Protocol

Subpatent Malaria in Pregnancy Study Group

Version 2.0

November 2019

WorldWide Antimalarial Resistance Network (WWARN)



**Suggested citation:** Protocol, Subpatent Malaria in Pregnancy Study Group: exploring the relationship between subpatent *Plasmodium falciparum* malaria infection and pregnancy outcome.

Version History

|  |  |  |
| --- | --- | --- |
| Version number  | Revision(s) & reason for amendment | Release date  |
| v1.0 |  | 16.05.2019  |
| v2.0 | Correction of title and co-authors and method one- and two-stage analyses | 11.11.2019 |
|  |  |  |

WorldWide Antimalarial Resistance Network (WWARN)

[www.wwarn.org](http://www.wwarn.org)

**Table of Contents**

[1. Protocol for a systematic review and meta-analysis of subpatent malaria and the relationship with pregnancy outcomes using aggregated and individual participant data 1](#_Toc8898336)

[2. Systematic review protocol Prospero registration number: 1](#_Toc8898337)

[3. For the Subpatent Malaria in Pregnancy Study Group: 1](#_Toc8898338)

[4. Amendments 3](#_Toc8898339)

[5. Support 3](#_Toc8898340)

[6. Introduction 3](#_Toc8898341)

[7. Objectives 4](#_Toc8898342)

[8. Inclusion criteria 5](#_Toc8898343)

[9. Information sources 6](#_Toc8898344)

[10. Search strategy 6](#_Toc8898345)

[11. Study records 7](#_Toc8898346)

[12. Data items 8](#_Toc8898347)

[13. Outcomes and prioritization 9](#_Toc8898348)

[14. Risk of bias assessment 11](#_Toc8898349)

[15. Data synthesis 11](#_Toc8898350)

[16. Meta-bias 13](#_Toc8898351)

[17. Confidence in cumulative evidence 14](#_Toc8898352)

[18. Ethics and dissemination 14](#_Toc8898353)

[19. References 14](#_Toc8898354)

## Protocol for a systematic review and meta-analysis of subpatent malaria and the relationship with pregnancy outcomes using aggregated and individual participant data

## Systematic review protocol Prospero registration number:

CRD42015027342 (URL: <http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42015027342>)

## For the Subpatent Malaria in Pregnancy Study Group:

van Eijk AM,1\* Meshnick SR,2 Hill J, 1 Stepniewska K,3 Ter Kuile FO,1 Mayor A4

Affiliations:

1 Liverpool School of Tropical Medicine, Liverpool, UK

2 Gillings School of Public Health, University of North Carolina, Chapel Hill, NC, USA

3 Worldwide Antimalarial Resistance Network (WWARN) and Centre for Tropical Medicine, Oxord, UK

4 CRESIB Barcelona Centre for International Health Research, Barcelona, Spain

\*Corresponding author:

AM van Eijk

Department of Clinical Sciences,

Liverpool School of Tropical Medicine,

Pembroke Place, Liverpool, UK

anna.vaneijk@lstmed.ac.uk or amvaneijk@gmail.com

Email: meshnick@email.unc.edu, jenny.hill@lstmed.ac.uk, kasia.stepniewska@wwarn.org, feiko.terkuile@lstmed.ac.uk, alfredo.mayor@isglobal.org

**Guarantor**: Steven R. Meshnick

**Names full study group in alphabetical order last name (in progress)**:

Manfred Accrombessi, Ayola A. Adegnika, Pedro Alonso, John Aponte, Eliana María Arango Flórez, Myriam Arévalo-Herrera, Emmanual Arinaitwe, Paulo Arnaldo, Per Ashorn, Ulla Ashorn, Azucena Bardaji, Inoni Betuela, Praveen K Bharti, Genton Blaise, Francis Bohissou, Camila Bôtto-Menezes, Vera Braun, Valerie Briand, Jessica Briggs, Matthew Cairns, Eugenia Castellanos, Daniel Chandramohan, Enesia Chaponda, Matthew Chico, Chetan Chitnis, Lauren Cohee, Michel Cot, Gilless Cottrell, Umberto D’Alessandro, Lise Denoeud-Ndam, Meghna Desai, Grant Dorsey, Maha A Elbadry, Sonia Enosse, Yuemei Fan, Nadine Fievet, Bohissou Francis, Raquel Gonzalez, Brian Greenwood, Julie Gutman, Jenny Hill, Linda Kalilani, Johanna H. Kattenberg, Kassoum Kayento, Carole Khairallah, Christopher L King, Dhanpat Kumar Kochar, Swati Kochar, Felix Koukouikila-Koussounda, Sarah H Landis, Miriam Laufer, Rose F. G. Leke, Eusebio Macete, Sonia Maculuve, Mwayi Madanitsa, Ken Maleta, Indu Malhotra, Rella Manego, Flor Ernestina Martínez-Espinosa, Achille Massougbodji, Alfredo Mayor, Michela Menegon, Clara Menendez, Petra F. Mens, Martin Meremikwu, Steven R. Meshnick, Frank Mockenhaupt, Ghyslain Mombo-Ngoma, Dominic Mosha, Ivo Müeller, Alain Nahum, Paul Natureeba, Nicaise Ndam, Francine Ntoumi, Olabisi Oduwole, Bernard A Okech, Maria Ome-Kaius, Kephas Otieno, Norma Padilla, Michael Ramharter, Stephen Rogerson, Anna Rosanes, Maria Ruperez, Sergi Sanz, Henk D. Schallig, Susana Scott, Esperanca Sevene, Carlo Severini, Kasia Stepniewska, Harry Tagbor, Diane Wallace Taylor, Steve Taylor, Feiko O ter Kuile, Halidou Tinto, Maminata Traoré, Holger Unger, Anna Maria van Eijk, Ana Maria Vasquez Cardona, Annie Walker-Abbey, Blair Wylie, Stephanie Yanow, Maria Yazdanbakhsh

**Abbreviations:**

|  |  |
| --- | --- |
| IPTp | Intermittent preventive treatment in pregnancy |
| ITN | Insecticide treated net |
| LAMP | Loop-mediated isothermal amplification |
| PCR | Polymerase chain reaction |
| RDT | Rapid diagnostic malaria test |
| SP | Sulfadoxine-pyrimethamine |

**Terms used**:

|  |  |
| --- | --- |
| Subpatent infections | Malaria infections which are positive by PCR or LAMP but not by microscopy or RDT |
| Submicroscopic infections | Malaria infections which are detected by PCR or LAMP but not by microscopy |
| Maternal anaemia | Haemoglobin <11 g/dl or < 10 g/dl |
| Maternal moderate-to-severe anaemia | Haemoglobin <8 g/dl |
| Low birth weight | <2500 gram at birth |
| Prematurity | < 37 weeks of gestation at delivery  |
| Small for gestational age | Birthweight below the 10-percentile of a reference population(INTERGROWTH-21st Project)1 |

1. Amendments

This protocol was amended in November and December 2016 to include an individual participant data component in addition to the use of aggregated data, and in January 2019 to reflect additional changes and add LAMP. The protocol was further amended in November 2019 to correct the title and add co-authors to the list, and change methods of one-stage analyses, to make clear that subpatent and patent malaria will be included as one variable with as reference value no malaria in the one-stage model.

## Support

This review was funded by grants from The Bill and Melinda Gates Foundation to the Liverpool School of Tropical Medicine for the Malaria in Pregnancy Consortium and to the WorldWide Antimalarial Resistance Network. The funder had no role in the development of the protocol and is not involved in any other aspect of the project, such as the design of the project’s protocol and analysis plan, the collection and analyses. The funder will have no input on the interpretation or publication of the study results.

## Introduction

Approximately 31 million pregnant women in malaria-endemic regions in Africa were estimated to be at risk of infection with malaria in 2015;2 no up-to-date estimates are available for regions outside of Africa, but for 2007 the number of malaria exposed pregnancies was estimated to be 94 million.3 During pregnancy, *Plasmodium falciparum* infected red blood cells expressing VAR2CSA are selected from circulation by selective cytoadherence to chondroitin sulfate proteoglycan receptors expressed in the placenta, leading to sequestration, accumulation of maternal immune cells in the placenta and increased levels of inflammatory cytokines. Early infections (in the first trimester) can affect the vasculature of the placenta.4 Adverse effects of malaria in the mother include maternal anaemia and clinical illness, and in the infant this can lead to prematurity, low birth weight due to fetal growth restriction or stillbirth. Prevention of malaria is recommended, in addition to early detection and treatment.5 Traditionally, malaria has been diagnosed using microscopy; more recent rapid diagnostic tests (RDTs) have been employed. In the context of studies, polymerase chain reaction (PCR) tests have been introduced; however, this method is generally too expensive and labour-intensive for clinical use in malaria-endemic areas. PCR can detect low-level infections which are not detectable by microscopy or RDTs and thus “subpatent”.6 PCR has enabled the examination of malaria in pregnancy in greater depth, including detection of different species, multiplicity of infection, and molecular markers for drug resistance.7 A review of submicroscopic malaria among non-pregnant persons for mono-infections with *P. falciparum* (106 studies)showed that microscopy detected, on average, 54.1% (95% confidence interval [CI], 50.3–58.2%) of all PCR-detected infections, whereby the percentage of infections detected by microscopy decreased as transmission level of malaria decreased.6 Loop-mediated isothermal amplification or LAMP is a more recent technology introduced to detect malaria; LAMP is an isothermal amplification method designed to detect a target nucleic acid without requiring sophisticated equipment, and provides high sensitivity but with rapid results: reactions can be performed in as little as 5–10 minutes and with limited resources. So far a limited number of studies on malaria in pregnancy have been using LAMP, but use is expanding.

Among pregnant women it is not clear if subpatent infections cause adverse pregnancy outcomes: some studies detected an association between subpatent infections and adverse pregnancy outcomes,8-10 but not others.11 12 In addition, it is not clear if factors which are known to affect microscopic malaria prevalence in pregnant women, such as gravidity, age, season, HIV infections, and level of malaria transmission have a similar effect on subpatent infections. A systematic review could potentially elucidate the clinical importance of subpatent infections for malaria in pregnancy and what factors may be affecting the relationship between subpatent infections and pregnancy outcome. We propose an individual participant data meta-analysis from studies with information on subpatent malaria to answer these questions.

## Objectives

The objectives of the review are as follows:

1. Evaluation of the association between subpatent malaria and adverse pregnancy outcomes
	1. Primary Objectives:
		1. To compare birth weight and low birth weight among women with subpatent and no malaria at delivery
		2. To compare haemoglobin and anaemia among women with subpatent and no malaria during pregnancy and at delivery
		3. To compare gestational age and preterm delivery among women with subpatent and no malaria
	2. Secondary objectives:
		1. To compare the outcomes above among women with subpatent malaria with women with patent malaria
		2. To compare fever among women with subpatent malaria among women with patent and no malaria
		3. To evaluate the association between subpatent malaria and birth outcome (stillbirth vs. life birth)
		4. To evaluate the association between subpatent malaria and small-for-gestational age (for datasets which provide birth weight and gestational age assessment)
	3. Tertiary objectives (if sufficient data available)
		1. To evaluate the effect of subpatent malaria over time on adverse outcomes among cohort studies with enough information
2. Evaluation of the prevalence of and risk factors for subpatent malaria
	1. Primary Objectives:
		1. To identify risk factors of subpatent malaria infections in pregnancy and compare these to risk factors of patent malaria
	2. Secondary objectives (if sufficient data available):
		1. To evaluate the association between patent and subpatent malaria of the placenta and histology findings
	3. Tertiary objective (if sufficient data available):
		1. To evaluate subpatent malaria over time among cohort studies with enough information

**PICOTS Framework for Objective A**

|  |  |
| --- | --- |
| **Components** | **Characteristics** |
| **Population** | Pregnant women in malarious areas |
| **Condition** | Positive malaria test by PCR or LAMP and negative by microscopy or RDT (subpatent malaria infection)  |
| **Control** | No malaria by PCR or LAMP |
| **Outcomes** | Pregnancy outcomes:1. Birth weight
2. Low birth weight
3. Gestational age at birth
4. Prematurity
5. Maternal Haemoglobin at birth
6. Maternal: Any Anaemia at birth
7. Maternal: Moderate-to-Severe Anaemia at birth
8. Maternal Haemoglobin during pregnancy
9. Maternal: Any Anaemia during pregnancy
10. Maternal: Moderate-to-Severe Anaemia during pregnancy
 |
| **Timing** | Published from 1997 onwards |
| **Setting** | Any survey, cohort or trial among pregnant women in a malarious area with information on subpatent malaria and pregnancy outcome. No restriction for language or continent will be made. |

**PICOTS Framework for Objective B**

|  |  |
| --- | --- |
| **Components** | **Characteristics** |
| **Population** | Pregnant women in malarious areas |
| **Condition** | Pregnant |
| **Control** | Not applicable |
| **Outcomes** | Subpatent malaria infection at any of these time points:* During pregnancy
* In the maternal blood at delivery
* In the placenta
 |
| **Timing** | Published from 1997 onwards |
| **Setting** | Any survey, cohort or trial among pregnant women in a malarious area with information on submicroscopic or subpatent malaria. No restriction for language or continent will be made. |

**Methodology:** Systematic review and meta-analysis of the relationship between submicroscopic infection and pregnancy outcome using

1. Individual patient data analysis (IPD)
2. Meta-analysis combining IPD and aggregated data from the literature

## Inclusion criteria

* Studies published from 1997 onwards (after the introduction of PCR in studies related to malaria in pregnancy)
* Related to pregnant women and malaria
* Information available about microscopy and PCR, or RDT and PCR, or microscopy and LAMP or RDT and LAMP in pregnant women or women at the time of delivery, in the same population
* English language material. For articles retrieved in other languages, an attempt may be done to verify content for inclusion if a person can be identified who masters that language.
* No restriction to location or continent

**Exclusion criteria**

* Before 1997
* Animal studies
* Case-reports or series
* No information on outcomes of interest in relationship to malaria testing (PCR and microscopy, or PCR and RDT)
* Selected groups, e.g. studies where a selection of the study population is made based on the presence of fever, or on the malaria status of the participants (e.g. studies where PCR is only conducted among women with a positive blood smear or a positive RDT result). If a subsample has been tested by PCR, the study can only be included if the subsample was randomly selected

Notes: Studies with only an abstract available will not a-priori be excluded; an attempt may be done to contact the authors to obtain more information if the abstract does not provide enough information for inclusion. Studies where the dataset is not obtained may be included as part of an aggregated analysis.

## Information sources

The Malaria in Pregnancy library will be used as the main data base for a systematic literature search.13 The Malaria in Pregnancy Library is updated every 4 months using over 20 resources, including PubMed, Google Scholar and the Global Health Database. However, to check for completeness, searches will be additionally repeated in PubMed, Google Scholar, and the Global Health database; these searches will be used to assess if there is material which was not identified in the malaria in pregnancy library. We will search 'grey literature' (e.g. reports), conference abstracts, manually review reference lists of selected publications as well as records recommended by contacting experts, to encompass a broad range of available literature. Searches will be regularly updated after the Malaria in Pregnancy Library update (three times a year).

## Search strategy

For the Malaria in Pregnancy library the search will include “(Polymerase Chain Reaction) OR PCR OR subpatent OR sub-patent OR submicroscopic OR sub-microscopic OR LAMP OR (loop-mediated isothermal amplifcation)”. The citations from the Malaria in Pregnancy and all articles retrieved will be screened, to ensure that all eligible information will be obtained. We will import citations from the malaria in pregnancy database into a spreadsheet; duplicates will be removed, and the last date of the search documented. For all other databases, an electronic literature search applying the PICOTS framework will be conducted. A multi-concept Boolean search strategy will be applied using keywords and MeSH terms for the other databases. Results will be imported in excel and compared with the results from the MiP library.

**Malaria in Pregnancy library\***

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Framework** | **Search terms** | **Number of articles** |
| **P** | **Population** |  |  |
| **I** | **Intervention or condition** | “Polymerase chain reaction” OR PCR OR subpatent OR submicroscopic OR Lamp OR loop-mediated isothermal amplification ORsub-patent OR sub-microscopic OR “loop mediated isothermal amplification” |  |
| **C** | **Control** | - |  |
| **O** | **Outcome** | All articles will be screened on useful information with regards to: -Prevalence-Birth weight, low birth weight-Gestational age, prematurity-Maternal haemoglobin, anaemia-Other (stillbirth, fever) |  |
| **T** | **Timing** | AND ("1997/01/01"[PDat] : "2019/06/31"[PDat]) |  |
| **S** | **Setting** | No restrictions on language or location |  |

\*Note that search terms related to malaria and pregnancy are not needed for the Malaria in Pregnancy library

**PubMed**:

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Framework** | **Search terms** | **Number of articles** |
| **P** | **Population** | Pregnant OR Pregnancy OR gravidity |  |
| **I** | **Intervention or condition** | (Malaria OR plasmodium) AND ((polymerase chain reaction [Mesh Term] OR PCR OR Subpatent OR submicroscopic) OR LAMP OR (loop-mediated isothermal amplification)) |  |
| **C** | **Control** | - |  |
| **O** | **Outcome** | Objective 1: AND (birth weight OR low birth weight OR haemoglobin OR hemoglobin OR anemia OR anaemia OR preterm OR prematurity)Objective 2: Prevalence |  |
| **T** | **Timing** | AND ("1997/01/01"[PDat]: "2019/06/31"[PDat]) |  |
| **S** | **Setting** | No restrictions on language or location |  |

**Google Scholar: (**PCR or polymerase chain reaction) and (Pregnant OR Pregnancy OR gravidity) AND (Malaria OR Plasmodium), (LAMP or loop-mediated isothermal amplifcation) and (Pregnant OR Pregnancy OR gravidity) AND (Malaria OR Plasmodium)

## Study records

**Screening and eligibility**

Studies will be recorded by site and time-period; multiple publications from the same study will be identified by authorship, location, time period and type of study and will be assigned to the same study. AMvE will independently screen the titles and abstracts yielded by the search against the inclusion criteria using full reports, where possible. AMvE will decide whether these meet the inclusion criteria; in collaboration with AM, they will seek additional information from study authors where necessary to resolve questions about eligibility. AMvE and AM will resolve disagreement through discussion. We will record the reasons for excluding studies. Neither of the review authors will be blind to the journal titles or to the study authors or institutions

**Individual patient data study**

For the IPD analyses, principal investigators of potential studies will be approached and provided with an excel sheet containing the variables of interest (supplement 1). They will be requested to compile a dataset (cvs, excel or stata) with variables of interest and an anonymous identifier and upload this dataset, or an unselected dataset in WWARN; alternatively, the investigators can do this for them if the WWARN agreement has been signed. Potential collaborators for the IPD analysis will be contacted by email and will be approached at least twice. If no response is obtained after at least two emails, the study will not be included in the IPD. The data sets will be converted into Stata, and variables will be recoded and transformed where necessary to ensure a uniform format across datasets. The information from the dataset will be checked against the information from available publications with regards to prevalence of malaria and outcomes where possible; if there are striking discrepancies or other issues we will contact the contributors to ask for clarifications. All datasets are merged into a final dataset for analysis.

**Aggregated study**

Studies which cannot be included in the IPD analysis because no data has been received will be included in aggregated analysis where information is available from the publication.

1. Data extraction and a quality assessment will be done by Ave and AM using a standardized pretested data extraction form. We will resolve disagreement through discussion. Where information is incomplete, the authors of the study involved will be contacted to assess if additional information can be obtained.
2. EPI-info and spreadsheets will be used for data extraction using pre-tested forms. Data will be exported into Stata (version 14.2) for analysis. Data from reports from studies with multiple publications will be combined in one entry, to avoid duplication.

## Data items

**Variables of interest**

Data to be collected as co-variates include variables with a known association with malaria from the literature or known confounders of malaria and birth outcome; an assessment of need for inclusion and likelihood of availability was made by the authors. The prevalence of malaria is known to be higher during and after the rainy season, and in rural areas.14 15 Among pregnant women, malaria is more common among primi- or secundigravidae, women infected with HIV and young women (<20 years).16 Intermittent preventive treatment, chemoprophylaxis, insecticide treated nets and indoor residual spraying can prevent malaria in pregnancy and the adverse effects.17-20 Anaemia, low birth weight, and prematurity have multicausal explanations, and malaria is one of them. Haemoglobin levels are known to change during pregnancy and are affected by maternal nutritional status, HIV status and iron and folate acid deficiency.21 22 Birthweight is affected by maternal nutritional status and haemoglobin level; overall, birthweight is higher among boys.1 Premature delivery can be related to (recent) fever from any cause and has been more common among male infants.23 24

**IPD study**

Variables requested include (see supplement 1) participant identification number, visit date, malaria test results by microscopy, PCR or RDT, including species and count (microscopy), gravidity, age, gestational age (and method of assessment), ITN use, IPTp use, recent antimalarial treatment, a history of fever or documented fever, haemoglobin and treatment arm if in a trial. At the time of delivery, these also include birth outcome, birthweight, and newborn sex. Additional variables include HIV-status, residence (urban or rural), smoking, IRS, iron and folate supplementation, and maternal nutritional status (height, weight, mid upper arm circumference), where available.

**Aggregated study**

Data extracted will include time period of the study, number of participants, location of the study, design, study population (the population that was studied), inclusion and exclusion criteria, sample size, characteristics (age, gravidity, urban/rural, HIV-status, ITN use, antimalarial use for the treatment or prevention of malaria, smoking, nutrition), prevention strategy (e.g. in trials which qualify for inclusion), details of malaria tests used (microscopy, RDT or PCR), malaria species, time of malaria detection, number of women by test result, and number of women with outcomes by test result (see supplement 2). We will extract information on prevalence of infection by RDT or microscopy and subpatent infection by age, gravidity, HIV-infection, season, urban-rural location, and other factors that may come up. We will evaluate if results from trials can be included by their separate treatment arm.

**External study-level data**

External data will be added to this file, such as GPS location from [Google Earth](https://www.google.com/earth/), [country level HIV-prevalence data](http://aidsinfo.unaids.org) among women aged 15-49 years and SP-molecular markers in the region (from the [WWARN SP Molecular Surveyor](http://www.wwarn.org/dhfr-dhps-surveyor/#0) or local sources), matched close in location and time. Using the GPS coordinates and the midpoint of the study years, the *Plasmodium falciparum* parasite rate in 2-10 year olds as an indicator of malaria transmission intensity and ITN coverage25 will be obtained from the [Malaria Atlas Project](https://map.ox.ac.uk). For studies with no information on ITN use or use of IPTp at the time of delivery, we will obtain an estimate using national surveys ([demographic and health surveys](https://dhsprogram.com), [malaria indicator surveys](http://www.malariasurveys.org) or [multiple indicator cluster surveys](http://mics.unicef.org)) closest in time for the administrative region. Economic status is an additional factor which can affect malaria risk, level of protection (better housing or means for prevention) and impact (access to treatment, nutrition). In the absence of a uniform indicator of socio-economic status across datasets, we will explore if an average level of socio-economic status can be obtained by geographic location and time-period for each study.

**Standardizing data**

Variables will be recoded where needed so the data can be combined in one data set. If season at time of study was not reported in the dataset, information on seasons in the study site will be obtained from the study authors or the internet.

**IPD integrity**

We will use standard checks to identify missing data, assess data validity and consistency. We will verify the amount of missing data, check the order of dates and assess data validity and consistency. If there are queries, we will approach the study authors for clarification.

## Outcomes and prioritization

The exposures of interest are defined in table 1. As outcomes will be assessed (see table 2): maternal haemoglobin level and maternal anaemia during pregnancy (any and moderate-to-severe anaemia) or at the time of delivery, infant birth weight and infant low birth weight, and gestational age and prematurity. In addition, other outcomes (secondary) will be examined, such as stillbirths, small for gestational age and fever if enough data is available. We will record for each outcome how and when it was measured. The prevalence of subpatent malaria is the outcome for the second objective. If there are sufficient studies with information we will evaluate the relationship between subpatent malaria and histology. If there are sufficient cohort studies with follow up information, we will evaluate the course of subpatent malaria during pregnancy and its effect on previous mentioned outcomes (haemoglobin/anaemia; birthweight/low birth weight; gestational age/preterm delivery).

**Table 1: Definitions used for exposure**

|  |  |
| --- | --- |
| **Term** | **Definition** |
| Patent malaria | Malaria detected by RDT and PCR, or microscopy and PCR. This can be in the maternal blood during pregnancy or at delivery, or in the placental blood at the time of delivery. |
| Subpatent malaria | Malaria detected by PCR or LAMP but not by RDT, or malaria detected by PCR and LAMP but not by microscopy (submicroscopic). This can be in the maternal blood during pregnancy or at delivery, or in the placental blood at the time of delivery.Initial analysis will use PCR and microscopy by compartment, and PCR and RDT by compartment (placenta or peripheral). Later analyses will include combinations of tests and compartment. |
| No Malaria | No malaria detected by RDT and PCR or LAMP, or by microscopy and PCR or LAMP. |

**Table 2: Definitions for outcomes**

|  |  |
| --- | --- |
| **Outcome** | **Definitions and notes** |
| Low birth weight (LBW) (primary outcome) | A birth weight < 2500 gramsBirth weight should preferably be measured within 24 hoursIf weight is not available at birth, weight measured within 7 days will be used and analysis will be adjusted for time of measurement if possible  |
| Preterm or premature (PT) (primary outcome) | A gestational age at the time of delivery of <37 weeksGestational age should preferably be measured by ultrasound earlier in the pregnancy. However, many methods are in use, e.g. fundal height, first day of the last menstrual period, or a scoring system at the time of delivery. All methods will be accepted and adjusted for in the analysis, where possible. |
| Maternal anaemia (HB11 or HB8) (primary outcome) | Any anaemia is defined as a haemoglobin <11 g/dl. Moderate-to- Severe anaemia is defined as haemoglobin <8 g/dl. If only a haematocrit is available, this will be divided by 3 to get an approximation of the estimate in g/dl26 |
| Fever (data permitting) (secondary outcome) | -History of fever for 1-7 days before the visit-History of fever for an undefined period during pregnancy reported at a visit-Documented fever (≥37.5 °C body temperature) at the visit-Combination of a history of fever or documented fever |
| Small for gestational age (SGA) (secondary outcome) | SGA will be defined as ≤ 10th percentile using the reference values by gestational age and gender of the Intergrowth-21st standard, as reported by Villar et al (2014).1  |
| Stillbirth (SB) (secondary outcome) | Infant born dead at any gestational age as defined by the study involved |

## Risk of bias assessment

Several tools, item-lists and scales have been developed to assess the risk of biased for studies comparing the same results across randomized controlled trials for the same intervention and for observational studies involving interventions.27 However, these tools are less suitable for IPD analyses where the exposure is not the intervention of a trial, or for IPD analyses of observational studies. In the absence of an existing tool, and after reviewing the literature,28 we decided to assess bias using an adaptation of the Newcastle Ottawa scale with criteria specific for the research questions and for each exposure and outcome examined.29 For each included study, we will evaluate the individual study publications, data available, or contact individual study collaborators to identify the following items to categorize studies as being either at lower or higher or unknown risk of bias: inclusion criteria described and adequate, exposed and non-exposed from the same population, measurement of important confounders available (maternal age, gravidity, rural versus urban residence, HIV infection, and malaria treatment or prevention), clearly described measurement of malaria microscopy, PCR, RDT, LAMP, birthweight, gestational age, and haemoglobin, availability of exposure and outcomes compared to number enrolled (if <75%, higher risk of bias), and assessment of outcome (independent and blind as lower risk). Large multi-country studies will be evaluated by country. Studies will be defined as at lower risk of bias if all or all-but-one eligible items were determined to be at a lower risk of bias. Additionally, enrolment by treatment arm over time will be checked for trials taking part in the IPD to confirm the randomization procedures. The quality score will be used as a co-variate as part of sensitivity analyses. Because participant care in trials and cohorts may differ from surveys, and enrolment in antenatal clinics may potentially include a larger segment of the target population than enrolment in maternities, the variables “study design” and “location of enrolment for the study (antenatal clinic, maternity or other)” will be evaluated for inclusion in multivariate analyses.

Risk of bias assessment will be conducted by two persons not blinded to the studies; where disagreement occurs, a joint review of the study involved will be conducted to come to an agreement. Where an agreement cannot be reached a third person will be involved.

## Data synthesis

**Study characteristics**

For studies included in IPD or aggregated analysis, study characteristics will be extracted into a spreadsheet. These include the main article, study country and location, study period, design, timepoints of data (pregnancy, delivery or both), compartment of malaria tests (maternal, placental, cord), malaria tests available and details of malaria tests used (microscopy, RDT or PCR), outcomes available, location of recruitment (ANC, maternity or community), inclusion and exclusion criteria, study arms if a trial, and methods for the assessment of fever, haemoglobin, birthweight, and gestational age if applicable, in addition to other publications from the same study. In addition, all variables available by study will be listed and a summary of these variables.

**Analyses**

The data synthesis will be specified in a data-analysis plan before the start of the analysis. Table 3 shows as an example the proposed analyses for submicroscopic malaria by timepoint, source of blood for malaria test, and reference group, for the primary objectives. We will start with a two-stage approach, to explore the initial results in a forest plot (Stata: IPDmetan), using random effects meta-analysis. We will use the “admetan” option in stata to add results from aggregated studies. We will continue with a one stage approach with a regression analysis of the IPD studies only, including a variable indicating subpatent malaria and patent malaria compared to no malaria as the main exposure of interest for the outcome, and allowing for the inclusion of co-variates. For continuous variables such as haemoglobin and gestational age, we will evaluate mean differences using the xtmixed procedure in Stata, and place a random effect on the intercept, to allow for heterogeneity in risk across studies. For binary variables, meglm will be used with log as link function to obtain risk ratios and the random effects option. Alternative models such as melogit or xtgee will be explored if convergence issues emerge. We will assume that the effects of the covariates will be the same across all sites. Subgroup analyses will be conducted by HIV status, gravidity, level of malaria transmission and level of malaria prevention.

Statistical heterogeneity will be tested using the Chi2 test (significance level: 0.1) and I2 statistic (0% to 40%: low heterogeneity; 30% to 60%: may represent moderate heterogeneity; 50% to 90%: may represent substantial heterogeneity; 75% to 100%: considerable heterogeneity),30 or the standard deviation of the random effect as the measure of heterogeneity in mixed level models. We will try to explain the source of heterogeneity by subgroup analysis, sensitivity analysis and regression analysis.

The co-variates examined in each analysis will differ by outcome examined and depend on availability (see analysis plan), but will include age, gravidity, season, setting (urban vs. rural), HIV infection, use of malaria prevention (ITNs, IPTp or IRS), use of haematinics, use of antimalarials in pregnancy, gestational age and method, or trimester of pregnancy (for haemoglobin during pregnancy). For birthweight and low birth weight, additional co-variates that will be examined include gender of the newborn, smoking, and maternal anthropometry where available.

There are different types of missing data: 1) the variable may not have been collected; 2) the variable was collected but incomplete; 3) the variable was collected but not included in the dataset. In the last case, we will contact the original authors of the study to obtain the relevant missing data. If the variable of interest was not collected, we will try to assess if we can obtain information from external sources (e.g. ITN use, HIV-prevalence). If the variable was collected but incomplete, imputation may be used as part of sensitivity analyses. We will examine if the non-availability of a co-variate is associated with characteristics of a study (e.g. malaria endemicity), to assess if this is a potential source of bias.

**Table 3: Example of number of analyses for microscopy vs. PCR for primary objectives\***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Test result** | **Time point** | **Compartment** | **Reference group** | **Outcomes** |
| BS negative, PCR positive | Pregnancy | Maternal | No malaria by BS and PCR | 1. Haemoglobin
 |
| 1. Anaemia
 |
| Malaria by BS and PCR | 1. Haemoglobin
 |
| 1. Anaemia
 |
| Delivery | Maternal | No malaria by BS and PCR | 1. Haemoglobin
 |
| 1. Anaemia
 |
| 1. Birthweight
 |
| 1. Low birthweight
 |
| 1. Gestational age
 |
| 1. Preterm delivery
 |
| Malaria by BS and PCR | 1. Haemoglobin
 |
| 1. Anaemia
 |
| 1. Birthweight
 |
| 1. Low birthweight
 |
| 1. Gestational age
 |
| 1. Preterm delivery
 |
| Placental | No malaria by BS and PCR | 1. Haemoglobin
 |
| 1. Anaemia
 |
| 1. Birthweight
 |
| 1. Low birthweight
 |
| 1. Gestational age
 |
| 1. Preterm delivery
 |
| Malaria by BS and PCR | 1. Haemoglobin
 |
| 1. Anaemia
 |
| 1. Birthweight
 |
| 1. Low birthweight
 |
| 1. Gestational age
 |
| 1. Preterm delivery
 |

Abbreviations: BS: blood smear (microscopy)

\*Same diagram applies to RDT and PCR, or LAMP and microscopy, or LAMP and RDT if there is sufficient data. In addition, combination of results of different compartments will be examined and histology results and PCR at the time of delivery (peripheral and placental) will be examined.

To be able to make full use of all available data, an additional two-stage model will be explored where for each study, dependent on the number of available co-variates, an adjusted estimate will be obtained and these adjusted estimates will be combined used meta-analyses. The outcomes for each type of analyses (one-stage or two stage) will be compared.

## Meta-bias

It is possible that some extreme outcomes may be more likely to have been published than some expected outcomes (publication bias), although it is also conceivable that this may have occurred the other way around. In addition, some outcomes may have been more likely to have been published than others because of the perceived importance by the authors. We will examine publication bias using forest plots. We will assess which outcomes have been frequently mentioned and which outcomes may have been neglected, either in the collection or the reporting of data in order to get an impression of the metabias that may exist (e.g. publication bias across studies, selective reporting within studies). In addition, we will try to reduce this bias by asking authors additional information where likely to be available in a format that can be included in this review.

## Confidence in cumulative evidence

In the absence of a grading system comparable to GRADE, the system used for meta-analyses of trials, we will describe the strength of the body of evidence across the domains of risk of bias, consistency, directness, precision and publication bias. Additional domains may be considered where appropriate.

## Ethics and dissemination

All individual studies have ethical approval from relevant local ethics committees and results are or will be available in one or more publications. Interim and final IPD results will be presented to the working group participants prior to publication and public dissemination for review. Results of the study will be published in peer-reviewed journals and presented at national and international conferences, whereby all members of the working group will be listed in slides or in the publication.

## References

1. Villar J, Cheikh Ismail L, Victora CG, 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;384:857-68. doi: 10.1016/S0140-6736(14)60932-6

2. Walker PG, Floyd J, ter KF, et al. 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 Medicine* 2017;14:e1002243. doi: 10.1371/journal.pmed.1002243 [doi];PMEDICINE-D-15-03688 [pii]

3. Dellicour S, Tatem AJ, Guerra CA, et al. Quantifying the number of pregnancies at risk of malaria in 2007: a demographic study. *PLoS Medicine* 2010;7:e1000221. doi: 10.1371/journal.pmed.1000221 [doi]

4. Moeller SL, Nyengaard JR, Larsen LG, et al. Malaria in early pregnancy impedes the development of the placental vasculature. *J Infect Dis* 2018 doi: 10.1093/infdis/jiy735

5. Desai M, Dellicour S. Effects of malaria and its treatment in early pregnancy. *Lancet Infectious Diseases* 2012;12:359-60. doi: S1473-3099(11)70345-0 [pii];10.1016/S1473-3099(11)70345-0 [doi]

6. Okell LC, Ghani AC, Lyons E, et al. Submicroscopic infection in Plasmodium falciparum-endemic populations: a systematic review and meta-analysis. *J Infect Dis* 2009;200:1509-17. doi: 10.1086/644781

7. Arango E, Maestre A, Carmona-Fonseca J. Effect of submicroscopic or polyclonal Plasmodium falciparum infection on mother and gestation product: systematic review. *Revista Brasileira de Epidemiologia* 2010;13:373-86.

8. Mockenhaupt FP, Rong B, Till H, et al. Submicroscopic Plasmodium falciparum infections in pregnancy in Ghana. *Tropical Medicine and International Health* 2000;5:167-73.

9. Adegnika AA, Verweij JJ, Agnandji ST, et al. Microscopic and sub-microscopic Plasmodium falciparum infection, but not inflammation caused by infection, is associated with low birth weight. *American Journal of Tropical Medicine and Hygiene* 2006;75:798-803.

10. Cottrell G, Moussiliou A, Luty AJ, et al. Submicroscopic Plasmodium falciparum infections are associated with maternal anemia, premature births, and low birth weight. *Clinical Infectious Diseases* 2015;60:1481-8.

11. Mockenhaupt FP, Bedu-Addo G, von Gaertner C, et al. Detection and clinical manifestation of placental malaria in southern Ghana. *Malaria Journal* 2006;5:119.

12. Walker-Abbey A, Djokam RR, Eno A, et al. Malaria in pregnant Cameroonian women: the effect of age and gravidity on submicroscopic and mixed-species infections and multiple parasite genotypes. *American Journal of Tropical Medicine and Hygiene* 2005;72:229-35.

13. van Eijk AM, Hill J, Povall S, et al. The Malaria in Pregnancy Library: a bibliometric review. *Malar J* 2012;11:362.

14. Aimone AM, Perumal N, Cole DC. A systematic review of the application and utility of geographical information systems for exploring disease-disease relationships in paediatric global health research: the case of anaemia and malaria. *Int J Health Geogr* 2013;12:1. doi: 10.1186/1476-072X-12-1

15. Reiner RC, Jr., Geary M, Atkinson PM, et al. Seasonality of Plasmodium falciparum transmission: a systematic review. *Malar J* 2015;14:343. doi: 10.1186/s12936-015-0849-2

16. van Eijk AM, Ayisi J, ter Kuile FO, et al. Risk factors for malaria in pregnancy in an urban and peri-urban population in western Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2002;96:586-92.

17. Radeva-Petrova D, Kayentao K, ter Kuile FO, et al. Drugs for preventing malaria in pregnant women in endemic areas: any drug regimen versus placebo or no treatment. *Cochrane Database for Systematic Reviews* 2014;10:CD000169. doi: 10.1002/14651858.CD000169.pub3 [doi]

18. Kayentao K, Garner P, van Eijk AM, et al. Intermittent preventive therapy for malaria during pregnancy using 2 vs 3 or more doses of sulfadoxine-pyrimethamine and risk of low birth weight in Africa: systematic review and meta-analysis. *JAMA* 2013;309:594-604. doi: 1570286 [pii];10.1001/jama.2012.216231 [doi]

19. Gamble C, Ekwaru PJ, Garner P, et al. Insecticide-treated nets for the prevention of malaria in pregnancy: A systematic review of randomised controlled trials. *PLoS Medicine* 2007;4:e107. doi: 10.1371/journal.pmed.0040107

20. Association between indoor residual spraying of insecticide and improved birth outcomes among HIV-infected pregnant women in Uganda. ASTMH 66th Annual Meeting, November 5-9, 2017, Baltimore, Maryland USA.

21. Dewey KG, Oaks BM. U-shaped curve for risk associated with maternal hemoglobin, iron status, or iron supplementation. *Am J Clin Nutr* 2017;106:1694S-702S. doi: 10.3945/ajcn.117.156075

22. van Eijk AM, Ayisi J, ter Kuile FO, et al. Human immunodeficiency virus seropositivity and malaria as risk factors for third-trimester anemia in asymptomatic pregnant women in western Kenya. *American Journal of Tropical Medicine and Hygiene* 2001;65:623-30.

23. Ingemarsson I. Gender aspects of preterm birth. *BJOG* 2003;110 Suppl 20:34-8.

24. McGregor JA, Leff M, Orleans M, et al. Fetal gender differences in preterm birth: findings in a North American cohort. *Am J Perinatol* 1992;9:43-8. doi: 10.1055/s-2007-994668

25. Bhatt S, Weiss DJ, Mappin B, et al. Coverage and system efficiencies of insecticide-treated nets in Africa from 2000 to 2017. *Elife* 2015;4 doi: 10.7554/eLife.09672

26. Carneiro IA, Drakeley CJ, Owusu-Agyei S, et al. Haemoglobin and haematocrit: is the threefold conversion valid for assessing anaemia in malaria-endemic settings? *Malar J* 2007;6:67. doi: 10.1186/1475-2875-6-67

27. Page MJ, McKenzie JE, Higgins JPT. Tools for assessing risk of reporting biases in studies and syntheses of studies: a systematic review. *BMJ Open* 2018;8:e019703. doi: 10.1136/bmjopen-2017-019703

28. Cates JE, Unger HW, Briand V, et al. Malaria, malnutrition, and birthweight: A meta-analysis using individual participant data. *PLoS Medicine* 2017;14:e1002373. doi: 10.1371/journal.pmed.1002373 [doi];PMEDICINE-D-17-00777 [pii]

29. Hartling L, Milne A, Hamm MP, et al. Testing the Newcastle Ottawa Scale showed low reliability between individual reviewers. *J Clin Epidemiol* 2013;66:982-93. doi: 10.1016/j.jclinepi.2013.03.003

30. Deeks JJ, Higgins JP, Altman DG. Analysing data and undertaking meta-analyses. In: Higgins JP, Green S, eds. Cochrane handbook for systematic reviews of interventions (version 510): The Cochrane Collaboration 2011.

Supplement 1: Variables of interest for the individual patient data analysis

|  |
| --- |
| **Name study:**  |
| ***Pregnancy or enrolment*** |
| ID |
| Study |
| Country of study |
| Date of test |
| Malaria by microscopy |
| Species by microscopy |
| Count by microscopy |
| Malaria by PCR |
| Species by PCR |
| Malaria by RDT |
| Species by RDT |
| LAMP |
| Gravidity |
| Age |
| Fever (documented fever or history of fever) |
| History of recent antimalarial treatment |
| Gestational age |
| Net use |
| ITN use |
| IPTp use (or other type of malaria prevention) |
| Treatment arm if part of a trial |
| Hemoglobin |
|  |
| ***Delivery*** |
| ID |
| Date of delivery |
| Peripheral malaria by microscopy |
| Peripheral species by microscopy |
| Peripheral count by microscopy |
| Peripheral malaria by PCR |
| Peripheral species by PCR |
| Peripheral malaria by RDT |
| Peripheral species by RDT |
| Placental malaria by microscopy |
| Placental species by microscopy |
| Placental count by microscopy |
| Placental malaria by PCR |
| Placental species by PCR |
| Placental malaria by RDT |
| Placental species by RDT |
| LAMP |
| Gravidity |
| Age |
| Fever (documented fever or history of fever) |
| History of recent antimalarial treatment |
| bednet use |
| ITN use |
| IPTp use (or other type of malaria prevention) |
| Birth outcome (stillbirth, live birth) |
| Birthweight |
| Newborn sex |
| Maternal haemoglobin |
|  |
| **Optional Additional variables (if available)** |
| *Enrolment* |
| HIV status |
| Rural residence |
| Smoker |
| Indoor residual spraying in household |
| Malaria season |
|  |
| Maternal nutritional status (weight, height, Mid upper arm circumference) |
|  |
| *Delivery* |
| Gestational age (how measured) |
| Prematurity |
| HIV status |
| Rural residence |
| Smoker |
| Placental histology (all stages) |
| Indoor residual spraying in household |
| Malaria season |
| Malaria species |
| Maternal nutritional status (weight, height, Mid upper arm circumference) |
| Iron supplementation |
| Folic acid supplementation |
|  |
| ***Additional information*** |
| Cluster adjusted study |
| Number of anc visits |
| Cord or newborn malaria tests |

Supplement 2: data extraction form for aggregated analyses















