not applicable	Not applicable	Open-label	0/27	8/8	
McGready, 2003b	Observational cohort	Not applicable	Not applicable	Unclear	6/27	7/8	
Rijken, 2008	Observational cohort	Not applicable	Not applicable	Unclear	5/50 (delivery)	8/8	
Kalilani, 2013	Observational cohort	Not applicable	Not applicable	Unclear	120/450 (delivery) including those without malaria	7/8	

Appendix Table 4-1 continued.

Study	Study design	Randomisation	Allocation concealment	Blinding	Loss to follow-up for efficacy assessment	NOS	Reason for exclusion
Studies those IPD were not shared							
NCT01054248	RCT	Computer generated permuted block of 15	Sealed envelope	Open-label Microscopists were blinded.	INA	-	Not published
Onyamboko, 2015	RCT	INA	INA	Open-label	INA	-	Not published
NCT02909712	RCT	INA	INA	Assessors	INA	-	Not completed (IPD not sought)
Adam, 2004a	RCT	Yes, but the method is not specified.	Unclear	Unclear	7/25 (20mg/kg/day) 2/26 (30mg/kg/day)	-	Data not accessible.
Adam, 2004c	Single-arm	Not applicable	Not applicable	Unclear	0/28	8/8	Data not accessible.
Adam, 2006	Single-arm	Not applicable	Not applicable	Unclear	0/32	8/8	Data not accessible.
Adam, 2012	PK study	Not applicable	Not applicable	Unclear	0/12	7/8	Data not accessible.
Bounyasong, 2001	RCT	Yes, but the method is not specified.	Unclear	Unclear	1/30 (Q) 2/30 (ASSP)	-	No responses.
Carmona-Fonseca, 2013	RCT	Ballot system	Yes	Unclear	0/15 (AL) 0/15 (ASMQ)	-	Not shared by November 2018.
Kaye, 2008	RCT	Computer-generated random number	Sealed envelope	Open-label	5/57 (AL) 5/57 (CD)	-	Not shared by November 2018.
Mutabingwa, 2009	RCT	Block randomisation	Sealed envelope	Open-label Microscopists were blinded.	8/83 (ASAQ) 8/80 (AQSP) 4/81 (CD) 2/28 (SP)	-	Data not accessible.
Mutagonda, 2017	PK study	Not applicable	Not applicable	Unclear	10/92	8/8	Premature to share.

AAP: artesunate-atovaquone-proguanil, AL: artemether-lumefantrine, AQ: amodiaquine, AS: artesunate, CHQ: chloroquine, CD: chlorproguanil-Dapsone, DP: dihydroartemisinin-piperaquine, INA: information not available (registered trial). MQ: mefloquine, NOS: Newcastle-Ottawa scale. PK: pharmacokinetic, Q: quinine, QC: quinine-clindamycin, RCT: randomised controlled trial, SP: sulfadoxine-pyrimethamine.

Appendix Table 4-2. Sensitivity analysis of PCR-corrected efficacy on day 28, 42 and 63 by Kaplan-Meier estimates pooled by random effects model, including aggregated data of unshared studies

Characteristic	PCR-corrected treatment success*					
	Day 28		Day 42		Day 63	
	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)
Treatment						
AL	1311	96.9 (94.6-98.3)	929	95.5 (92.6-97.5)	598	93.6 (89.1-96.7)
ASAQ	881	99.6 (98.9-99.9)	782	99.2 (98.5-99.7)	611	98.8 (97.2-99.6)
ASMQ	1025	99.9 (99.6-100)	948	99.5 (98.3-99.9)	717	99.2 (97.0-99.9)
DP	827	99.5 (98.3-99.9)	811	99.5 (97.5-99.9)	717	98.6 (96.1-99.7)
ASSP	191	99.3 (97.1-99.9)	120	99.2 (95.7-99.9)	113	99.2 (95.7-99.9)
AS	169	95.9 (88.4-99.1)	140	95.6 (87.3-99.1)	91	95.3 (85.7-99.2)
AC	106	99.5 (97.4-99.9)	73	98.6 (85.4-100)	39	97.5 (71.4-100)
AAP	58	99.9 (98.4-100)	49	98.9 (91.5-100)	40	98.5 (91.4-99.9)
Q	205	93.5 (70.3-99.8)	164	86.8 (56.6-99.3)	128	84.9 (51.6-99.3)
QC	43	99.9 (97.6-100)	37	99.9 (97.0-100)	28	99.9 (96.3-100)

* Estimated by pooling the Kaplan-Meier survival estimates or proportions of patients with treatment success in each study by random effects method, including aggregated data from publication for the studies that IPD were not shared.

AAP: Artesunate with atovaquone-proguanil, AC: artesunate with clindamycin, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, PCR: polymerase chain reaction, Q: Quinine monotherapy, QC: Quinine with clindamycin.

Appendix Table 4-3. Sensitivity analysis of the risk of PCR-corrected treatment failure without splitting time

Characteristic	Proportion	Univariable*		Multivariable*	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Treatment					
AL	68/1278	Reference		Reference	
ASAQ	12/841	0.27 (0.14-0.52)	<0.001	0.27 (0.14-0.52)	<0.001
ASMQ	25/1028	0.58 (0.35-0.96)	0.03	0.56 (0.34-0.94)	0.03
DP	14/874	0.35 (0.18-0.69)	0.002	0.35 (0.18-0.68)	0.002
ASSP	4/173	2.33 (0.45-12.18)	0.31	2.05 (0.38-11.03)	0.40
AS	15/230	0.64 (0.33-1.22)	0.17	0.64 (0.34-1.23)	0.18
AC	6/142	0.38 (0.16-0.94)	0.04	0.37 (0.15-0.91)	0.03
AAP	2/91	0.33 (0.06-1.68)	0.18	0.31 (0.06-1.61)	0.17
Q	31/244	5.87 (2.50-13.76)	<0.001	6.11 (2.57-14.54)	<0.001
QC	1/67	0.48 (0.04-5.13)	0.54	0.48 (0.04-5.24)	0.55
EGA	178/4965	0.99 (0.96-1.02)	0.45		
Age (years)	178/4968	0.97 (0.95-1.00)	0.02		
Gravidity 1	78/1763	Reference			
2	36/1118	0.64 (0.43-0.97)	0.03		
≥3	60/1989	0.59 (0.41-0.83)	0.003		
Parity 0	89/2130	Reference		Reference	
1	31/1040	0.59 (0.39-0.89)	0.01	0.59 (0.39-0.89)	0.01
≥2	56/1733	0.62 (0.44-0.89)	0.01	0.62 (0.44-0.89)	0.01
Height (cm)	153/4256	1.01 (0.98-1.04)	0.41		
Weight (kg)	177/4943	1.00 (0.98-1.02)	0.80		
BMI	153/4243	0.99 (0.92-1.06)	0.74		
Fever (temperature >37.5)	32/504	1.00 (0.66-1.50)	0.98		
no fever	142/4350	Reference			
Haemoglobin on day 0 (g/dL)	175/4917	0.93 (0.84-1.04)	0.20		
HIV infection	1/45	0.98 (0.12-7.88)	0.99		
negative/not confirmed	177/4923	Reference			
Parasitaemia (log ₁₀ /μL)	178/4968	1.99 (1.67-2.38)	<0.001	1.93 (1.61-2.32)	<0.001
Hyperparasitaemia	6/79	0.77 (0.32-1.85)	0.56		
No	172/4889	Reference			
Presence of gametocytes	12/197	1.35 (0.73-2.50)	0.33		
no gametocytes	157/4629	Reference			
Mixed infection	5/54	1.73 (0.68-4.41)	0.25		
mono-infection	173/4914	Reference			
Intercalated vivax infection	10/233	0.59 (0.29-1.20)	0.15		
No vivax infection	168/4735	Reference			
Malaria transmission intensity					
Low	81/1319	Reference			
Moderate	49/2594	0.85 (0.33-2.20)	0.73		
High	48/1055	1.17 (0.36-3.84)	0.80		

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, HR: hazard ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for treatment, previous antimalarial exposure and baseline parasitaemia (on log₁₀ scale).

Appendix Table 4-4. Sensitivity analysis of the risk of PCR-corrected treatment failure for different antimalarial treatments in two periods after treatment using a model censoring intercalated *P. vivax* infection

Treatment	Proportion	Univariable		Multivariable*	
		HR (95% CI)	p-value	HR (95% CI)	p-value
day0-28					
AL	39/1278	Reference		Reference	
ASAQ	3/841	0.14 (0.04-0.47)	0.001	0.15 (0.04-0.49)	0.002
ASMQ	1/1028	0.05 (0.01-0.35)	0.003	0.05 (0.01-0.35)	0.003
DP	4/874	0.18 (0.06-0.57)	0.003	0.19 (0.06-0.59)	0.004
ASSP	2/173	1.46 (0.21-10.10)	0.70	1.22 (0.17-8.82)	0.84
AS	12/230	0.81 (0.39-1.71)	0.58	0.84 (0.40-1.77)	0.64
AC	1/142	0.08 (0.01-0.63)	0.02	0.08 (0.01-0.61)	0.01
AAP	0/91	No failure			
Q	24/244	7.27 (2.89-18.28)	<0.001	7.43 (2.91-18.98)	<0.001
QC	0/67	No failure			
day 29-					
AL	25/1022	Reference		Reference	
ASAQ	9/804	0.40 (0.18-0.87)	0.02	0.39 (0.18-0.84)	0.02
ASMQ	24/971	1.12 (0.62-2.02)	0.72	1.05 (0.58-1.91)	0.86
DP	9/809	0.52 (0.23-1.18)	0.12	0.50 (0.22-1.13)	0.10
ASSP	2/135	8.81 (0.73-107.01)	0.09	7.35 (0.62-87.54)	0.11
AS	2/131	0.36 (0.08-1.65)	0.19	0.36 (0.08-1.64)	0.19
AC	3/80	1.71 (0.46-6.37)	0.42	1.50 (0.41-5.53)	0.54
AAP	1/49	0.49 (0.05-4.42)	0.53	0.45 (0.05-4.05)	0.47
Q	7/168	3.63 (1.11-11.86)	0.03	3.65 (1.11-11.98)	0.03
QC	0/41	No failure			

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, HR: hazard ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for previous antimalarial exposure, parity and baseline parasitaemia (on log₁₀ scale).

Appendix Table 4-5. Sensitivity analysis of baseline characteristics associated with the risk of PCR-corrected treatment failure using a model censoring intercalated *P. vivax* infection

Characteristic	Proportion	Univariable*		Multivariable*	
		HR (95% CI)	p-value	HR (95% CI)	p-value
EGA	168/4965	0.99 (0.96-1.01)	0.31		
Age (years)	168/4968	0.96 (0.94-0.99)	0.01		
Gravidity 1	77/1763	Reference			
2	35/1118	0.65 (0.43-0.98)	0.04		
≥3	52/1989	0.54 (0.37-0.78)	0.001		
Parity 0	88/2130	Reference		Reference	
1	30/1040	0.61 (0.40-0.93)	0.02	0.61 (0.40-0.93)	0.02
≥2	48/1733	0.57 (0.39-0.82)	0.003	0.57 (0.39-0.82)	0.003
Height (cm)	143/4256	1.01 (0.98-1.03)	0.70		
Weight (kg)	167/4943	1.00 (0.98-1.02)	0.96		
BMI	143/4243	0.98 (0.92-1.05)	0.62		
Fever (temperature >37.5)	31/504	1.09 (0.71-1.66)	0.70		
no fever	133/4350	Reference			
Haemoglobin on day 0 (g/dL)	165/4917	0.91 (0.82-1.02)	0.10		
HIV infection	1/45	1.08 (0.13-8.71)	0.94		
negative/not confirmed	167/4923	Reference			
Parasitaemia (log ₁₀ /μL)	168/4968	2.09 (1.74-2.51)	<0.001	2.00 (1.65-2.41)	<0.001
Hyperparasitaemia	6/79	0.77 (0.32-1.87)	0.56		
No	162/4889	Reference			
Presence of gametocytes	11/197	1.54 (0.81-2.91)	0.19		
no gametocytes	149/4629	Reference			
Mixed infection	5/54	2.30 (0.88-6.03)	0.09		
mono-infection	163/4914	Reference			
Malaria transmission intensity					
Low	71/1319	Reference			
Moderate	49/2594	0.83 (0.31-2.23)	0.71		
High	48/1055	1.15 (0.33-3.96)	0.83		

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, HR: hazard ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for treatment, previous antimalarial exposure and their interactions with time after treatment (0-28 days and after 28 days), and baseline parasitaemia (on log₁₀ scale).

Appendix Table 4-6. Univariable and multivariable analysis of the risk of PCR-corrected treatment failure among women treated with AL

Characteristic	Proportion	Univariable*		Multivariable*	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Lumefantrine dose (mg/kg)	67/1250	0.99 (0.96-1.02)	0.59	0.99 (0.96-1.02)	0.59
Fat intake	24/314	3.24 (0.71-14.76)	0.13		
No	2/114	Reference			
EGA	68/1262	1.00 (0.96-1.05)	0.94		
Age group <20	32/460	1.61 (0.88-2.98)	0.12		
20-24	16/400	Reference			
25-29	8/222	0.81 (0.34-1.91)	0.63		
≥30	12/180	1.39 (0.65-2.97)	0.39		
Gravidity 1	28/438	Reference			
2	14/275	0.74 (0.39-1.43)	0.38		
≥3	22/478	0.72 (0.40-1.30)	0.28		
Parity 0	33/547	Reference			
1	15/250	0.97 (0.52-1.82)	0.93		
≥2	18/427	0.68 (0.37-1.23)	0.20		
Height (cm)	63/1065	1.01 (0.97-1.06)	0.66		
Weight (kg)	67/1250	0.92 (0.78-1.09)	0.36		
BMI	63/1065	0.94 (0.81-1.10)	0.46		
Fever (temperature >37.5)	12/119	1.32 (0.68-2.57)	0.41		
no fever	55/1097	Reference			
Haemoglobin on day 0 (g/dL)	67/1236	0.96 (0.80-1.14)	0.62		
HIV infection	0/13	omitted			
negative/not confirmed	68/1249				
Parasitaemia (log ₁₀ /μL)	68/1262	2.03 (1.54-2.69)	<0.001	2.03 (1.54-2.69)	<0.001
Hyperparasitaemia	3/24	0.77 (0.21-2.77)	0.69		
No	65/1238	Reference			
Presence of gametocytes	5/53	1.75 (0.68-4.55)	0.25		
no gametocytes	60/1173	Reference			
Mixed infection	1/13	1.26 (0.17-9.56)	0.83		
mono-infection	67/1249	Reference			
Intercalated vivax infection	4/36	1.41 (0.43-4.59)	0.57		
No vivax infection	64/1226	Reference			
Malaria transmission intensity					
Low	20/200	Reference			
Moderate	30/760	0.66 (0.31-1.40)	0.28		
High	18/302	0.96 (0.41-2.24)	0.93		

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, HR: hazard ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for dose, parasitaemia and previous antimalarial exposure.

Appendix Table 4-7. Sensitivity analysis of PCR-uncorrected efficacy on day 28, 42 and 63 using Kaplan-Meier estimates pooled by random effects model

Low transmission intensity

Treatment	PCR-uncorrected treatment success*					
	Day 28		Day 42		Day 63	
	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)
AL	174	92.7 (63.2-99.9)	157	87.7 (60.4-99.1)	110	78.9 (58.3-93.7)
ASAQ	25	99.9 (96.1-100.0)	25	99.9 (96.1-100.0)	9	99.9 (91.8-100.0)
ASMQ	177	99.2 (90.9-100.0)	171	99.2 (90.9-100.0)	160	98.2 (94.4-99.6)
DP	71	92.1 (84.1-97.0)	63	82.5 (72.7-90.4)	44	76.1 (51.6-94.1)
ASSP	121	99.2 (95.7-99.9)	120	99.2 (95.7-99.9)	113	99.2 (95.7-99.9)
AS	177	90.9 (85.9-94.7)	147	89.9 (79.3-96.4)	95	83.3 (74.8-90.2)
AC	108	97.5 (83.7-99.9)	83	88.7 (65.6-98.8)	44	70.9 (40.3-94.8)
AAP	59	99.9 (98.4-100.0)	50	98.9 (91.5-100.0)	41	93.4 (82.5-98.6)
Q	128	66.4 (42.2-88.6)	112	65.5 (41.2-88.2)	86	59.7 (36.1-84.2)
QC	43	99.9 (97.3-100.0)	37	97.5 (87.6-99.9)	28	94.2 (82.2-99.1)

* Estimated by pooling the Kaplan-Meier survival estimates in each study by random effects method.

AAP: Artesunate with atovaquone-proguanil, AC: artesunate with clindamycin, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, PCR: polymerase chain reaction, Q: Quinine monotherapy, QC: Quinine with clindamycin.

Moderate transmission intensity

Treatment	PCR-uncorrected treatment success*					
	Day 28		Day 42		Day 63	
	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)
AL	726	92.2 (86.9-95.9)	559	84.7 (74.0-92.7)	402	77.4 (58.6-91.9)
ASAQ	470	99.2 (97.2-99.9)	458	97.6 (95.8-98.8)	425	93.0 (90.3-95.1)
ASMQ	491	99.4 (98.4-99.8)	475	99.1 (96.1-99.9)	389	87.2 (77.4-94.1)
DP	732	99.6 (98.7-99.9)	723	99.1 (97.4-99.8)	647	90.1 (84.1-94.5)
ASSP	13	80.2 (55.8-95.9)	0		0	
Q	111	73.0 (45.2-94.2)	76	62.1 (27.6-94.6)	57	54.4 (18.7-95.0)

* Estimated by pooling the Kaplan-Meier survival estimates in each study by random effects method.

AAP: Artesunate with atovaquone-proguanil, AC: artesunate with clindamycin, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, PCR: polymerase chain reaction, Q: Quinine monotherapy, QC: Quinine with clindamycin.

High transmission intensity

Treatment	PCR-uncorrected treatment success*					
	Day 28		Day 42		Day 63	
	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)
AL	308	86.2 (82.1-89.8)	236	69.7 (57.5-81.1)	94	42.0 (36.2-48.4)
ASAQ	282	99.0 (97.0-99.7)	309	89.3 (82.1-94.5)	181	70.7 (57.5-82.8)
ASMQ	334	98.9 (94.5-99.9)	318	90.8 (83.6-95.7)	176	68.2 (44.5-89.2)
DP	56	99.9 (97.7-100.0)	55	99.9 (97.7-100.0)	46	86.3 (75.2-94.1)
ASSP	25	96.0 (81.1-99.8)	0		0	
Q	64	42.0 (27.7-59.8)	12	31.1 (18.3-49.6)	2 ^a	27.8 (10.9-60.0) ^a

* Estimated by pooling the Kaplan-Meier survival estimates in each study by random effects method. ^a: including results between day 45 day 63.

AAP: Artesunate with atovaquone-proguanil, AC: artesunate with clindamycin, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, PCR: polymerase chain reaction, Q: Quinine monotherapy, QC: Quinine with clindamycin.

Appendix Table 4-8. Univariable and multivariable logistic regression of the risk of developing abdominal pain on day 1-7

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
AL	103/1177 (8.8%)	Reference		Reference	
ASAQ	99/827 (12.0%)	2.01 (1.39-2.92)	<0.001	2.03 (1.40-2.96)	<0.001
ASMQ	133/891 (14.9%)	1.43 (1.02-2.00)	0.04	1.44 (1.03-2.02)	0.03
DP	80/831 (9.6%)	1.05 (0.72-1.54)	0.79	1.08 (0.74-1.58)	0.69
ASSP	5/5 (100.0%)	Omitted			
AS	49/200 (24.5%)	1.19 (0.64-2.22)	0.58	1.19 (0.64-2.23)	0.58
AC	0/9 (0.0%)	Omitted			
AAP	21/73 (28.8%)	2.24 (0.83-6.04)	0.11	2.26 (0.80-6.34)	0.12
Q	54/228 (23.7%)	1.78 (1.07-2.98)	0.03	1.81 (1.07-3.04)	0.03
QC	18/59 (30.5%)	1.33 (0.49-3.59)	0.58	1.32 (0.48-3.62)	0.59
Abdominal pain on day 0	265/774 (34.2%)	5.56 (4.46-6.94)	<0.001	5.73 (4.59-7.16)	<0.001
no	287/3516 (8.2%)	Reference		Reference	
EGA	562/4297 (13.1%)	0.99 (0.97-1.01)	0.32		
Age (years)	562/4300 (13.1%)	1.00 (0.98-1.01)	0.82		
Gravidity 1	199/1538 (12.9%)	Reference			
2	118/987 (12.0%)	0.78 (0.60-1.02)	0.07		
≥3	245/1772 (13.8%)	0.86 (0.68-1.07)	0.18		
Parity 0	233/1849 (12.6%)	Reference			
1	120/895 (13.4%)	0.93 (0.71-1.20)	0.57		
≥2	209/1549 (13.5%)	0.90 (0.72-1.13)	0.36		
Height (cm)	429/3758 (11.4%)	0.99 (0.97-1.01)	0.27		
Weight (kg)	562/4283 (13.1%)	0.99 (0.97-1.00)	0.05		
BMI	429/3754 (11.4%)	0.96 (0.92-1.00)	0.07		
Fever (>37.5°C)	78/350 (22.3%)	1.25 (0.90-1.72)	0.18		
no fever	479/3906 (12.3%)	Reference			
Haemoglobin on day 0 (g/dL)	559/4262 (13.1%)	0.97 (0.91-1.04)	0.45		
HIV infection	9/37 (24.3%)	1.55 (0.67-3.59)	0.31		
negative/not confirmed	553/4263 (13.0%)	Reference			
Parasitaemia (log ₁₀ /μL)	562/4300 (13.1%)	1.15 (1.02-1.29)	0.02		
Hyperparasitaemia	9/47 (19.1%)	0.86 (0.38-1.98)	0.73		
no	553/4253 (13.0%)	Reference			
Presence of gametocytes	23/154 (14.9%)	0.78 (0.47-1.29)	0.33		
no gametocytes	536/4138 (13.0%)	Reference			
Mixed infection	7/32 (21.9%)	0.92 (0.35-2.44)	0.87		
mono-infection	555/4268 (13.0%)	Reference			
Malaria transmission intensity					
Low	198/768 (25.8%)	1.43 (0.70-2.90)	0.32	1.43 (0.70-2.90)	0.32
Moderate	219/2476 (8.8%)	Reference		Reference	
High	145/1056 (13.7%)	3.41 (1.89-6.15)	<0.001	3.41 (1.89-6.15)	<0.001

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, EGA: estimated gestational age, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for treatment and abdominal pain on day 0.

Appendix Table 4-9. Univariable and multivariable logistic regression of the risk of developing anorexia on day 1-7

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
AL	116/1228 (9.4%)	Reference		Reference	
ASAQ	114/853 (13.4%)	3.80 (2.31-6.25)	<0.001	3.88 (2.36-6.38)	<0.001
ASMQ	117/881 (13.3%)	2.39 (1.52-3.77)	<0.001	2.41 (1.53-3.79)	<0.001
DP	62/829 (7.5%)	1.33 (0.79-2.23)	0.28	1.32 (0.79-2.22)	0.29
ASSP	No data	Omitted			
AS	133/206 (64.6%)	1.29 (0.71-2.35)	0.40	1.34 (0.73-2.44)	0.34
AC	2/9 (22.2%)	Omitted			
AAP	48/79 (60.8%)	3.30 (1.02-10.71)	0.05	2.96 (0.95-9.20)	0.06
Q	57/227 (25.1%)	4.66 (2.15-10.10)	<0.001	4.29 (1.99-9.23)	<0.001
QC	49/64 (76.6%)	3.34 (1.17-9.49)	0.02	3.41 (1.19-9.79)	0.02
Anorexia on day 0	441/798 (55.3%)	9.20 (7.20-11.75)	<0.001	8.80 (6.86-11.28)	<0.001
no	255/3576 (7.1%)	Reference		Reference	
EGA	698/4373 (16.0%)	1.01 (0.99-1.03)	0.38		
Age group <20					
20-24	142/1470 (9.7%)	Reference		Reference	
25-29	207/1332 (15.5%)	1.50 (1.12-2.02)	0.01	1.61 (1.19-2.16)	0.002
≥30	153/838 (18.3%)	1.43 (1.04-1.99)	0.03	1.55 (1.11-2.16)	0.01
Gravidity 1	196/736 (26.6%)	1.65 (1.19-2.27)	0.002	1.78 (1.28-2.47)	0.001
2	189/1553 (12.2%)	Reference			
≥3	139/1001 (13.9%)	0.96 (0.71-1.31)	0.81		
Parity 0	351/1793 (19.6%)	1.18 (0.91-1.52)	0.21		
1	222/1867 (11.9%)	Reference			
≥2	146/907 (16.1%)	1.09 (0.81-1.47)	0.55		
Height (cm)	310/1569 (19.8%)	1.29 (1.01-1.66)	0.05		
Weight (kg)	531/3804 (14.0%)	1.00 (0.98-1.02)	1.00		
BMI	696/4359 (16.0%)	0.99 (0.98-1.01)	0.26		
Fever (>37.5°C)	142/375 (37.9%)	1.50 (1.07-2.11)	0.02		
no fever	552/3953 (14.0%)	Reference			
Haemoglobin on day 0 (g/dL)					
	695/4339 (16.0%)	1.00 (0.92-1.07)	0.92		
HIV infection	7/37 (18.9%)	2.94 (1.11-7.82)	0.03		
negative/not confirmed	691/4339 (15.9%)	Reference			
Parasitaemia (log ₁₀ /μL)	698/4376 (16.0%)	1.20 (1.05-1.36)	0.01	1.23 (1.07-1.40)	0.003
Hyperparasitaemia	24/53 (45.3%)	2.64 (1.20-5.83)	0.02		
no	674/4323 (15.6%)	Reference			
Presence of gametocytes	44/155 (28.4%)	1.17 (0.71-1.95)	0.54		
no gametocytes	631/4187 (15.1%)	Reference			
Mixed infection	17/35 (48.6%)	0.91 (0.36-2.25)	0.83		
mono-infection	681/4341 (15.7%)	Reference			
Malaria transmission intensity					
Low	405/804 (50.4%)	3.01 (1.09-8.32)	0.03	2.83 (1.05-7.65)	0.04
Moderate	161/2517 (6.4%)	Reference		Reference	
High	132/1055 (12.5%)	3.49 (1.93-6.33)	<0.001	3.32 (1.82-6.04)	<0.001

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, EGA: estimated gestational age, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for treatment and anorexia on day 0.

Appendix Table 4-10. Univariable and multivariable logistic regression of the risk of developing dizziness on day 1-7

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
AL	106/1198 (8.8%)	Reference		Reference	
ASAQ	217/857 (25.3%)	16.00 (10.21-25.08)	<0.001	16.04 (10.21-25.18)	<0.001
ASMQ	324/1025 (31.6%)	17.08 (11.12-26.21)	<0.001	17.03 (11.08-26.16)	<0.001
DP	36/834 (4.3%)	2.60 (1.44-4.70)	0.002	2.58 (1.43-4.67)	0.002
ASSP	10/121 (8.3%)	8.96 (3.47-23.17)	<0.001	8.82 (3.40-22.85)	<0.001
AS	121/206 (58.7%)	1.30 (0.76-2.24)	0.34	1.37 (0.79-2.36)	0.26
AC	3/11 (27.3%)	0.81 (0.13-5.12)	0.82	0.90 (0.15-5.48)	0.91
AAP	50/78 (64.1%)	4.36 (1.41-13.51)	0.01	4.60 (1.55-13.65)	0.01
Q	143/228 (62.7%)	9.08 (5.46-15.09)	<0.001	10.25 (6.08-17.28)	<0.001
QC	55/64 (85.9%)	7.89 (2.74-22.70)	<0.001	8.22 (2.86-23.68)	<0.001
Dizziness on day 0	351/494 (71.1%)	5.01 (3.73-6.74)	<0.001	4.79 (3.54-6.47)	<0.001
no	708/4122 (17.2%)	Reference		Reference	
EGA	1064/4619 (23.0%)	0.99 (0.97-1.00)	0.14	0.98 (0.97-1.00)	0.04
Age (years)	1065/4622 (23.0%)	1.02 (1.00-1.03)	0.04		
Gravidity 1	302/1660 (18.2%)	Reference			
2	231/1073 (21.5%)	1.13 (0.89-1.43)	0.33		
≥3	518/1860 (27.8%)	1.27 (1.04-1.56)	0.02		
Parity 0	358/1999 (17.9%)	Reference		Reference	
1	223/974 (22.9%)	1.15 (0.91-1.45)	0.26	1.14 (0.90-1.45)	0.27
≥2	470/1618 (29.0%)	1.30 (1.07-1.59)	0.01	1.33 (1.09-1.63)	0.01
Height (cm)	778/4044 (19.2%)	1.00 (0.98-1.01)	0.60		
Weight (kg)	1064/4605 (23.1%)	0.99 (0.98-1.00)	0.16		
BMI	777/4040 (19.2%)	0.96 (0.92-1.00)	0.03		
Fever (>37.5°C)	174/438 (39.7%)	1.15 (0.88-1.51)	0.32		
no fever	889/4164 (21.3%)	Reference			
Haemoglobin on day 0 (g/dL)	1055/4581 (23.0%)	1.04 (0.97-1.10)	0.27		
HIV infection	10/37 (27.0%)	1.22 (0.50-2.97)	0.66		
negative/not confirmed	1055/4585 (23.0%)	Reference			
Parasitaemia (log ₁₀ /μL)	1065/4622 (23.0%)	1.02 (0.92-1.14)	0.65		
Hyperparasitaemia	28/60 (46.7%)	2.19 (1.06-4.54)	0.03	2.26 (1.08-4.74)	0.03
no	1037/4562 (22.7%)	Reference		Reference	
Presence of gametocytes	57/161 (35.4%)	0.82 (0.53-1.26)	0.37		
no gametocytes	985/4423 (22.3%)	Reference			
Mixed infection	21/35 (60.0%)	1.84 (0.78-4.35)	0.16		
mono-infection	1044/4587 (22.8%)	Reference			
Malaria transmission intensity					
Low	522/1060 (49.2%)	3.10 (1.79-5.36)	<0.001	3.14 (1.79-5.50)	<0.001
Moderate	210/2535 (8.3%)	Reference		Reference	
High	333/1027 (32.4%)	2.99 (1.47-6.06)	0.002	2.92 (1.44-5.95)	0.003

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, EGA: estimated gestational age, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for treatment and dizziness on day 0.

Appendix Table 4-11. Univariable and multivariable logistic regression of the risk of developing headache on day 1-7

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
AL	275/1229 (22.4%)	Reference		Reference	
ASAQ	137/853 (16.1%)	1.56 (1.14-2.13)	0.01	1.56 (1.14-2.14)	0.01
ASMQ	199/1003 (19.8%)	1.21 (0.92-1.60)	0.17	1.21 (0.92-1.60)	0.17
DP	161/831 (19.4%)	1.22 (0.91-1.64)	0.17	1.24 (0.92-1.66)	0.16
ASSP	16/121 (13.2%)	1.13 (0.50-2.54)	0.77	1.13 (0.50-2.57)	0.76
AS	154/208 (74.0%)	1.05 (0.53-2.08)	0.88	1.03 (0.52-2.02)	0.94
AC	2/9 (22.2%)	Omitted			
AAP	53/78 (67.9%)	2.94 (1.05-8.25)	0.04	2.86 (1.00-8.19)	0.05
Q	36/228 (15.8%)	0.71 (0.37-1.37)	0.31	0.71 (0.36-1.38)	0.31
QC	41/63 (65.1%)	1.25 (0.46-3.42)	0.67	1.21 (0.44-3.32)	0.71
Headache on day 0	793/1818 (43.6%)	5.56 (4.64-6.66)	<0.001	5.56 (4.64-6.66)	<0.001
no	278/2802 (9.9%)	Reference		Reference	
EGA	1074/4620 (23.2%)	1.00 (0.99-1.02)	0.86		
Age (years)	1074/4623 (23.2%)	1.01 (0.99-1.02)	0.25		
Gravidity 1	325/1664 (19.5%)	Reference			
2	225/1073 (21.0%)	1.01 (0.80-1.27)	0.93		
≥3	507/1857 (27.3%)	1.20 (0.99-1.46)	0.06		
Parity 0	398/2001 (19.9%)	Reference			
1	220/974 (22.6%)	1.07 (0.86-1.34)	0.55		
≥2	439/1615 (27.2%)	1.22 (1.01-1.48)	0.04		
Height (cm)	912/4051 (22.5%)	1.00 (0.99-1.02)	0.86		
Weight (kg)	1072/4606 (23.3%)	1.00 (0.99-1.01)	0.50		
BMI	910/4047 (22.5%)	1.01 (0.98-1.05)	0.35		
Fever (>37.5°C)	172/435 (39.5%)	1.23 (0.93-1.64)	0.14		
no fever	893/4139 (21.6%)	Reference			
Haemoglobin on day 0 (g/dL)	1072/4584 (23.4%)	0.98 (0.92-1.04)	0.44		
HIV infection	5/37 (13.5%)	1.43 (0.49-4.12)	0.51		
negative/not confirmed	1069/4586 (23.3%)	Reference			
Parasitaemia (log ₁₀ /μL)	1074/4623 (23.2%)	1.00 (0.90-1.11)	0.98		
Hyperparasitaemia	26/62 (41.9%)	0.92 (0.48-1.74)	0.79		
no	1048/4561 (23.0%)	Reference			
Presence of gametocytes	44/165 (26.7%)	0.69 (0.43-1.10)	0.12		
no gametocytes	1005/4422 (22.7%)	Reference			
Mixed infection	23/34 (67.6%)	2.12 (0.76-5.92)	0.15		
mono-infection	1051/4589 (22.9%)	Reference			
Malaria transmission intensity					
Low	464/1048 (44.3%)	1.53 (0.67-3.52)	0.32	1.53 (0.67-3.52)	0.32
Moderate	427/2519 (17.0%)	Reference		Reference	
High	183/1056 (17.3%)	2.82 (1.59-5.00)	<0.001	2.82 (1.59-5.00)	<0.001

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, EGA: estimated gestational age, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for treatment and headache on day 0.

Appendix Table 4-12. Univariable and multivariable logistic regression of the risk of developing musculoskeletal pain on day 1-7

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
AL	139/1184 (11.7%)	Reference		Reference	
ASAQ	109/827 (13.2%)	2.80 (1.87-4.20)	<0.001	2.83 (1.88-4.24)	<0.001
ASMQ	96/883 (10.9%)	1.31 (0.89-1.93)	0.17	1.26 (0.85-1.86)	0.25
DP	85/831 (10.2%)	1.14 (0.76-1.71)	0.53	1.13 (0.75-1.69)	0.56
ASSP	No data	Omitted			
AS	124/203 (61.1%)	1.13 (0.62-2.08)	0.69	1.06 (0.58-1.94)	0.85
AC	1/9 (11.1%)	Omitted			
AAP	30/72 (41.7%)	2.41 (0.72-8.04)	0.15	1.77 (0.56-5.62)	0.33
Q	26/228 (11.4%)	2.43 (0.94-6.29)	0.07	1.87 (0.73-4.77)	0.19
QC	27/62 (43.5%)	0.73 (0.27-1.94)	0.52	0.71 (0.26-1.88)	0.49
Musculoskeletal pain on day 0	440/1027 (42.8%)	8.35 (6.66-10.46)	<0.001	7.76 (6.17-9.76)	<0.001
no	193/3268 (5.9%)	Reference		Reference	
EGA	637/4296 (14.8%)	1.02 (1.01-1.04)	0.01	1.02 (1.00-1.04)	0.03
Age (years)	637/4299 (14.8%)	1.02 (1.00-1.04)	0.02		
Gravidity 1	168/1532 (11.0%)	Reference			
2	119/984 (12.1%)	1.01 (0.74-1.37)	0.96		
≥3	350/1780 (19.7%)	1.35 (1.06-1.73)	0.02		
Parity 0	201/1843 (10.9%)	Reference		Reference	
1	126/894 (14.1%)	1.10 (0.82-1.47)	0.52	1.08 (0.80-1.45)	0.61
≥2	310/1555 (19.9%)	1.47 (1.16-1.87)	0.002	1.45 (1.14-1.85)	0.003
Height (cm)	545/3760 (14.5%)	1.00 (0.98-1.02)	0.96		
Weight (kg)	636/4282 (14.9%)	1.00 (0.99-1.02)	0.53		
BMI	544/3756 (14.5%)	1.02 (0.99-1.06)	0.23		
Fever (>37.5°C)	116/350 (33.1%)	1.57 (1.11-2.22)	0.01	1.58 (1.11-2.24)	0.01
no fever	521/3905 (13.3%)	Reference		Reference	
Haemoglobin on day 0 (g/dL)	637/4262 (14.9%)	0.99 (0.92-1.06)	0.74		
HIV infection	5/37 (13.5%)	1.41 (0.43-4.64)	0.57		
negative/not confirmed	632/4262 (14.8%)	Reference			
Parasitaemia (log ₁₀ /μL)	637/4299 (14.8%)	1.06 (0.94-1.21)	0.34		
Hyperparasitaemia	17/49 (34.7%)	1.46 (0.64-3.31)	0.37		
no	620/4250 (14.6%)	Reference			
Presence of gametocytes	29/154 (18.8%)	0.81 (0.46-1.42)	0.47		
no gametocytes	599/4134 (14.5%)	Reference			
Mixed infection	18/34 (52.9%)	1.55 (0.57-4.21)	0.39		
mono-infection	619/4265 (14.5%)	Reference			
Malaria transmission intensity					
Low	306/768 (39.8%)	4.04 (1.39-11.73)	0.01	3.39 (1.19-9.64)	0.02
Moderate	207/2475 (8.4%)	Reference		Reference	
High	124/1056 (11.7%)	2.56 (1.47-4.46)	0.001	2.68 (1.53-4.69)	0.001

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, EGA: estimated gestational age, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for treatment and musculoskeletal pain on day 0.

Appendix Table 4-13. Univariable and multivariable logistic regression of the risk of developing nausea on day 1-7

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
AL	75/1225 (6.1%)	Reference		Reference	
ASAQ	112/827 (13.5%)	6.56 (3.93-10.94)	<0.001	6.04 (3.68-9.91)	<0.001
ASMQ	208/1033 (20.1%)	6.22 (3.86-10.05)	<0.001	5.68 (3.58-9.00)	<0.001
DP	61/830 (7.3%)	3.14 (1.82-5.41)	<0.001	2.88 (1.70-4.88)	<0.001
ASSP	10/121 (8.3%)	1.54 (0.60-3.96)	0.37	1.30 (0.51-3.35)	0.59
AS	79/203 (38.9%)	1.15 (0.64-2.06)	0.63	1.23 (0.69-2.17)	0.48
AC	1/10 (10.0%)	Reference		Reference	
AAP	29/72 (40.3%)	6.39 (2.27-18.00)	<0.001	4.70 (1.83-12.07)	0.001
Q	76/227 (33.5%)	8.35 (4.16-16.77)	<0.001	7.29 (3.78-14.08)	<0.001
QC	29/61 (47.5%)	1.87 (0.70-4.98)	0.21	2.07 (0.80-5.39)	0.14
Nausea on day 0	307/599 (51.3%)	7.36 (5.83-9.28)	<0.001	7.60 (6.02-9.61)	<0.001
no	369/3987 (9.3%)	Reference		Reference	
EGA	680/4606 (14.8%)	0.99 (0.97-1.00)	0.11		
Age (years)	680/4609 (14.8%)	1.02 (1.01-1.04)	0.004	1.02 (1.01-1.04)	0.01
Gravidity 1	203/1666 (12.2%)	Reference			
2	149/1078 (13.8%)	0.87 (0.67-1.14)	0.32		
≥3	327/1862 (17.6%)	1.13 (0.90-1.41)	0.28		
Parity 0	245/2005 (12.2%)	Reference			
1	152/977 (15.6%)	1.04 (0.80-1.34)	0.78		
≥2	281/1620 (17.3%)	1.18 (0.94-1.47)	0.14		
Height (cm)	539/4063 (13.3%)	1.00 (0.98-1.01)	0.66		
Weight (kg)	680/4592 (14.8%)	0.99 (0.98-1.01)	0.31		
BMI	539/4059 (13.3%)	0.99 (0.95-1.02)	0.45		
Fever (>37.5°C)	115/418 (27.5%)	1.24 (0.92-1.67)	0.15		
no fever	563/4141 (13.6%)	Reference			
Haemoglobin on day 0 (g/dL)	675/4568 (14.8%)	0.99 (0.92-1.06)	0.69		
HIV infection	5/37 (13.5%)	1.48 (0.52-4.23)	0.46		
negative/not confirmed	675/4572 (14.8%)	Reference			
Parasitaemia (log ₁₀ /μL)	680/4609 (14.8%)	1.00 (0.89-1.12)	0.99		
Hyperparasitaemia	12/59 (20.3%)	0.93 (0.42-2.03)	0.85		
no	668/4550 (14.7%)	Reference			
Presence of gametocytes	34/166 (20.5%)	0.77 (0.48-1.23)	0.28		
no gametocytes	644/4416 (14.6%)	Reference			
Mixed infection	10/33 (30.3%)	1.01 (0.40-2.54)	0.99		
mono-infection	670/4576 (14.6%)	Reference			
Malaria transmission intensity					
Low	338/1015 (33.3%)	3.98 (2.04-7.78)	<0.001	3.95 (2.04-7.66)	<0.001
Moderate	188/2538 (7.4%)	Reference		Reference	
High	154/1056 (14.6%)	2.82 (1.59-5.02)	<0.001	2.76 (1.56-4.91)	0.001

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, EGA: estimated gestational age, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for treatment and nausea on day 0.

Appendix Table 4-14. Univariable and multivariable logistic regression of the risk of developing tinnitus on day 1-7

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
AL	41/1150 (3.6%)	Reference		Reference	
ASAQ	12/827 (1.5%)	5.09 (1.04-24.89)	0.04	4.24 (0.89-20.29)	0.07
ASMQ	37/1007 (3.7%)	7.06 (1.72-28.99)	0.01	6.22 (1.56-24.82)	0.01
DP	15/830 (1.8%)	6.20 (1.23-31.19)	0.03	5.75 (1.19-27.77)	0.03
ASSP	4/121 (3.3%)	2.05 (0.26-15.96)	0.49	1.67 (0.22-12.79)	0.62
AS	76/205 (37.1%)	1.64 (0.77-3.49)	0.20	1.73 (0.81-3.71)	0.16
AC	1/10 (10.0%)	0.26 (0.02-3.86)	0.33	0.28 (0.02-4.19)	0.36
AAP	26/75 (34.7%)	5.05 (0.94-27.26)	0.06	6.29 (1.33-29.86)	0.02
Q	168/228 (73.7%)	211.99 (63.05-712.78)	<0.001	249.84 (80.90-771.56)	<0.001
QC	54/63 (85.7%)	63.56 (17.33-233.10)	<0.001	71.91 (19.45-265.86)	<0.001
Tinnitus on day 0	182/287 (63.4%)	48.00 (29.52-78.07)	<0.001	50.95 (31.13-83.40)	<0.001
no	250/4227 (5.9%)	Reference		Reference	
EGA	434/4513 (9.6%)	1.00 (0.97-1.03)	0.94		
Age (years)	434/4516 (9.6%)	1.02 (0.99-1.04)	0.26		
Gravidity 1	118/1624 (7.3%)	Reference			
2	108/1055 (10.2%)	1.54 (0.97-2.44)	0.07		
≥3	208/1834 (11.3%)	1.33 (0.89-1.99)	0.17		
Parity 0	145/1956 (7.4%)	Reference			
1	103/960 (10.7%)	1.37 (0.88-2.14)	0.17		
≥2	186/1595 (11.7%)	1.23 (0.83-1.83)	0.31		
Height (cm)	202/3971 (5.1%)	0.99 (0.95-1.03)	0.49		
Weight (kg)	434/4499 (9.6%)	0.99 (0.97-1.02)	0.52		
BMI	202/3967 (5.1%)	0.99 (0.91-1.08)	0.88		
Fever (>37.5°C)	95/409 (23.2%)	0.97 (0.61-1.54)	0.90		
no fever	339/4092 (8.3%)	Reference			
Haemoglobin on day 0 (g/dL)	430/4476 (9.6%)	1.07 (0.96-1.21)	0.23		
HIV infection	9/37 (24.3%)	1.09 (0.34-3.48)	0.88		
negative/not confirmed	425/4479 (9.5%)	Reference			
Parasitaemia (log ₁₀ /μL)	434/4516 (9.6%)	1.06 (0.88-1.28)	0.51		
Hyperparasitaemia	13/55 (23.6%)	0.52 (0.18-1.47)	0.22		
no	421/4461 (9.4%)	Reference			
Presence of gametocytes	47/159 (29.6%)	1.75 (0.92-3.34)	0.09		
no gametocytes	384/4350 (8.8%)	Reference			
Mixed infection	11/34 (32.4%)	1.04 (0.31-3.49)	0.94		
mono-infection	423/4482 (9.4%)	Reference			
Malaria transmission intensity					
Low	356/1018 (35.0%)	4.26 (1.98-9.17)	<0.001	4.26 (1.98-9.17)	<0.001
Moderate	54/2472 (2.2%)	Reference		Reference	
High	24/1026 (2.3%)	5.90 (1.73-20.08)	0.005	5.90 (1.73-20.08)	0.005

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, EGA: estimated gestational age, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for treatment and tinnitus on day 0.

Appendix Table 4-15. Univariable and multivariable logistic regression of the risk of developing vomiting on day 1-7

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
AL	33/1223 (2.7%)	Reference		Reference	
ASAQ	172/853 (20.2%)	11.38 (6.95-18.62)	<0.001	11.56 (7.04-19.00)	<0.001
ASMQ	207/1025 (20.2%)	10.15 (6.28-16.41)	<0.001	10.34 (6.38-16.76)	<0.001
DP	70/830 (8.4%)	4.09 (2.41-6.96)	<0.001	4.24 (2.49-7.21)	<0.001
ASSP	5/121 (4.1%)	1.54 (0.50-4.76)	0.45	1.54 (0.50-4.75)	0.45
AS	30/200 (15.0%)	2.08 (1.03-4.19)	0.04	2.07 (1.03-4.17)	0.04
AC	0/9 (0.0%)	Omitted			
AAP	8/70 (11.4%)	5.12 (1.77-14.79)	0.003	4.96 (1.66-14.80)	0.004
Q	40/227 (17.6%)	9.85 (4.88-19.88)	<0.001	9.61 (4.70-19.63)	<0.001
QC	16/60 (26.7%)	9.21 (3.20-26.55)	<0.001	8.73 (3.00-25.37)	<0.001
Vomiting on day 0	157/351 (44.7%)	6.61 (5.05-8.64)	<0.001	6.46 (4.93-8.46)	<0.001
no	421/4245 (9.9%)	Reference		Reference	
EGA	581/4615 (12.6%)	0.98 (0.96-1.00)	0.03	0.98 (0.96-1.00)	0.03
Age (years)	581/4618 (12.6%)	1.01 (0.99-1.02)	0.41		
Gravidity 1	185/1663 (11.1%)	Reference			
2	127/1074 (11.8%)	0.93 (0.71-1.20)	0.57		
≥3	254/1852 (13.7%)	1.11 (0.89-1.39)	0.36		
Parity 0	232/2001 (11.6%)	Reference			
1	123/972 (12.7%)	0.95 (0.74-1.23)	0.71		
≥2	211/1612 (13.1%)	1.05 (0.84-1.31)	0.65		
Height (cm)	500/4055 (12.3%)	0.99 (0.98-1.01)	0.48		
Weight (kg)	581/4601 (12.6%)	1.00 (0.98-1.01)	0.41		
BMI	500/4051 (12.3%)	0.98 (0.95-1.02)	0.29		
Fever (>37.5°C)	78/429 (18.2%)	1.21 (0.89-1.65)	0.23		
no fever	502/4140 (12.1%)	Reference			
Haemoglobin on day 0 (g/dL)	577/4578 (12.6%)	1.05 (0.98-1.12)	0.19		
HIV infection	3/37 (8.1%)	0.58 (0.16-2.14)	0.41		
negative/not confirmed	578/4581 (12.6%)	Reference			
Parasitaemia (log ₁₀ /μL)	581/4618 (12.6%)	0.99 (0.88-1.11)	0.89		
Hyperparasitaemia	7/59 (11.9%)	0.72 (0.29-1.80)	0.48		
no	574/4559 (12.6%)	Reference			
Presence of gametocytes	21/163 (12.9%)	1.01 (0.61-1.68)	0.96		
no gametocytes	543/4402 (12.3%)	Reference			
Mixed infection	3/32 (9.4%)	0.73 (0.20-2.66)	0.64		
mono-infection	578/4586 (12.6%)	Reference			
Malaria transmission intensity					
Low	157/1024 (15.3%)	1.46 (0.86-2.49)	0.16	1.48 (0.87-2.54)	0.15
Moderate	254/2538 (10.0%)	Reference		Reference	
High	170/1056 (16.1%)	1.80 (1.09-2.96)	0.02	1.82 (1.10-3.01)	0.02

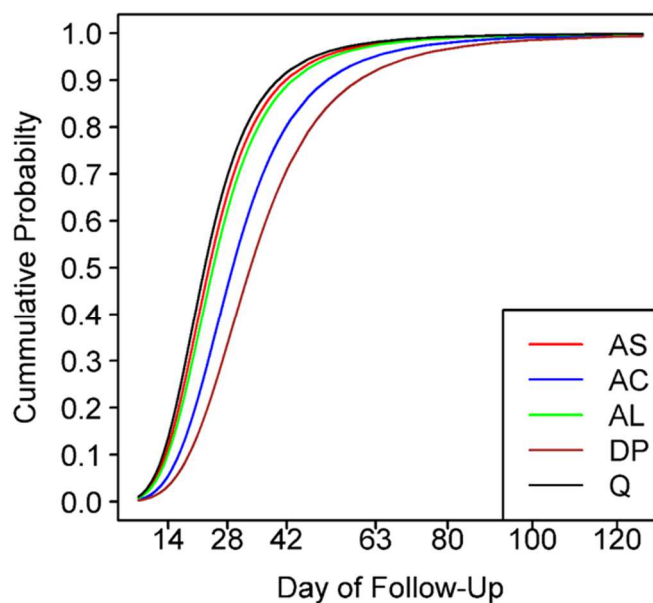
AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, EGA: estimated gestational age, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for treatment and vomiting on day 0.

Appendix Table 4-16. Univariable and multivariable logistic regression of the risk of developing fatigue on day 1-7

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
AL	139/1199 (11.6%)	Reference		Reference	
ASAQ	309/853 (36.2%)	12.74 (8.80-18.44)	<0.001	12.65 (8.70-18.38)	<0.001
ASMQ	221/954 (23.2%)	2.70 (1.90-3.82)	<0.001	2.62 (1.84-3.71)	<0.001
DP	133/831 (16.0%)	2.23 (1.53-3.26)	<0.001	2.16 (1.48-3.16)	<0.001
ASSP	49/121 (40.5%)	4.18 (2.12-8.26)	<0.001	3.93 (1.97-7.84)	<0.001
AS	67/141 (47.5%)	1.01 (0.58-1.78)	0.97	0.97 (0.55-1.70)	0.91
AC	0/3 (0.0%)	Omitted			
AAP	No data	Omitted			
Q	18/144 (12.5%)	2.79 (1.19-6.59)	0.02	2.43 (1.02-5.78)	0.05
QC	No data	Omitted			
Fatigue on day 0	545/1015 (53.7%)	7.80 (6.37-9.54)	<0.001	8.07 (6.57-9.91)	<0.001
no	391/3231 (12.1%)	Reference		Reference	
EGA	936/4243 (22.1%)	1.00 (0.98-1.02)	0.96		
Age (years)	936/4246 (22.0%)	1.02 (1.01-1.04)	0.004	1.03 (1.01-1.04)	0.001
Gravidity 1	275/1553 (17.7%)	Reference			
2	205/993 (20.6%)	1.04 (0.82-1.33)	0.72		
≥3	432/1671 (25.9%)	1.26 (1.03-1.55)	0.03		
Parity 0	347/1871 (18.5%)	Reference			
1	193/891 (21.7%)	1.05 (0.83-1.33)	0.69		
≥2	372/1453 (25.6%)	1.29 (1.05-1.58)	0.014		
Height (cm)	887/3910 (22.7%)	1.01 (1.00-1.02)	0.17		
Weight (kg)	936/4233 (22.1%)	1.00 (0.99-1.01)	0.63		
BMI	887/3910 (22.7%)	1.00 (0.96-1.03)	0.76		
Fever (>37.5°C)	134/371 (36.1%)	1.13 (0.84-1.52)	0.41		
no fever	800/3856 (20.7%)	Reference			
Haemoglobin on day 0 (g/dL)	935/4212 (22.2%)	0.96 (0.90-1.02)	0.19	0.93 (0.87-1.00)	0.04
HIV infection	9/37 (24.3%)	2.18 (0.88-5.43)	0.09		
negative/not confirmed	927/4209 (22.0%)	Reference			
Parasitaemia (log ₁₀ /μL)	936/4246 (22.0%)	0.96 (0.86-1.07)	0.45		
Hyperparasitaemia	21/48 (43.8%)	1.66 (0.81-3.42)	0.17		
no	915/4198 (21.8%)	Reference			
Presence of gametocytes	38/128 (29.7%)	1.25 (0.78-2.00)	0.35		
no gametocytes	871/4085 (21.3%)	Reference			
Mixed infection	10/27 (37.0%)	0.87 (0.32-2.36)	0.78		
mono-infection	926/4219 (21.9%)	Reference			
Malaria transmission intensity					
Low	267/702 (38.0%)	2.33 (1.08-5.05)	0.03	2.11 (0.96-4.63)	0.06
Moderate	380/2518 (15.1%)	Reference		Reference	
High	289/1026 (28.2%)	4.04 (2.36-6.92)	<0.001	4.10 (2.39-7.04)	<0.001

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, EGA: estimated gestational age, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. *Adjusted for treatment and weakness on day 0.

Appendix 5-1



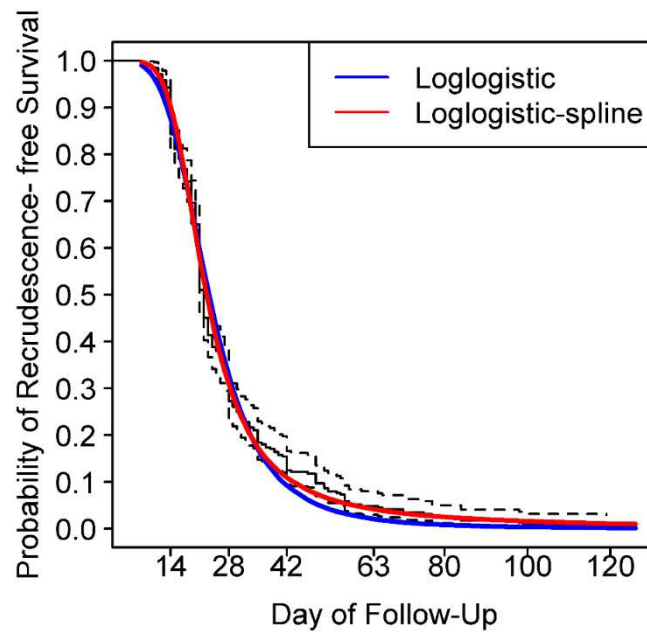
Appendix Figure 5-1. Cumulative probability of recrudescence over time by treatment in pregnant women on the Thailand-Myanmar border, 1994–2010

AC, artesunate with clindamycin; AL, artemether-lumefantrine; AS, artesunate monotherapy; DP, dihydroartemisinin-piperaquine; Q, quinine monotherapy.

Appendix 5-2. A sensitivity analysis by the flexible parametric models

Using the variables in the final model, sensitivity analyses were conducted whether other parametric models gave a better fit to the data. Among the AFT models (without knots), log-logistic model was confirmed to have the smallest AIC (Appendix Table 5-1). When Royston and Parmar's flexible parametric models (restricted cubic splines with knots) were applied, log-logistic model with 1 knot was with the smallest AIC. Because flexible parametric models lose the interpretability as AFT model, we preferred the original log-logistic model (without knots) as the final model. Instead, the prediction by log-logistic model with 1 knot was compared with our final model.

Graphically, the improvement of the fit to the data was mainly seen in the right-hand tail of the survival curve, particularly after 42 days (Appendix Figure 5-2). The predicted cumulative probability of recrudescence (the proportion of recrudescence detected) at day 28, 42 and 63 were calculated based on this model (Appendix Table 5-2). As expected from the graphical presentation, the gap between the final model and the sensitivity analysis model became wider when the follow-up days became longer. The predicted cumulative proportion of treatment failure detected by 42 or 63 were consistently lower by 1–7% than those by the final model.



Appendix Figure 5-2. Survival curves of multivariable model by log-logistic distribution model and splined log-logistic distribution model with one knot
Kaplan-Meier curve is shown with a black line with 95% confidence interval.

Appendix Table 5-1. Akaike information criterion (AIC) comparing different accelerated failure time models and flexible parametric models with covariates

Model	Number of knot	AIC
Exponential	0	857.3
Weibull	0	658.7
	1	504.7
	2	495.6
Log normal	0	525.5
	1	492.6
	2	493.4
Log logistic	0	513.8
	1	492.1
	2	493.5

AIC: Akaike information criterion.

Appendix Table 5-2. Predicted cumulative proportions of treatment failure detected by days 28, 42 and 63 after antimalarial treatment by a model using log-logistic distribution with one knot

Treatment	Predicted cumulative proportion of treatment failure detected by		
	Day 28	Day 42	Day 63
	Proportion (95% CI)	Proportion (95% CI)	Proportion (95% CI)
Quinine	0.71 (0.67–0.76)	0.90 (0.87, 0.92)	0.96 (0.94–0.98)
AS	0.67 (0.55–0.78)	0.88 (0.81–0.93)	0.95 (0.92–0.98)
AL	0.65 (0.44–0.81)	0.87 (0.74–0.94)	0.95 (0.88–0.98)
AC	0.49 (0.29–0.69)	0.78 (0.60–0.89)	0.91 (0.80–0.96)
DP	0.37 (0.13–0.68)	0.68 (0.36–0.89)	0.86 (0.60–0.96)

AC: artesunate with clindamycin. AL: artemether-lumefantrine. AS: artesunate monotherapy. CI: confidence interval. DP: dihydroartemisinin-piperaquine.

Appendix 5-3. A sensitivity analysis by a model without censoring by *P. vivax* infection

Using the final multivariable log-logistic model, another sensitivity analysis was conducted ignoring *P. vivax* intercalated infection. In this analysis, those who had *P. vivax* intercalated infection were not censored on the date of *P. vivax* intercalated infection, and the time to recrudescence was analysed for all episodes regardless of the *P. vivax* intercalated infection.

The predicted cumulative proportion of treatment failure detected by 28, 42, 63 days were consistently lower by 1–4% than those by the final model (Appendix Table 5-3).

Appendix Table 5-3. Predicted cumulative proportions of treatment failure detected by days 28, 42 and 63 after antimalarial treatment by a model using log-logistic distribution without censoring *P. vivax* intercalated infection

Treatment	Predicted cumulative proportion of treatment failure detected by		
	Day 28	Day 42	Day 63
	Proportion (95% CI)	Proportion (95% CI)	Proportion (95% CI)
Quinine	0.69 (0.64–0.73)	0.91 (0.88–0.93)	0.98 (0.97–0.98)
AS	0.62 (0.50–0.74)	0.88 (0.81–0.93)	0.97 (0.95–0.98)
AL	0.58 (0.37–0.76)	0.86 (0.72–0.94)	0.97 (0.92–0.99)
AC	0.42 (0.24–0.64)	0.77 (0.58–0.89)	0.94 (0.86–0.97)
DP	0.34 (0.13–0.65)	0.70 (0.40–0.89)	0.91 (0.75–0.97)

AC: artesunate with clindamycin. AL: artemether-lumefantrine. AS: artesunate monotherapy. CI: confidence interval. DP: dihydroartemisinin-piperaquine.

Appendix 5-4. A model including mefloquine monotherapy

A sensitivity analysis was conducted including mefloquine monotherapy (n=15), which is neither an artemisinin-based nor quinine-based treatment. Mefloquine monotherapy data available for this additional analysis included episodes from 1994–1996 when high grade resistance to mefloquine was already prevalent in the area.^{253,374}

In the univariable analysis, treatment was associated with time to recrudescence (not shown). With quinine as the reference, the time to recrudescence (95% confidence interval [CI]) was longer by 1.04-times (0.90 to 1.20) after AS, 1.08-times (0.86 to 1.35) after AL, 1.30-times (1.03 to 1.63) after AC, 1.46-times (1.02 to 2.08) after DP and 1.09-times (0.86 to 1.38) after mefloquine.

In the multivariable analysis, treatment was associated with time to recrudescence adjusted by baseline parasitemia. The adjusted interval to recrudescence was longer by 1.05-times (95% CI: 0.91 to 1.21) after AS, 1.09-times (0.87 to 1.36) after AL, 1.29-times (1.03 to 1.63) after AC, 1.48-times (1.04 to 2.12) after DP, and 1.08-times (0.86 to 1.37) after mefloquine than that after quinine.

The peak time of recrudescence was day 20 for quinine, day 21 for AS, AL and mefloquine, day 25 for AC and day 29 for DP. The predicted cumulative proportions of recrudescence detected by commonly used follow-up days (day 28, 42 and 63) for the other drugs were almost unchanged by including mefloquine monotherapy, with differences of up to 1 % (0.01) (Appendix Table 5-4).

Appendix Table 5-4. Predicted cumulative proportions of treatment failure detected by days 28, 42 and 63 after antimalarial treatment including mefloquine monotherapy by a model using log-logistic distribution

Treatment	Predicted cumulative proportion of treatment failure detected by		
	Day 28	Day 42	Day 63
	Proportion (95% CI)	Proportion (95% CI)	Proportion (95% CI)
Quinine	0.69 (0.65–0.74)	0.92 (0.89–0.94)	0.98 (0.97–0.99)
AS	0.66 (0.53–0.76)	0.90 (0.84–0.94)	0.98 (0.96–0.99)
AL	0.62 (0.41–0.79)	0.89 (0.77–0.95)	0.97 (0.94–0.99)
AC	0.46 (0.26–0.67)	0.80 (0.63–0.91)	0.95 (0.89–0.98)
DP	0.33 (0.11–0.66)	0.70 (0.38–0.90)	0.92 (0.74–0.98)
Mefloquine	0.63 (0.41–0.80)	0.89 (0.77–0.95)	0.97 (0.94–0.99)

AC: artesunate with clindamycin. AL: artemether-lumefantrine. AS: artesunate monotherapy. CI: confidence interval. DP: dihydroartemisinin-piperaquine.

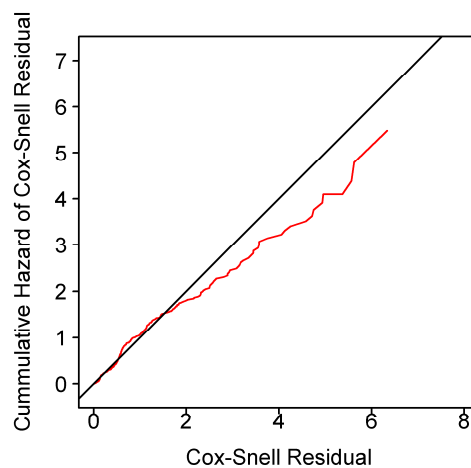
Appendix 5-5. Assessment of model fit and assumption

The overall model fit of the final model was assessed visually (Appendix Figure 5-3).

The final log-logistic model gave a reasonable fit to the data except the right-tail with less samples.

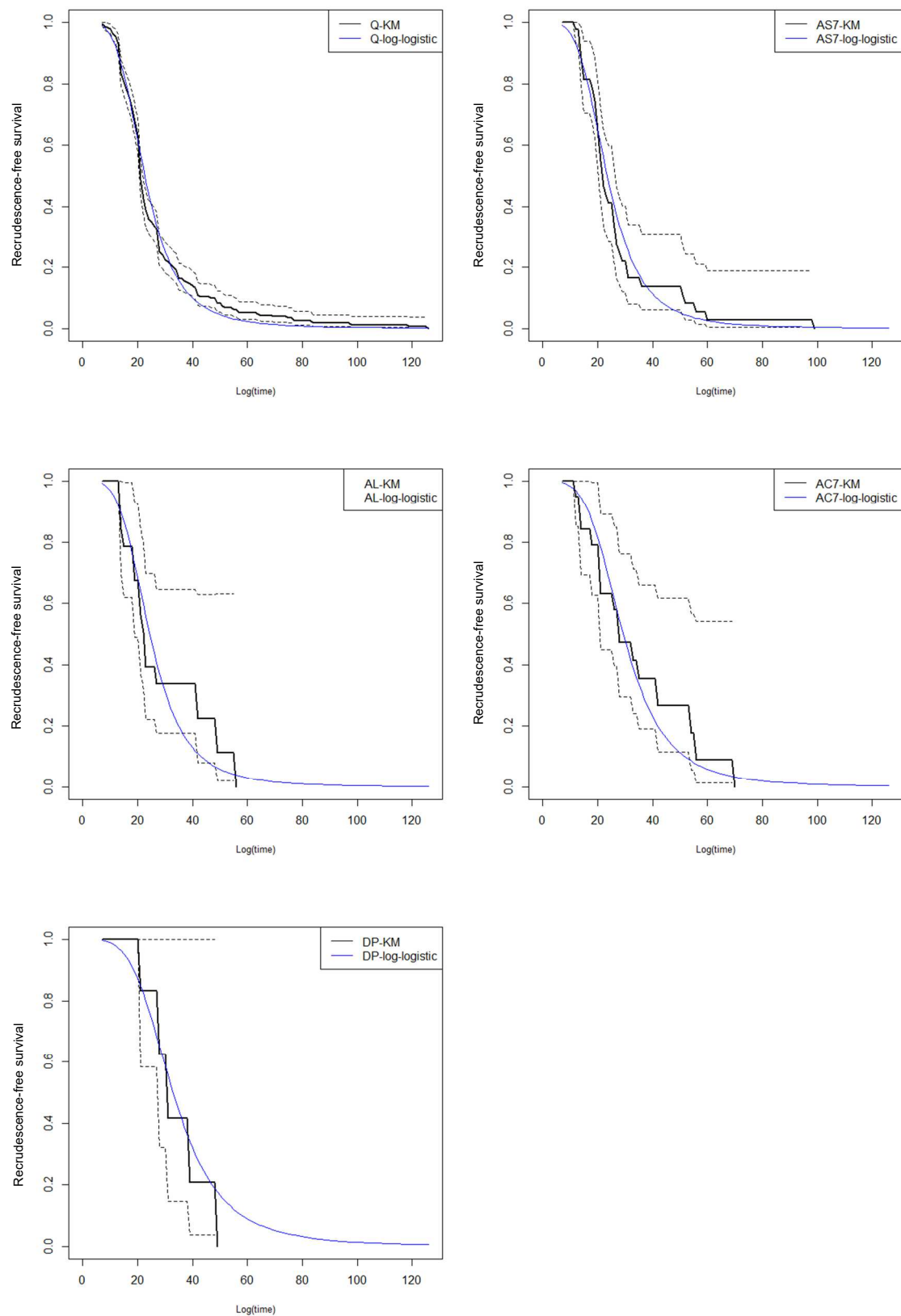
To assess the model fit of each treatment group, predicted survival (1-predicted cumulative probability of recrudescence) was plotted with Kaplan-Meier survival estimates (Appendix Figure 5-4). The predicted curves fit well to the data even for DP with the smallest number of recrudescence.

The assumption for the log-logistic model was checked by plotting log odds of survival against log of time (Appendix Figure 5-5). The lines are reasonably straight, suggesting that log-logistic model is a reasonable model.

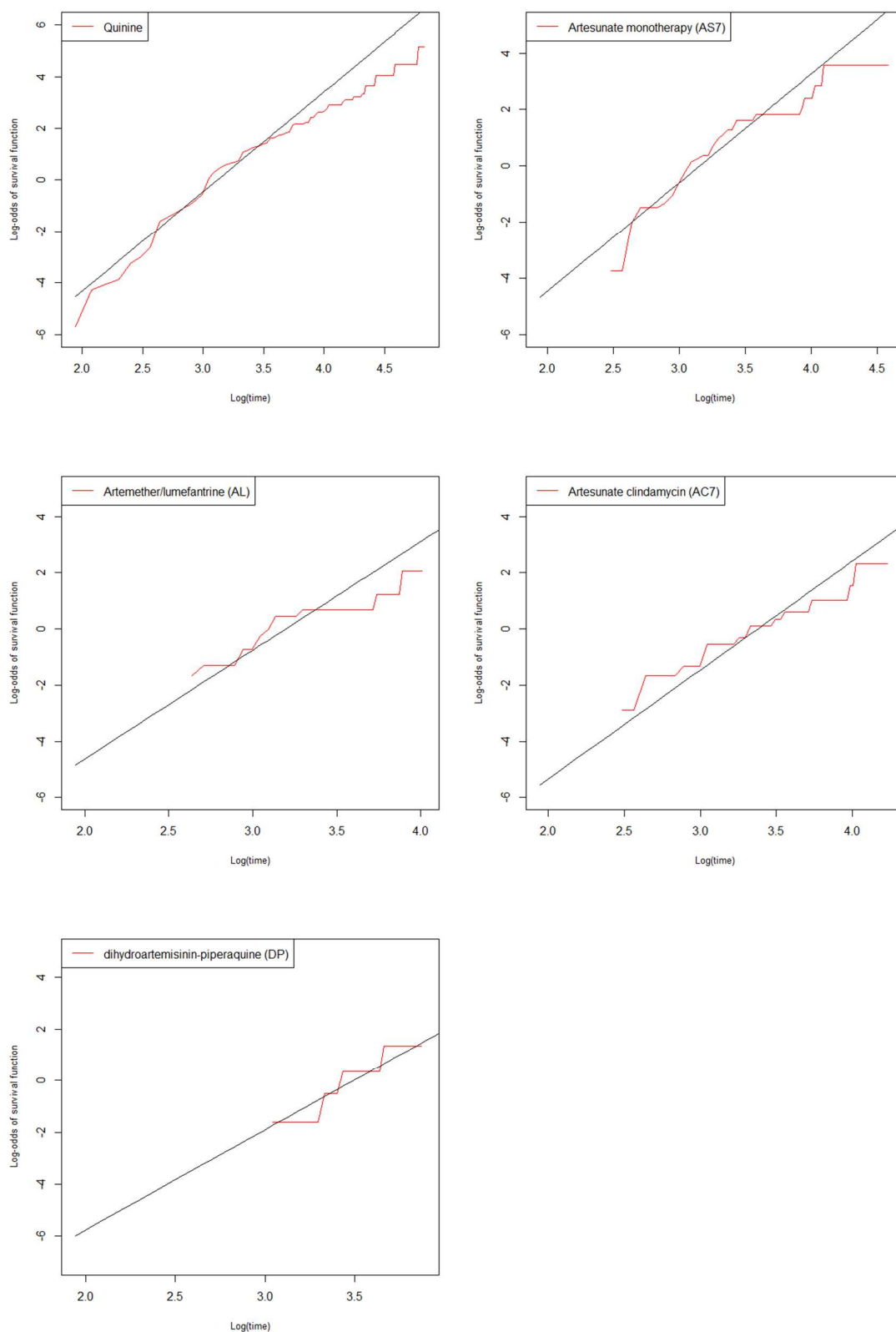


Appendix Figure 5-3. Cumulative hazard plot of the Cox-Snell residuals

Straight line is the reference line with an intercept of 0 and slope of 1.



Appendix Figure 5-4. Predicted overall recurrence-free survival based on the final log-logistic model compared with Kaplan-Meier survival estimates for each treatment



Appendix Figure 5-5. Assumption check for log-logistic model for each treatment
 Jagged lines are based on Kaplan-Meier estimates of survival curves and smooth lines are based on fitted values from the log-logistic model.

Appendix 6. Methods of multiple imputation

Multiple imputation was conducted in Chapter 6 because height (and BMI), which are known factors associated with pregnancy outcomes, were missing up to 15%. HIV status was also missing in up to 18%, even when the pregnant women at SMRU (where seroprevalence of HIV was <0.5%) were regarded as negative. Height is normally distributed and assumed to be conditionally missing at random. Height was not assessed in six studies and partially missing in another study. HIV status was not assessed in one study and most of the missing was from another large study. The latter study excluded women who were treated for HIV.

Imputation was conducted for 20 times under fully conditional specification with data augmentation for perfect prediction. All variables assessed in the univariable model and a variable for study site (as a fixed variable) were included in the imputation model. BMI was passively calculated based on the imputed height and weight. Imputation was conducted for each outcome. The variable for outcome (and Nelson-Aalen estimator for survival analyses) were also included in the imputation model. Pregnant women with missing outcome were not included in the imputation. Interaction term was not included in the imputation model. The imputed results were combined by Rubin's rules. Imputation and combining were conducted using Stata 15.1 MP (*mi* command).

Because missingness of height was more frequent in quinine and quinine with clindamycin, complete case analyses were biased when adjusted for height. The multivariable results by multiple imputation method were similar to univariable results by complete case analyses method (data not shown).

Appendix Table 6-1. Univariable and multivariable analyses of the risk of moderate to late preterm birth assuming day 6 on the week

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
AL	114/1043	Reference		Reference	
ASAQ	82/750	0.95 (0.69-1.31)	0.76	0.96 (0.69-1.33)	0.79
ASMQ	81/927	0.95 (0.69-1.32)	0.77	0.91 (0.65-1.28)	0.60
DP	102/807	1.19 (0.88-1.61)	0.26	1.13 (0.82-1.54)	0.45
ASSP	21/148	1.81 (0.88-3.72)	0.11	1.74 (0.81-3.75)	0.16
AS	15/174	1.57 (0.72-3.42)	0.26	1.51 (0.63-3.58)	0.35
AAP	4/76	0.62 (0.19-2.02)	0.43	0.69 (0.19-2.50)	0.58
Q	15/171	1.28 (0.63-2.61)	0.50	1.70 (0.79-3.68)	0.17
QC	4/51	1.18 (0.31-4.49)	0.80	1.45 (0.35-6.03)	0.61
Age (year)					
<20	210/1334	Reference		Reference	
20-24	106/1333	0.50 (0.39-0.65)	<0.001	0.60 (0.44-0.82)	0.001
25-29	68/804	0.55 (0.41-0.74)	<0.001	0.74 (0.48-1.14)	0.18
30-34	28/401	0.44 (0.29-0.67)	<0.001	0.57 (0.33-0.99)	0.04
≥35	26/275	0.71 (0.45-1.11)	0.13	0.94 (0.53-1.66)	0.84
Gravidity and previous history of pregnancy loss					
G1	210/1449	Reference		Reference	
G2 with no loss	58/706	0.56 (0.41-0.76)	<0.001	0.68 (0.48-0.97)	0.04
G≥3 with no loss	91/1129	0.55 (0.42-0.71)	<0.001	0.65 (0.43-0.99)	0.04
G2 with 1 loss	27/252	0.82 (0.53-1.28)	0.39	0.92 (0.58-1.45)	0.71
G≥3 with 1 loss	34/436	0.57 (0.39-0.84)	0.004	0.66 (0.41-1.06)	0.08
G≥3 with ≥2 losses	15/138	1.02 (0.57-1.81)	0.95	1.15 (0.61-2.19)	0.66
Height (cm)	399/3670	0.98 (0.97-1.00)	0.08	0.98 (0.96-1.00)	0.03
BMI	399/3670	0.97 (0.93-1.01)	0.10	0.94 (0.90-0.98)	0.008
Fever (>37.5°C)	35/375	1.05 (0.72-1.55)	0.80		
No fever	393/3734	Reference			
Haemoglobin on day 0 (g/dL)					
	435/4123	0.93 (0.86-1.00)	0.06		
Parasitaemia (log ₁₀ /μL)	438/4147	1.08 (0.95-1.22)	0.23		
Presence of gametocyte	21/154	1.68 (1.03-2.73)	0.04		
Without gametocyte	407/3936	Reference			
Mixed infection	2/29	1.02 (0.23-4.51)	0.98		
Monoinfection	436/4118	Reference			
HIV infection	4/31	1.69 (0.52-5.49)	0.38	1.57 (0.48-5.16)	0.46
not confirmed	376/3476	Reference		Reference	
Gestational period					
<14 weeks	5/38	4.22 (1.50-11.86)	0.01	4.31 (1.51-12.31)	0.01
14-19.9 weeks	53/866	Reference		Reference	
20-23.9 weeks	101/1029	1.84 (1.29-2.62)	0.001	1.86 (1.30-2.65)	0.001
24-27.9 weeks	90/921	1.99 (1.38-2.85)	<0.001	2.17 (1.51-3.14)	<0.001
28-36.9 weeks	189/1293	3.79 (2.70-5.32)	<0.001	4.52 (3.20-6.40)	<0.001
Malaria transmission					
Low	72/929	Reference		Reference	
Moderate	265/2247	1.40 (0.77-2.56)	0.27	1.61 (0.84-3.10)	0.15
High	101/971	1.31 (0.63-2.72)	0.47	1.40 (0.62-3.18)	0.42

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, G: gravidity, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. * Adjusted for treatment.

Appendix Table 6-2. Univariable and multivariable analyses of the risk of moderate to late preterm birth assuming day 6 on the week after different treatments in different gestational periods of pregnancy

Treatment	Proportion	Univariable		Multivariable*	
		OR (95% CI)	p-value	OR (95% CI)	p-value
14-19.9 weeks					
AL	11/250	Reference		Reference	
ASAQ	11/171	1.28 (0.54-3.06)	0.58	1.34 (0.56-3.21)	0.51
ASMQ	9/134	2.59 (1.02-6.62)	0.05	2.83 (1.10-7.27)	0.03
DP	13/173	1.56 (0.67-3.61)	0.30	1.63 (0.70-3.78)	0.26
ASSP	2/28	2.86 (0.54-15.22)	0.22	3.10 (0.58-16.66)	0.19
AS	1/35	1.79 (0.20-15.66)	0.60	2.19 (0.24-19.60)	0.48
AAP	2/17	4.94 (0.85-28.84)	0.08	6.30 (1.04-38.08)	0.04
Q	3/48	3.25 (0.78-13.54)	0.11	4.21 (1.00-17.77)	0.05
QC	1/10	5.55 (0.53-58.46)	0.15	6.40 (0.59-69.22)	0.13
20-23.9 weeks					
AL	25/271	Reference		Reference	
ASAQ	16/175	0.89 (0.45-1.75)	0.74	0.95 (0.48-1.88)	0.88
ASMQ	16/229	1.10 (0.56-2.17)	0.78	1.13 (0.57-2.23)	0.73
DP	29/208	1.47 (0.82-2.65)	0.19	1.41 (0.78-2.55)	0.25
ASSP	6/38	2.54 (0.81-8.03)	0.11	2.50 (0.78-7.97)	0.12
AS	4/32	3.34 (0.93-12.00)	0.07	3.83 (1.03-14.29)	0.05
AAP	0/15	0.22 (0.02-1.88)	0.17	0.23 (0.03-2.07)	0.19
Q	5/52	2.23 (0.71-7.00)	0.17	2.84 (0.89-9.06)	0.08
QC	0/9	0.91 (0.16-5.33)	0.92	1.00 (0.17-5.99)	1.00
24-27.9 weeks					
AL	23/218	Reference		Reference	
ASAQ	20/207	0.85 (0.44-1.62)	0.62	0.87 (0.45-1.68)	0.68
ASMQ	13/221	0.75 (0.36-1.56)	0.44	0.70 (0.34-1.45)	0.33
DP	19/158	1.15 (0.59-2.24)	0.68	1.15 (0.59-2.26)	0.68
ASSP	5/34	2.18 (0.66-7.17)	0.20	1.97 (0.59-6.58)	0.27
AS	6/26	5.69 (1.74-18.57)	0.004	5.96 (1.75-20.26)	0.004
AAP	0/14	0.22 (0.02-1.88)	0.17	0.23 (0.03-2.07)	0.19
Q	3/29	2.25 (0.56-8.98)	0.25	2.81 (0.68-11.60)	0.15
QC	1/14	1.29 (0.13-12.64)	0.83	1.73 (0.17-17.47)	0.64
28-36.9 weeks					
AL	54/297	Reference		Reference	
ASAQ	35/196	1.00 (0.60-1.65)	0.99	0.92 (0.55-1.54)	0.76
ASMQ	42/339	0.77 (0.48-1.22)	0.26	0.73 (0.46-1.16)	0.19
DP	41/259	0.91 (0.57-1.47)	0.70	0.85 (0.53-1.39)	0.52
ASSP	7/45	1.19 (0.42-3.39)	0.74	1.10 (0.38-3.16)	0.86
AS	3/73	0.36 (0.09-1.36)	0.13	0.35 (0.09-1.36)	0.13
AAP	1/28	0.22 (0.02-1.88)	0.17	0.23 (0.03-2.07)	0.19
Q	4/38	0.99 (0.30-3.26)	0.99	1.05 (0.31-3.54)	0.94
QC	2/18	0.91 (0.16-5.33)	0.92	1.00 (0.17-5.99)	1.00

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin.

* Adjusted for age, height, BMI, HIV status and gravidity combined with previous history of pregnancy loss.

Appendix Table 6-3. Univariable and multivariable analyses of the risk of small-for-gestational-age assuming day 6 on the week

Characteristic	Proportion	Univariable*		Multivariable	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment					
AL	307/969	Reference		Reference	
ASAQ	253/695	1.24 (0.99-1.56)	0.06	1.28 (1.01-1.63)	0.04
ASMQ	337/810	1.11 (0.89-1.38)	0.36	1.10 (0.87-1.38)	0.43
DP	230/703	0.98 (0.77-1.23)	0.84	0.98 (0.77-1.25)	0.86
ASSP	67/128	1.51 (0.92-2.49)	0.10	1.35 (0.79-2.31)	0.28
AS	56/154	1.22 (0.75-1.98)	0.42	1.17 (0.69-2.01)	0.55
AAP	25/55	1.61 (0.78-3.30)	0.20	1.43 (0.60-3.43)	0.42
Q	20/119	0.49 (0.27-0.89)	0.02	0.55 (0.27-1.11)	0.09
QC	15/41	1.09 (0.46-2.58)	0.84	0.92 (0.36-2.39)	0.87
Age (year)					
<20	494/1199	Reference			
20-24	431/1175	0.70 (0.58-0.83)	<0.001		
25-29	210/710	0.50 (0.40-0.61)	<0.001		
30-34	96/348	0.45 (0.35-0.60)	<0.001		
≥35	79/242	0.56 (0.41-0.75)	<0.001		
Gravidity and previous history of pregnancy loss					
G1	577/1312	Reference		Reference	
G2 with no loss	192/606	0.54 (0.44-0.67)	<0.001	0.57 (0.46-0.70)	<0.001
G≥3 with no loss	257/998	0.40 (0.33-0.48)	<0.001	0.47 (0.38-0.57)	<0.001
G2 with 1 loss	99/225	0.81 (0.60-1.10)	0.18	0.81 (0.59-1.09)	0.17
G≥3 with 1 loss	116/379	0.46 (0.36-0.59)	<0.001	0.54 (0.41-0.70)	<0.001
G≥3 with ≥2 losses	50/117	0.78 (0.52-1.16)	0.22	0.80 (0.53-1.21)	0.30
Height (cm)	1193/3315	0.95 (0.94-0.97)	<0.001	0.95 (0.94-0.96)	<0.001
BMI	1193/3315	0.92 (0.90-0.95)	<0.001	0.92 (0.90-0.95)	<0.001
Fever (>37.5°C)	124/324	1.03 (0.80-1.32)	0.83		
No fever	1178/3322	Reference			
Haemoglobin on day 0 (g/dL)					
	1306/3657	0.88 (0.84-0.93)	<0.001		
Parasitaemia (log ₁₀ /μL)	1310/3674	1.24 (1.14-1.35)	<0.001	1.13 (1.03-1.24)	0.01
Presence of gametocyte	45/133	0.90 (0.61-1.32)	0.59		
Without gametocyte	1246/3490	Reference			
Mixed infection	14/25	2.76 (1.21-6.31)	0.02	2.45 (1.04-5.76)	0.04
Monoinfection	1296/3649	Reference		Reference	
HIV infection	7/24	0.81 (0.32-2.03)	0.65	1.01 (0.41-2.49)	0.99
not confirmed	1078/3082	Reference		Reference	
Gestational period					
<14 weeks	10/31	0.66 (0.29-1.48)	0.31	0.60 (0.26-1.40)	0.24
14-19.9 weeks	288/758	Reference		Reference	
20-23.9 weeks	334/911	0.84 (0.68-1.04)	0.10	0.84 (0.68-1.04)	0.12
24-27.9 weeks	295/816	0.75 (0.61-0.93)	0.01	0.82 (0.65-1.02)	0.08
28-36.9 weeks	367/1119	0.58 (0.47-0.72)	<0.001	0.67 (0.54-0.84)	<0.001
≥37 weeks	16/39	0.98 (0.49-1.97)	0.96	1.30 (0.63-2.68)	0.48
Malaria transmission					
Low	277/726	Reference			
Moderate	680/2018	1.13 (0.73-1.75)	0.58		
High	353/930	0.90 (0.53-1.51)	0.69		

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, BMI: body mass index, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, G: gravidity, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin. * Adjusted for treatment.

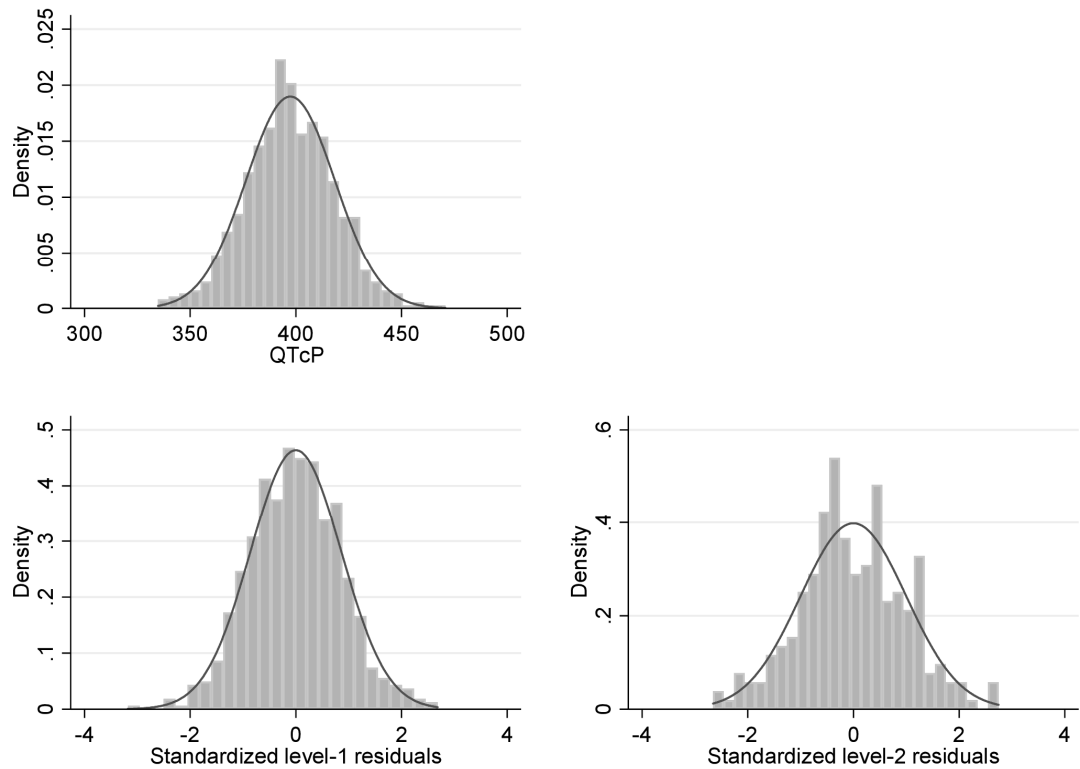
Appendix Table 6-4. Univariable and multivariable analyses of the risk of small-for-gestational-age assuming day 6 on the week after different treatments in different gestational periods of pregnancy

Treatment	Proportion	Univariable		Multivariable*	
		OR (95% CI)	p-value	OR (95% CI)	p-value
14-19.9 weeks					
AL	79/229	Reference		Reference	
ASAQ	63/161	1.29 (0.83-2.00)	0.26	1.24 (0.79-1.95)	0.35
ASMQ	52/105	1.07 (0.65-1.76)	0.79	1.07 (0.64-1.79)	0.79
DP	56/150	1.11 (0.70-1.74)	0.66	1.10 (0.69-1.75)	0.69
ASSP	11/25	0.78 (0.31-1.95)	0.60	0.68 (0.26-1.75)	0.43
AS	12/34	0.95 (0.41-2.17)	0.90	0.98 (0.41-2.35)	0.97
AAP	5/12	1.24 (0.33-4.68)	0.75	1.27 (0.30-5.30)	0.74
Q	8/35	0.50 (0.20-1.24)	0.13	0.57 (0.21-1.51)	0.26
QC	2/7	0.65 (0.11-3.82)	0.63	0.48 (0.07-3.14)	0.44
20-23.9 weeks					
AL	89/250	Reference		Reference	
ASAQ	60/164	1.05 (0.69-1.62)	0.81	1.17 (0.75-1.82)	0.49
ASMQ	91/209	0.95 (0.64-1.41)	0.80	0.98 (0.65-1.47)	0.91
DP	62/181	0.97 (0.63-1.47)	0.87	0.95 (0.62-1.47)	0.83
ASSP	14/33	0.90 (0.39-2.07)	0.81	0.77 (0.32-1.85)	0.56
AS	11/25	1.46 (0.59-3.61)	0.41	1.29 (0.50-3.34)	0.59
AAP	1/10	0.17 (0.02-1.51)	0.11	0.19 (0.02-1.82)	0.15
Q	4/34	0.25 (0.08-0.77)	0.02	0.28 (0.08-0.91)	0.03
QC	2/5	1.17 (0.17-7.96)	0.87	1.39 (0.19-10.02)	0.74
24-27.9 weeks					
AL	66/205	Reference		Reference	
ASAQ	70/193	1.25 (0.81-1.93)	0.30	1.29 (0.82-2.01)	0.27
ASMQ	86/196	1.23 (0.81-1.87)	0.34	1.15 (0.75-1.79)	0.52
DP	41/137	0.87 (0.53-1.42)	0.57	0.84 (0.51-1.40)	0.51
ASSP	16/27	2.01 (0.82-4.94)	0.13	1.68 (0.66-4.28)	0.28
AS	4/16	0.75 (0.22-2.57)	0.65	0.48 (0.13-1.78)	0.27
AAP	5/10	1.87 (0.47-7.48)	0.38	1.25 (0.28-5.62)	0.77
Q	4/22	0.56 (0.17-1.83)	0.34	0.67 (0.19-2.37)	0.54
QC	3/10	0.96 (0.21-4.39)	0.95	0.78 (0.16-3.85)	0.76
28-36.9 weeks					
AL	67/265	Reference		Reference	
ASAQ	60/177	1.45 (0.93-2.25)	0.10	1.50 (0.95-2.36)	0.08
ASMQ	108/295	1.30 (0.89-1.91)	0.17	1.27 (0.85-1.88)	0.24
DP	67/228	1.06 (0.69-1.60)	0.80	1.03 (0.67-1.59)	0.89
ASSP	26/41	3.13 (1.43-6.87)	0.00	2.91 (1.29-6.55)	0.01
AS	22/59	2.13 (1.05-4.32)	0.03	1.91 (0.91-4.01)	0.09
AAP	8/17	2.26 (0.72-7.10)	0.16	1.78 (0.51-6.19)	0.36
Q	3/22	0.59 (0.16-2.17)	0.43	0.62 (0.16-2.43)	0.50
QC	6/15	2.24 (0.65-7.74)	0.20	1.60 (0.43-6.02)	0.49

AAP: Artesunate with atovaquone-proguanil, AL: artemether-lumefantrine, AS: Artesunate monotherapy, ASAQ: Artesunate-amodiaquine, ASMQ: Artesunate-mefloquine, ASSP: Artesunate-sulfadoxine-pyrimethamine, CI: confidence interval, DP: Dihydroartemisinin-piperaquine, OR: odds ratio, Q: Quinine monotherapy, QC: Quinine with clindamycin.

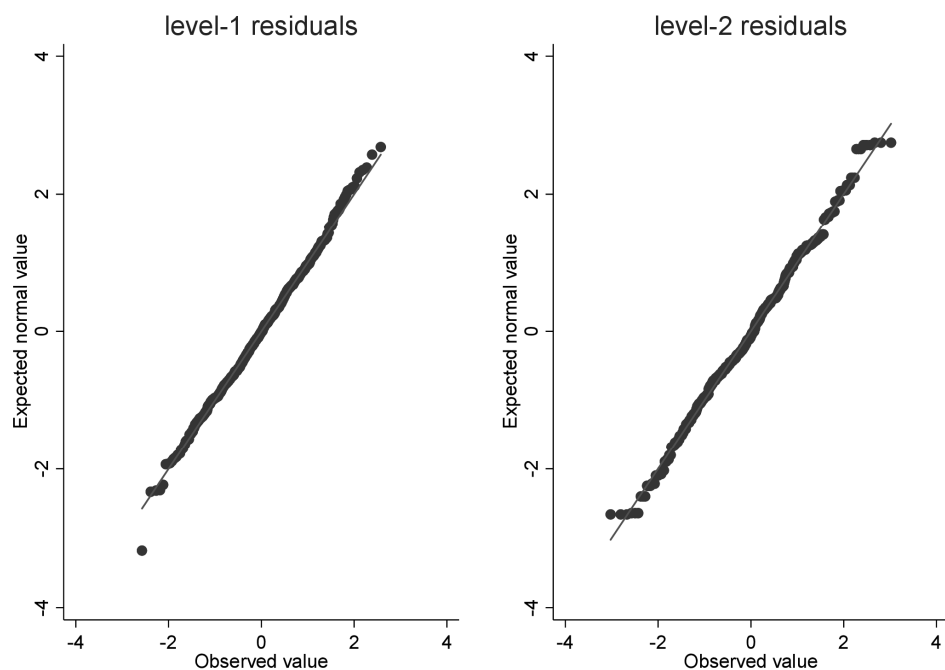
* Adjusted for height, BMI, parasitaemia on log₁₀ scale, malaria species, HIV status and gravidity combined with previous history of pregnancy loss.

Appendix 7



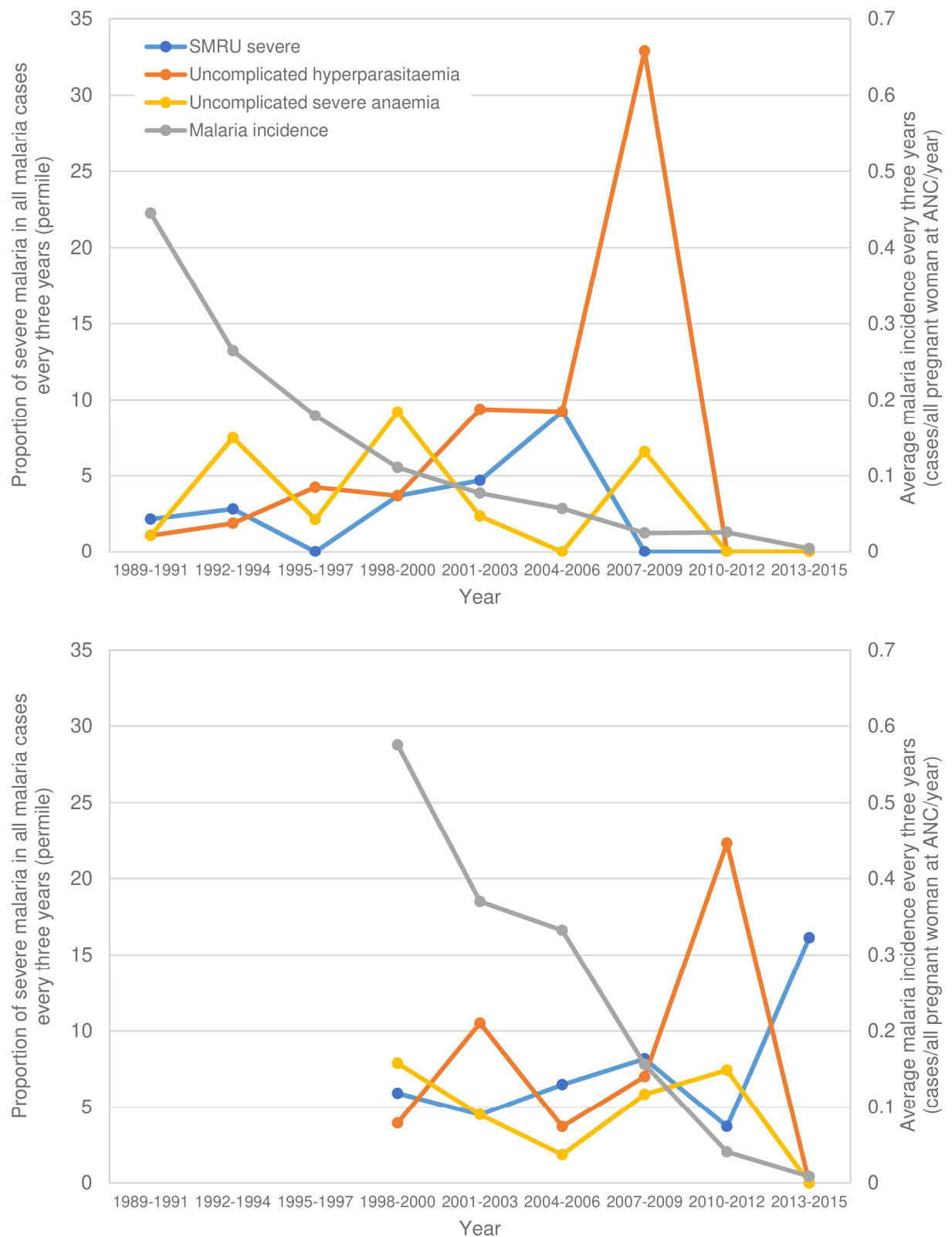
Appendix Figure 7-1. Histogram with normal distribution curve

Top: QTcP ($p=0.48$ by revised D'Agostino test). Bottom left: Level 1 residuals ($p=0.49$ by revised D'Agostino test). Bottom right: Level 2 residual ($p=0.92$ by revised D'Agostino test).



Appendix Figure 7-2. Normal quantile-quantile plot of level-1 (left) and level-2 (right) residuals

Appendix 8



Appendix Figure 8-1 Proportion of severe malaria among the malaria episodes in pregnancy (%) and the average annual malaria incidence (malaria cases/pregnant women attended ANC) over year in refugees (top) and migrants (bottom)

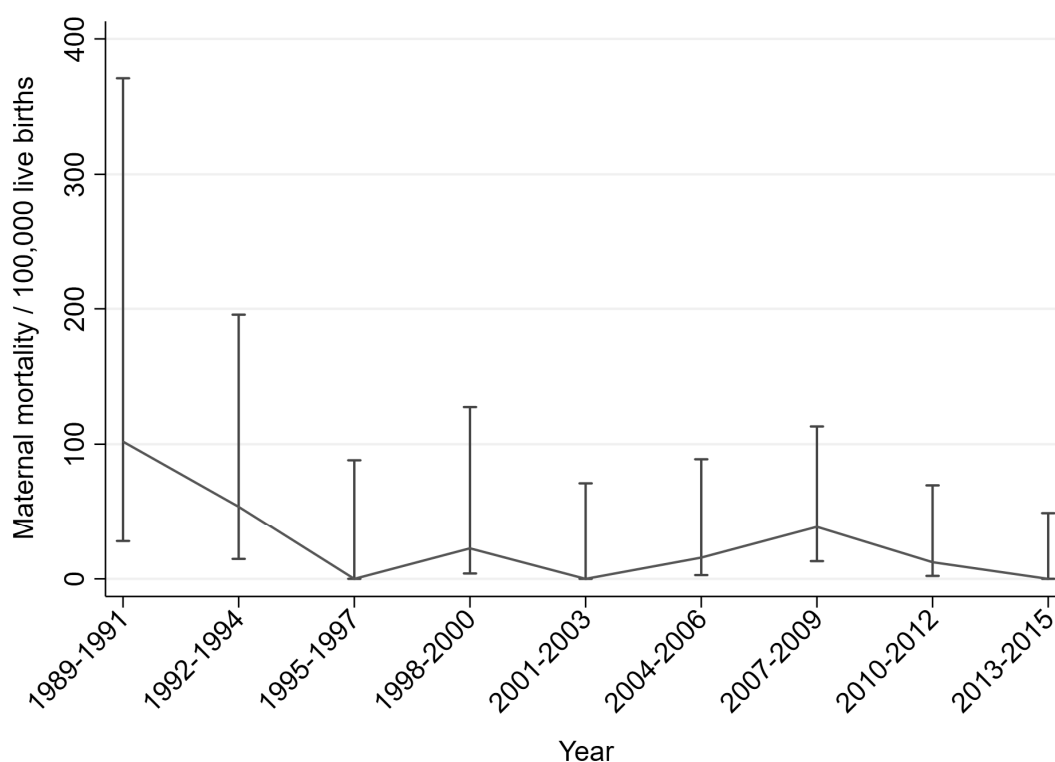
Appendix Table 8-1. Effect of continuity corrections on the risk ratio of a study with no maternal deaths changing the constraints of the total continuity correction

Characteristic	Pooled risk ratio		
	TCC=1	TCC=0.1	TCC=0.01
Mixed infection†	0.11 (0.00–58.16)	0.01 (0.00–4532647)	0.001 (0.00–1.4x10 ²⁴)
Severe anaemia only	0.15 (0.01–2.84)	0.02 (0.00–176.82)	0.002 (0.00–8.6x10 ⁹)
Renal failure	6.94 (2.15–22.42)	7.52 (2.24–25.29)	7.59 (2.25–25.62)
Convulsion	2.30 (0.38–13.96)	2.47 (0.39–15.62)	2.48 (0.39–15.81)
Hypoglycemia	7.28 (1.53–34.76)	7.77 (1.59–37.99)	7.83 (1.60–38.34)
Hypotension	3.62 (0.58–22.48)	3.99 (0.62–25.58)	4.04 (0.63–25.93)
Hyperparasitaemia only†	0.10 (0.01–2.33)	0.01 (0.00–167.34)	0.001 (0.00–1.6x10 ¹⁰)

Variables assessed only in the SMRU cohort (†) .

TCC=0.1 was used as the main results.

TCC: total continuity correction.



Appendix Figure 8-2. Maternal mortality (/ 100,000 live births) attributable to malaria by year

Appendix Table 8-2. Heterogeneity of the variables for meta-analysis

Characteristic	I ²	p-value
Fever >37.5 °C	0.0%	0.61
Presence of gametocytes†	-	-
Presence of schizonts†	-	-
Mixed infection†	-	-
Coma	0.0%	0.32
Severe anaemia	0.0%	0.33
Severe anaemia only	0.0%	1.00
Renal failure	54.1%	0.14
Respiratory insufficiency	84.3%	0.01
Convulsion	65.5%	0.09
Metabolic acidosis‡	-	-
Jaundice	14.0%	0.28
Hypoglycemia	0.0%	0.55
Hypotension	45.5%	0.18
Hyperparasitaemia	0.0%	0.33
Hyperparasitaemia only†	-	-
Prostration†	-	-
Age (year)	0.0%	0.80
Body weight (kg)	82.4%	0.02
EGA (week)	85.8%	0.01
Parity†	-	-
Day of fever (day)	0.0%	0.99
Parasitaemia (log ₁₀ (/μL))	0.0%	0.53

Variables assessed only in the SMRU cohort (†) or the SEQUAMAT cohort (§).
EGA: estimated gestational age.