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Evaluation of the safety and efficacy of dihydroartemisinin–piperaquine for intermittent preventive treatment of malaria in HIV-infected pregnant women: protocol of a multicentre, two-arm, randomised, placebo-controlled, superiority clinical trial (MAMAH project)

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ABSTRACT

Introduction Malaria infection during pregnancy is an important driver of maternal and neonatal health especially among HIV-infected women. Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine–pyrimethamine is recommended for malaria prevention in HIV-uninfected women, but it is contraindicated in those HIV-infected on cotrimoxazole prophylaxis (CTXp) due to potential adverse effects. Dihydroartemisinin–piperaquine (DHA–PPQ) has been shown to improve antimalarial protection, constituting a promising IPTp candidate. This trial's objective is to determine if monthly 3-day IPTp courses of DHA–PPQ added to daily CTXp are safe and superior to CTXp alone in decreasing the proportion of peripheral malaria parasitaemia at the end of pregnancy.

Methods and analysis This is a multicentre, two-arm, placebo-controlled, individually randomised trial in HIV-infected pregnant women receiving CTXp and antiretroviral treatment. A total of 664 women will be enrolled at the first antenatal care clinic visit in sites from Gabon and Mozambique. Participants will receive an insecticide-treated net, and they will be administered monthly IPTp with DHA-PPQ or placebo (1:1 ratio) as directly observed therapy from the second trimester of pregnancy. Primary study outcome is the prevalence of maternal parasitaemia at delivery. Secondary outcomes include prevalence of malaria-related maternal and infant outcomes and proportion of adverse perinatal outcomes. Participants will be followed until 6 weeks after the end of pregnancy and their infants until 1 year of age to also evaluate the impact of DHA–PPQ on mother-to-child transmission of HIV. The analysis will be done in the intention to treat and according to protocol cohorts, adjusted by gravidity, country, seasonality and other variables associated with malaria.

Ethics and dissemination The protocol was reviewed and approved by the institutional and national ethics committees of Gabon and Mozambique and the Hospital Clinic of Barcelona. Project results will be presented to all stakeholders and published in open-access journals.

Trial registration number NCT03671109.

Strengths and limitations of this study

- A major strength of this trial is its double-blind placebo-controlled design, which will allow to yield conclusive results about the efficacy of the study intervention.
- The inclusion of pregnant women from different sub-Saharan countries will provide a wide representation of different malaria endemicity areas and HIV subgroups.
- The study is also adequately powered to test the superiority hypothesis.

BACKGROUND

Malaria accounted for 229 million cases and 409 000 deaths in 2019 worldwide. The burden of disease concentrates in sub-Saharan Africa (SSA) where 94% of malaria cases and 95% of malaria deaths occurred in 2019.[1] For reasons not well understood,

pregnant women are at increased risk of the infection and of severe clinical episodes.[2–4] Especially in SSA, malaria in pregnancy constitutes an important driver of maternal and infant health and a significant cause of maternal and infant mortality and morbidity.[4–8] An estimated 20 million HIV-infected individuals in SSA live in malaria endemic areas and over 12 million are women of reproductive age.[9] In addition, approximately 1 million pregnancies each year are complicated by coinfection with malaria and HIV in this region.[10] Prevalence of malaria and HIV coinfection in pregnant women from SSA has been estimated to range between 0.94% and 37% in a recent review.[11]

Both malaria and HIV infections are among the leading causes of mortality and morbidity in African pregnant women and their children. Thus, modest effects of one infection on the other could lead to a substantial negative impact on the health of pregnant women and their infants.[12] The interaction between the two infections is particularly deleterious in pregnancy leading to increased risk and severity of both malaria infection and disease, as well as to increased HIV viral load, which may raise the frequency of mother-to-child transmission of HIV (MTCT-HIV).[13]

Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine–pyrimethamine (SP) given monthly at each antenatal care (ANC) clinic visit from the second trimester, along with long-lasting insecticide treated nets (LLITNs), is the cornerstone for malaria prevention recommended by the WHO in stable transmission areas in HIV-uninfected pregnant women.[14] In HIV-infected pregnant women living in areas with limited health resources and high HIV prevalence, universal cotrimoxazole prophylaxis (CTXp) is recommended to prevent opportunistic infections.[15] However, SP is contraindicated in women on CTXp due to potential adverse effects. Thus, even though IPTp-SP is a life-saving and highly cost-effective intervention, it cannot be administered to the most vulnerable group, HIV-infected women.[16–19] Consequently and paradoxically, the most susceptible women to malaria are currently the least protected.[20]

A placebo-controlled trial conducted in three sub-Saharan countries between 2010 and 2013 showed that an effective antimalarial added to CTXp and LLITNs in HIV-infected pregnant women has a significant impact in improving malaria prevention and maternal health through reductions in hospital admissions.[21] However, the antimalarial used (mefloquine) was not well tolerated, and most importantly, it was associated with a twofold increase in the frequency of MTCT-HIV, limiting its potential for IPTp. These findings indicate the need to find drug alternatives with better tolerability and safety profile to reduce malaria in this vulnerable group.[21]

Dihydroartemisinin–piperaquine (DHA–PPQ) is an artemisinin-based combination therapy recommended by the WHO for treatment of uncomplicated malaria in adults and children aged ≥6 months.[22] Among the available antimalarial drugs, DHA–PPQ constitutes one of the best candidates for IPTp because it is not used as first-line malaria treatment, it is well tolerated and it is recommended for treatment of clinical malaria in the second and third trimesters of pregnancy.[22]

DHA–PPQ has been safely used for case management in HIV-uninfected pregnant women in Thailand and in a multicentre trial in SSA.[23 24] Moreover, studies comparing IPTp–SP with IPTp–DHA–PPQ in HIV-uninfected pregnant women from Kenya and Uganda indicate that the drug could be an alternative to SP in terms of antimalarial efficacy and safety in pregnancy.[25 26] A recent meta-analysis of the safety and efficacy of repeated doses of DHA–PPQ for prevention and treatment of malaria including 11 studies (among adults, children and pregnant women) has concluded that monthly DHA–PPQ is well tolerated and effective for IPT and that additional data are needed in pregnancy and to further explore the cardiac safety with monthly dosing.[27] Scientific evidence shows that efficacy and safety findings from malaria control strategies evaluated in HIV-uninfected pregnant women cannot be directly extrapolated to HIV-infected women.[21] A trial comparing monthly IPTp with DHA–PPQ to CTXp among 200 HIV-infected Ugandan women did not find differences in the risk of histopathologically detected placental malarial infection and other outcomes between groups.[28] However, authors acknowledge the limitations of their results due to the low prevalence of malaria in the study area at the time of the trial.[28] Therefore, it is of highest public health priority to provide conclusive evidence as to whether the most vulnerable population (HIV-infected pregnant women) will benefit from the use of the currently most promising and available alternative drug for IPTp, DHA–PPQ.[20]

The objectives of the Improving Maternal heAlth by reducing Malaria in African HiV women (MAMAH) trial are: (1) to evaluate the safety, tolerability and efficacy of DHA-PPQ as IPTp for malaria prevention in HIV-infected pregnant women receiving daily CTXp and ARV drugs, (2) to assess the effect of DHA-PPQ as IPTp on MTCT-HIV and (3) to evaluate the effectiveness of CTXp in clearing malaria parasites in HIV-infected pregnant women.

METHODS

This is multicentre, two-arm, placebo-controlled, individually randomised superiority clinical trial with two study arms including HIV-infected pregnant women. The study will be carried out and reported according to Consolidated Standards of Reporting Trials guidelines.[29]

Study settings

The trial will be conducted in two countries from Central and South Eastern SSA (Gabon and Mozambique), where HIV prevalence among pregnant women ranges from 6% to 29%.[30 31] Malaria epidemiological indicators and HIV prevalence in pregnancy in study sites are shown in table 1. The trial sites have been selected to provide representation of different malaria endemicity and HIV

Table 1 Malaria and HIV epidemiology in the study sites

Site/country	Malaria transmission	High season	EIR	*Plasmodium falciparum* infection prevalence in women at delivery*	HIV prevalence in pregnant women	Frequency of MTCT of HIV
Manhiça† /Mozambique	Hypoendemic	September–March	21–50[47]	6%	29%	6%[48]
Lambaréné‡/ Gabon	Mesoendemic	October–May	21–50[49]	11%	6%	12%[50]
Libreville§/ Gabon	Mesoendemic	October–May	21–50[51]	NI	6%	12%[50]

*Data from 2010 to 2012 in women receiving either two IPTp doses of mefloquine or SP (Tuikue-Ndam *et al*, unpublished).
†The trial will be conducted at the ANC services of the Manhiça District Hospital, where the *Centro de Investigação em Saúde de Manhiça* is situated; the average monthly number of pregnant women attending first ANC clinic visit is 110.
‡In Lambaréné, the trial will be conducted at the ANC services and maternity of the Albert Schweitzer Hospital by the Centre de Recherches Médicales de Lambaréné; the average monthly number of pregnant women attending first ANC clinic visit is 115.
§In Libreville, the trial will be conducted at the ANC services of *the Centre hospitalier Régional Estuaire de Melen- Unité de Recherche Clinique sur le Paludisme* and the Jeanne Ebori Hospital; the average monthly number of pregnant women attending first ANC clinic visit is 150.
ANC, antenatal care; EIR, entomological inoculation rate; IPTp, intermittent preventive treatment in pregnancy; MTCT, mother-to-child transmission; NI, no information; SP, sulphadoxine–pyrimethamine.

subgroups (HIV-1 subtype C in Mozambique and HIV-1 CRF02_AG in Gabon, where HIV-2 also circulates[32 33]).

Study population

All pregnant women attending the study ANC services for the first time and/or who have not received IPTp during their current pregnancy will be screened for participation in the trial. Inclusion criteria are: (1) permanent resident in the study area, (2) gestational age at the first antenatal visit ≤28 weeks, (3) HIV seropositive status and (4) agreement to deliver in the study site's maternity(ies) wards. Exclusion criteria are: (1) planning to move out in the following 10 months from enrolment, (2) gestational age at the first antenatal visit >28 weeks of pregnancy, (3) known history of allergy to CTX, (4) known history of allergy or contraindications to DHA-PPQ and (5) participating in other intervention studies.

Informed consent

All participants will receive information about study procedures, including knowledge about malaria and HIV infection in pregnancy. A signed informed consent form (or thumb-printed with a witness whenever the woman is illiterate) will be obtained before any study tests or evaluations are carried out by study nurses in each site. The trial's informed consent is available as online supplemental material 1. If the participant is under the legal age of maturity, she will sign the assent form and her legal guardian will sign the informed consent according to national ethics local policies. The informed consent will cover the woman and the new born infant.

Recruitment and randomisation

After the study details are explained and informed consent is signed, pregnant women will be given a study number and automatically randomised to one of the study arms: (1) Daily CTX+monthly IPTp–DHA–PPQ or (2) daily CTX+monthly IPTp-placebo. Each participant will be uniquely identified in the study by a combination of her site code and participant number. Allocation of participants to study arms will be done centrally by the trial's sponsor (the Barcelona Institute for Global Health, ISGlobal) by block randomisation and stratified by country. This method will ensure balanced allocation to both arms during different malaria seasons in the two study countries. Each subject number will be related to a treatment number, which assigns them to one of the IPT arms. Study number allocation for each study participant will be concealed in opaque sealed envelopes that will be opened only after recruitment. At each site, the first participant will be assigned a patient number, and consecutive numbers will be assigned to subsequent women. A study identification card containing the individual study number and basic demographic information will be given to the participant in order to facilitate identification at all study contacts.

Figure 1 displays the study design.

Blinding

Study tablets (IPTp–DHA–PPQ and IPTp–placebo) will be identically packaged. Study personnel will prepare and deliver the medication to the participant. All study personnel, investigators, outcome assessors, data analysts and the participants will remain blinded throughout the trial. Unblinding is only envisaged in case of a medical emergency (in such case, the investigator on site will have to justify to the sponsor and the Data Safety Monitoring Board (DSMB) the need for unblinding).

Interventions

IPTp administration

Administration of the 3-day IPTp course will always be done under fasting conditions (following DHA–PPQ

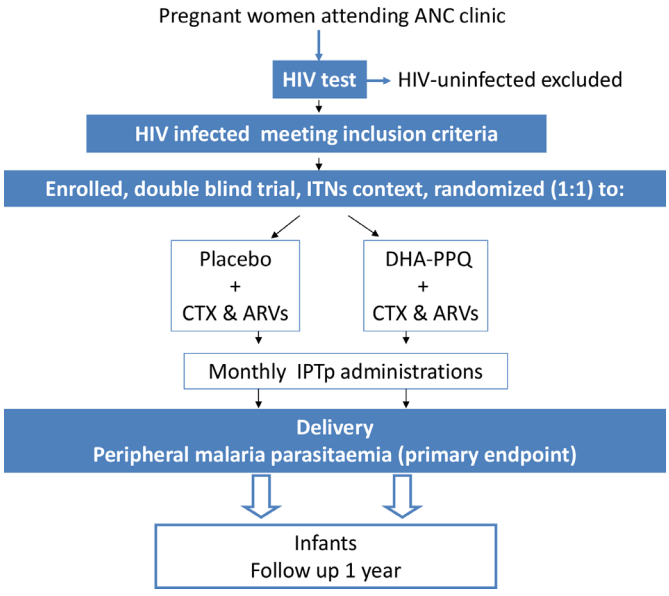

Figure 1 MAMAH trial design. ANC, antenatal care; ARVs, antiretroviral drugs; CTX, cotrimoxazole; DHA-PPQ, dihydroartemisinin–piperaquine; ITNs, insecticide- treated nets; MAMAH, Improving maternal Health by Reducing Malaria in African HIV Women.

administration recommendations) and direct observation by study personnel. The number of daily IPTp tablets to be administered will be based on bodyweight and according to the treatment guidelines set by WHO [target dose (range) of 4 (2–10) mg/kg/ day of DHA and 18 (16–27) mg/kg/day of PPQ given once a day for 3 days for adults]. Following physical examination, recruited women of gestational age ≥13 weeks will receive the assigned IPTp drug. In case of gestational age <13 weeks, first IPTp administration will be scheduled 1 month later. If participants report malaria treatment in the preceding 4 weeks, first IPTp administration will also be delayed 1 month. Administration of the second and third day treatment course will be done by study personnel either at the study health facility or household level. Women will be observed for 60 min after administration of the IPTp dose. Those women vomiting within the first 30 min of IPTp administration will be given a second full IPTp dose; women vomiting after 30–60 min of IPTp administration will be given an additional half dose of the drug. Subsequent doses of IPTp will be given coinciding with the next scheduled monthly ANC clinic visit, at least 1 month apart from the previous dose.

CTX administration

CTX (fixed combination drug containing 800 mg of trimethoprim and 160 mg of sulfamethoxazole) will be taken daily from enrolment until delivery as per national guidelines for prevention of opportunistic infections. Women will be asked to visit the ANC clinic monthly to receive the amount of CTX tablets for the whole month. At each ANC clinic visit, adherence to CTX prophylaxis will be assessed.

ARV therapy and concomitant medications

Treatment for prevention of HIV MTCT (option B+) will be given by the study health personnel, according to national HIV/AIDS control guidelines.[34] Any other concomitant treatment received by the study participants will be recorded in the study questionnaires.

Long-lasting insecticide treated nets

Regardless of gestational age at the time of recruitment, all women will receive a LLITN, and details about its use will be explained.

Study outcomes

The primary outcome of the trial will be the prevalence of maternal parasitaemia at delivery defined by the presence of *Plasmodium falciparum* asexual parasites of any density in peripheral blood (determined by microscopy). The secondary maternal and infant endpoints can be found in box 1.

Sample size

Based on previous estimations at the study sites and assuming a prevalence of peripheral parasitaemia at delivery of 7.5% with CTXp, it is estimated that 298 women per arm will be required to detect with 80% power a significant ($p<0.05$) decrease of 5% or more in the prevalence of peripheral parasitaemia in the CTXp +IPTp–DHA–PPQ group.[21] In order to allow for 10% losses to follow-up, it is calculated that 332 women/ study arm will need to be recruited (total n=664). Considering the prevalence of HIV infection among pregnant women in both sites (please refer to table 1), a total of 444 women are planned to be recruited in Mozambique and 220 women in Gabon to allow for a recruitment rate of 33 women/month on average. These estimations are based on recruitment rates of previous IPTp clinical trials conducted among pregnant women in the two study sites.[21 35 36]

Follow-up and measurements of outcomes

At baseline, the woman's demographic and obstetric information will be recorded in study specific case report forms (CRFs).

Physical and clinical examination at enrolment

The physical examination of the woman will include the following assessments: weight, height, gestational age by bimanual palpation and measurement of middle-upper arm circumference (MUAC). Ultrasound will be performed to determine gestational age at enrolment and confirm pregnancy viability.

Baseline biological samples

At enrolment, a venous blood sample (5 mL) will be collected for analysis of haemoglobin level, CD4 cell counts, HIV viral load and malaria PCR.

Follow-up and household visits

Women will be given an appointment to attend the subsequent ANC clinic visit 1 month after the first one.

Box 1 Study outcomes

Primary endpoint

Prevalence of maternal parasitaemia at delivery (defined by the presence of *Plasmodium falciparum* asexual parasites of any density in peripheral blood determined by microscopy).

Secondary endpoints

Maternal

Incidence of clinical malaria during pregnancy.
Incidence of all-cause admissions.
Incidence of all-cause outpatient attendances.
Frequency and severity of adverse events (including cardiotoxic signals).
Mean haemoglobin concentration at delivery.
Prevalence of submicroscopic *P. falciparum* peripheral parasitaemia at delivery.
Prevalence of anaemia at delivery (Hemoglobine(Hb) <11 g/dL).
Prevalence of severe anaemia at delivery (Hb <7 g/dL).
Mean CD4+ T cell counts levels at delivery.
Proportion of women with detectable HIV viral load at delivery.
Prevalence of placental *P. falciparum* infection (defined by the presence of parasites and/or pigment in the histological examination, or microscopic or submicroscopic in the impression smear from placental blood).
Prevalence of *P. falciparum* peripheral parasitaemia at the postpartum visit.
Maternal mortality rate.

Infant

Prevalence of *P. falciparum* parasitaemia in cord blood (microscopic and submicroscopic).
Prevalence of neonatal anaemia (Hb <12.5 g/dL in cord blood, and Hb <13 g/dL in neonatal blood).
Mean birth weight.
Prevalence of low birth weight (<2500 g).
Mean gestational age at birth.
Prevalence of prematurity.
Prevalence of embryo and fetal losses (miscarriages and stillbirths).
Prevalence of small for gestational age.
Frequency of congenital malformations.
Incidence of clinical malaria.
Neonatal mortality rate.
Frequency of mother-to-child transmission of HIV at one and at 12 months of age.
Infant mortality rate.

Participants will receive the standard ANC package of interventions, which includes iron and folate supplementation, following national guidelines. The subsequent IPTp doses will be given at least 4 weeks apart from the previous one. MAMAH participants will be asked to visit the study facilities in case of any illness. A malaria blood smear will be collected in those participants passively reporting sick and presenting with malaria related signs/symptoms (fever (≥37.5°C) or having history of fever in the past 24 hours, arthromyalgias or headache), as per national management guidelines. Women will be visited at home the day after recruitment to confirm residence status, assess drug tolerability and the correct use of the net. Adherence to CTX prophylaxis, ARV therapy and compliance with the LLITNs use will be assessed monthly at the ANC attendance.

Adverse events (AEs) monitoring and reporting

Active safety monitoring will consist in household visits to study participants 2 days after each IPTp administration to assess drug tolerability and record all AEs. In addition, a health facility-based passive surveillance system will be established to capture unscheduled visits of participants during follow-up. Information on unsolicited AEs will be collected at each scheduled and unscheduled visit. Any participant passively reporting being sick during the study visits will be referred to the clinical services as per routine system in place. A blood smear will be collected in those presenting with malaria-related signs/symptoms (fever (≥37.5°C) or having history of fever in the past 24 hours, arthromyalgias or headache). In case of malaria parasitaemia or anaemia, they will be treated following national guidelines. Serious adverse events will be reported by the site investigator within 24 hours of being made aware to the trial's safety monitor and the DSMB.

End of pregnancy and infant assessments

At the end of pregnancy, maternal blood (5 mL), placental and cord blood samples (5 mL) will be collected for haematological and parasitological examination. The newborn will be examined, weighed and measured, and his or her gestational age will be assessed by the modified Ballard method.[37]

Postpartum visit

Participants will be visited approximately after 6 weeks of end of pregnancy at the study health facility where a blood smear for malaria screening will be collected. A summary of study procedures is displayed in table 2.

Infants follow-up

Infants born to study participants will be followed up until 1 year of age. Mothers will be asked to bring their child to the study facilities at weeks 4–6 of age and at 6, 9 and 12 months after birth. At each scheduled visit, study infants will be physically examined, and their psychomotor and neurological development will be assessed following a standard protocol for African settings.[38–40] Weight, height and axillary temperature will be measured and recorded. A capillary blood sample will be taken from infants with fever (axillary temperature ≥37.5°C) or history of fever in the last 24 hours, or appearing pale, for malaria parasitaemia examination and haematological determination. In case of malaria parasitaemia or anaemia, they will be treated following national guidelines. At each scheduled visit, HIV DNA will be evaluated in the infant from dried blood spot on filter paper, polymerase chain reaction (for PCR). As per routine healthcare national programmes HIV-exposed infants will receive CTX prophylaxis starting 4–6 weeks after birth. Infants will also receive ARV, according to national guidelines. Infant's follow-up visits and study procedures are described in table 3.

Table 2 Schedule of enrolment, interventions and maternal assessments

Study period								
	Pre-enrolment	Allocation	First ANC clinic visit	Household visits	Monthly ANC visits	End of pregnancy	One month after end of pregnancy	Unscheduled visits
Timepoint	**0**	**0**	**1**	**+2days**	**+1 month and then monthly**			
Screening and enrolment:								
Eligibility screen	×							
Informed consent	×							
Randomisation		×						
Interventions:								
IPTp administration			×	×	×			
CTX administration			×	*	*	*	*	*
ARV administration			×	*	*	*	*	*
LLITN distribution			×					
Maternal assessments:								
Demographics, medical history			×					×
Socioeconomic characteristics				†		×		
Record of concomitant medication			×	×	×	×	×	×
Record of adverse events			×	×	×	×	×	×
Physical/clinical examination			×			×		×
Gestational age by ultrasound			×		×	×		×
Temperature							×	×
Blood pressure			×			×	×	×
Weight			×		×		×	×
Height			×					
MUAC			×				×	
RPR test			×					
CD4 count and HIV viral load			×			×		
Blood smear (malaria)			‡		‡	×	×	‡
Haemoglobin test			×			×	×	
Intrapartum samples (cord blood and placenta)						×		
Drug tolerability assessment			×	×	×			
Compliance with LLITNs check				×	×	×	×	×

*CTX and ARV adherence should be assessed at each scheduled visit.
†Only in the first household visit after the ANC visit of first IPTp administration.
‡Only in women passively reporting sick and presenting with malaria related signs/symptoms (fever (≥37.5°C) or having history of fever in the past 24 hours, arthromyalgia or headache), as per national management guidelines.
ANC, antenatal care; CTX, cotrimoxazole; IPTp, intermittent preventive treatment in pregnancy; LLITNs, long-lasting insecticide treated nets; MUAC, middle-upper arm circumference; RPR, Rapid Plasma Reagin test for syphilis.

Table 3 Schedule of infant visits and procedures

	Timepoints					
	Birth	1 month*	6 months	9 months	12 months	Unscheduled visits
Procedures:						
Medical history	×	×	×	×	×	×
Physical examination	×	×	×	×	×	×
Psychomotor development assessment	×	×	×	×	×	
Weight	×	×	×	×	×	×
Height	×	×	×	×	×	×
Temperature	×	×	×	×	×	×
Blood smear	×	†	†	†	†	†
Haemoglobin test	×	†	†	†	†	†
HIV PCR ‡		×	×	×	×	
Malaria PCR (filter paper)	×					
HIV prophylaxis adherence	§	§	§	§	§	§
HIV treatment adherence	§	§	§	§	§	§

*First visit will be scheduled 1 month after birth or coinciding with first EPI visit.
†Only if fever (≥37.5°C) or history of fever in the past 24 hours or signs suggestive of malaria.
‡HIV PCR test should also be repeated at month 18 after birth.
§Adherence should be assessed at each visit.
EPI, Expanded Program on Immunization; PCR, Polymerase chain reaction.

Laboratory tests

Parasitological and haematological determinations

Thick and thin blood smears will be stained with Giemsa's stain and examined for *Plasmodium* spp, following standard procedures. Also, blood haemoglobin will be determined following local Standard Operating Procedures (SOPs).

Detection of HIV and quantitative determination of viral load

Quantitative PCR HIV viral load will be determined from the venous blood samples drawn at enrolment and delivery. Additionally, vertical transmission of HIV will be determined by qualitative DNA PCR performed on samples drawn from infants at 1 month and 12 months of age.

Immunological determinations related to HIV status

CD4 +T cell count will be determined by flow cytometry after staining of whole blood with CD3, CD8 and CD4 fluorochrometo-labelled antibodies and acquisition using FACSCalibur (BD Biosciences) and TruCOUNT tubes (Becton Dickinson, San Jose, California, USA) *or MiniVIdas device*. HIV viral load will be determined from plasma cryopreserved at −80°C using the devices in place *in the study sites (such as* COBAS AMPLICOR, AmpliPrep (Roche Diagnostics) *or GeneXpert)*.

Placental samples analysis

A tissue sample (approximately 2 cm^3) will be collected from the maternal surface of the placenta and will be immediately placed in 25 mL of 10% neutral buffered formalin and kept at 4°C until processed and embedded in paraffin wax by standard techniques. Paraffin sections 4 µm thick from the placental tissue, will be stained with H&E, Giemsa's stain and the periodic acid-Schiff technique. Placentas will be classified histologically as: (1) not infected, (2) active infection and (3) past infection, depending on the presence or absence of parasites, pigment or both.[41] Impression smears will be prepared from the placental blood for parasitological examination. Blood from the placenta will also be collected onto filter paper for PCR determination of malaria parasites.

Data management

Data will be collected using paper CRFs developed for the trial by study personnel at each scheduled and unscheduled visit. The quality of the data recorded in the study source documents and CRFs will be monitored regularly following the principles of Good Clinical Practices by the trials' clinical monitor.[42] Data will be double entered into the study database using the OpenClinica open source software (V.3.14) for clinical data management (www.OpenClinica.com) at each study site. Automatic quality checks will be performed to ensure CRF completeness. The database system will be designed to protect the confidentiality (sensitive data will be automatically encrypted) and integrity of the data and will include authorisation, authentication, auditing and availability features to safeguard the access and usage of the data. Concomitant medications registered into the database will be coded using the WHO Drug Reference List, which employs the Anatomical Therapeutic Chemical classification system. Medical history/current medical conditions and AEs

will be coded using the Medical dictionary for regulatory activities (MedDRA) terminology.

Analysis plan

The following populations of analysis have been defined: (A) intention to treat (ITT): it includes all randomised pregnant women who have data on outcome (target population for the efficacy analysis); (B) according to protocol (ATP): it includes participants who fulfil all the inclusion–exclusion criteria, took monthly IPTp–DHA–PPQ/placebo study doses, received a LLITN, received CTXp and ARV drugs and from whom data is available for the analysis; (C) safety: it includes participants who received at least one dose of IPTp–DHA–PPQ/placebo and had at least one postbaseline safety assessment. No interim analyses of data are envisaged. The primary analysis of the trial will be the comparison of the proportion of pregnant women with peripheral parasitaemia at delivery in the ITT and ATP cohorts, adjusted by gravidity, country, seasonality and other variables associated with the prevalence of malaria. Proportions will be compared between groups using the Fisher's exact test. Crude and adjusted analysis will be done using Poisson regression with a log link and robust estimate of the covariance (Huber method), using the method proposed by Zou.[43 44] Relative risk ratio (RR) or reduction of the RR (1−RR*100%) if RR lower than 1 will be presented. Continuous variables will be compared between groups using t-test and the Wilcoxon rank values according to variables' characteristics. The differences in rank values will be assessed by looking at the expected rank sums in each group under the null hypothesis. Adjustment for covariates and possible cofounders (such as country, gestational age, gravidity, anaemia, literacy, MUAC, viral load and CD4 +T cell count) will be done using ordinary least square regression. Subgroup analysis is not envisaged. Incidence of clinical malaria, overall admissions and outpatient attendances will be estimated as the number of episodes over the time at risk. Time at risk will be estimated as the time from the start of follow-up until the end of follow-up (visit 1 month after end of pregnancy for mothers) or withdrawal due to censoring or death, whatever occurs first. The total number of events will be compared between groups using negative binomial regression models that take into account a possible extra Poisson variation due to different frailty of the subjects. The comparison will be expressed as relative RR (RRate). Data analysis will be performed using Stata (Stata Corp).

Patient and public involvement

Patients will not be directly involved in the design, conduct, reporting or dissemination plans of the study. However, a Community Advisory Board (CAB) has been set at each study site to strengthen communication and interaction between the local study community and research teams. The CAB also oversees and guides the study team on key issues such as potential risks and burdens for participants or host communities that may be hidden from researchers and how to minimise them.[45]

Ethics and dissemination

The study is conducted in accordance with the European Medicines Agency (EMA)/International Council for Harmonisation (ICH) Guideline on Good Clinical Practice and in total agreement with the applicable international, European Union (EU) and national law of all the participating countries.[46] The study protocol (V.1.0, 2 May 2018) and the informed consent forms have been reviewed and approved by the institutional and national ethics committees of Gabon (042/2018/PR/SG/CNER) and Mozambique (504/CBNS/18) and the Hospital Clinic of Barcelona (HCB/2018/0650, Spain). An independent DSMB monitors regularly the safety of study participants. The trial is registered on clinicaltrials.gov (https://clinicaltrials.gov/ct2/show/NCT03671109).

The findings of the clinical trial will be submitted for publication in a peer-reviewed journal within 12 months of study completion through an open access mechanism, or otherwise made available publicly in compliance with H2020 open access requirements. Primary project raw data will be published in the project website. At no stage will data containing personal information of research participants be released.

A project communication has been developed in order to ensure timely, accurate and effective dissemination of project results. After concluding the trial's data analysis, findings will be made available to all partners, key stakeholders and Ministries of Health. The project members will actively disseminate information to the scientific community through reports, presentations at scientific forums and publications in international open-access journals. Trial results will also be shared with the WHO Global Malaria Program and Malaria Policy Advisory Groups.

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