Assessing the association between receipt of antimalarial drugs and adverse pregnancy outcomes using pooled data

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Abstract

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Malaria is a major cause of maternal morbidity and mortality and neonatal death in areas of malaria transmission. Worldwide, it is responsible for up to 100,000 neonatal deaths and 10,000 maternal deaths annually. There is a need for better information on the safety of antimalarial drugs for women and their fetuses. Artemisinin-based combination therapies (ACT) are the most effective antimalarials, but are associated with teratogenic and embryotoxic effects in animal models when administered in early pregnancy. The limited observational studies in humans reported to-date have been reassuring. We conducted a systematic review and meta-analysis of the occurrence of adverse pregnancy outcomes among women treated with artemisinin monotherapy or artemisinin-based combination therapy during the 2nd or 3rd trimester relative to pregnant women who received non-artemisinin antimalarials or no antimalarial treatments in pregnancy. Pooled odds ratios (POR) were calculated using Mantel-Haenszel fixed effects models with a 0.5 continuity correction for zero events. To assess the risk of congenital malformations after receipt of an ACT during pregnancy, we pooled data from five recently completed randomized-controlled trials (RCT) and one multi-site, observational cohort study from

the Malaria in Pregnancy Consortium (MIPc). We calculated prevalence risk ratios (PRR) comparing ACT exposed infants to ACT unexposed infants. From the meta-analysis, the pooled odds ratios (POR) (95% confidence interval (CI)) for stillbirth, fetal loss, and congenital anomalies when comparing artemisinin versus quinine were 0.49 (95% CI 0.24-0.97, I^2=0%, 3 studies); 0.58 (95% CI 0.31-1.16, I^2=0%, 6 studies); and 1.00 (95% CI 0.27-3.75, I^2=0%, 3 studies), respectively. From the pooled analysis, there was no increased risk of congenital anomalies associated with receipt of an ACT during pregnancy compared to no such use (PRR 0.91, 95%CI 0.52-1.58). The PRR for congenital anomalies among the infants of women who received an ACT in the 1st, 2nd, and 3rd trimester compared to infants with no exposure to an ACT were 1.54 (95% CI 0.55-4.34), 0.79 (95%CI 0.47-1.34) and 1.08 (95%CI 0.57-2.07), respectively. Evidence from these studies supports the current WHO guidelines that call for the use of ACTs in 2nd and 3rd trimester to treat malaria. The findings from the analysis of the first trimester exposures and the risk of congenital anomalies are inconclusive due to the small number of malformations observed. Additional pharmacovigilance is recommended to obtain more reassurance of safety.

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If you want to go quickly, go alone. If you want to go far, go together. ~African Proverb

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Introduction

I. Defining the burden of malaria in pregnancy

Each year, approximately 50 million women will become pregnant in areas of stable *P. falciparum* malaria transmission and an additional 70.5 million will become pregnant in areas of low transmission or areas with only *P. vivax* malaria (1,2). Due to changes in the immune system during pregnancy, pregnant women are at increased risk for malaria (3). In areas of stable transmission, primagravid women and younger women are at highest risk (3). In areas of low transmission or epidemic transmission only, increasing age and parity do not confer protection against malaria infections. The clinical presentation of malaria in pregnancy depends on the acquired immunity of the woman, and therefore differs by epidemiologic setting. In areas of stable transmission, pregnant women who are semi-immune may be symptomless, although they are more likely to present with fever than non-pregnant women (3). In areas of stable transmission, 26% of maternal anemia can be attributed to malaria infection. Anemia can lead to maternal death if severe, and for the fetus it can lead to low birth weight and prematurity (4). In areas of low transmission, pregnant women are more likely to present with fever or severe malaria than their counterparts in areas of high transmission. In 2007, it was estimated that malaria was responsible for up to 100,000 neonatal deaths due to being born premature, small for gestational age (SGA) and 10,000 maternal deaths (3,4). Furthermore, malaria infection in pregnancy is associated with an increased risk of miscarriage and stillbirth (3).

II. WHO recommendations for the treatment of malaria in pregnancy

For the treatment of uncomplicated malaria in the 2nd or 3rd trimester of pregnancy the WHO recommends the use of artemisinin-based combination therapies (ACT) (5). These drugs combine one of the fast acting artemisinin-based compounds - including artesunate, artemether, and dihydroartemisinin - with a second antimalarial from a different drug class in an effort to prevent drug resistance. The combination drugs can be one of the following mefloquine, lumefantrine, amodiaquine, sulfadoxine/pyrimethamine, piperaquine, and chlorproquanil/dapsone. Currently, the WHO recommends treating uncomplicated malaria in the 1st trimester with 7 days of quinine+clindamycin, or quinine monotherapy if clindamycin is unavailable (5). The WHO only recommends the use of ACTs in the first trimester if quinine is unavailable or if the life of the woman is in danger. The WHO recommends use of intravenous or intramuscular artesunate for at least 24 hours for the treatment of severe malaria in pregnancy regardless of trimester. After the patient can tolerate oral medication, they should receive a three-day treatment with an ACT.

III. Animal studies suggest that artemisinins are embyotoxic and teratogenic

Studies of rats, rabbits and primates have found artemisinins to be associated with embryotoxicity and teratogenicity (6). A review of all antimalarial drugs used in pregnancy noted artesunate is very toxic to rat and rabbit embryos, with fetal reabsorption in rats reported at doses between 28-223 mg/kg/day given orally on days 9-14 of gestation (7). A high prevalence of congenital anomalies, including bent and/or shortened long bones and treatment related heart defects, has been observed in rat litters (7). Primate studies demonstrated similar embryo toxicity, with 55% and 100% embryo

lethality for 12 and 30 mg/kg/d oral doses respectively (8). In addition, observations of three live embryos from the 30mg group noted reduction in blood cells in the vasculature and the presence of cardiac chambers that were distended with thin walls (8). The primary embryonic targets for artemisinins are the primitive erythroblasts proliferating during gestational periods of 10-14 days in the rat and 18-40 days in monkeys. In humans this corresponds to a embryo sensitive period of 4-10 weeks post conception (9). The animal studies led researchers to hypothesize that exposure to ACTs in the first trimester could lead to and increased risk of cardiovascular and skeletal anomalies as well as an increased risk of miscarriage. In addition, several organs are still under development in the 2nd trimester and therefore sensitive to teratogens, including the uterus (18 weeks), brain (until birth), eyes (24-36 weeks), and ears (18 weeks) (10), although animal data did not observe these types of anomalies after artemisinin exposure (11).

IV. Evidence from human studies is limited

Given the high morbidity and mortality associated with malaria in pregnancy, safe and efficacious drugs are needed for treatment and prevention. However, because of ethical concerns, except in certain circumstances, randomized clinical trials for new drugs do not include pregnant women as study subjects (7,12). Five cohort studies and three randomized controlled trials have studied the safety of artemisinins in pregnancy (13–19), but all had small sample sizes and thus limited power to examine safety outcomes. Four of the five cohort studies reported exposures to artemisinins during the first trimester from the same cohort of women attending health centers in the refugee camps along the Thailand-Burma border. No study found a statistically significant increased rate of spontaneous abortions, stillbirths, neonatal deaths, prematurity, or congenital anomalies.

In one study, the highest prevalence of congenital anomalies was observed in women exposed to artesunate during the first trimester (4.5%) but it was not statistically significantly higher than the prevalence of 1% observed in women not exposed to artemisinins during the first trimester (16). Previous WHO guidelines for the treatment of malaria in pregnancy in 2002 and 2006 were developed based on the findings for 607 and 1000 2nd or 3rd trimester exposures, respectively (20,21). In the absence of high quality data from clinical trials or prospective cohort studies, most safety data on antimalarials in pregnancy come from spontaneous or passive reporting of adverse events by health care providers who prescribe or dispense these drugs to their patients. Many developing countries have adopted post-market safety systems that rely exclusively on passive surveillance, but this strategy has failed for malaria due, in part, to low level of spontaneous or passive reporting of adverse drug reactions (ADRs) to ACTs from malaria endemic countries (22). Furthermore, it is often difficult to differentiate severe adverse events (SAEs) due to antimalarials from the adverse health effects of malaria infections. Specifically, it is difficult to differentiate if a miscarriage occurred due to the malaria infection or due to the use on an ACT to treat the malaria infection. Moreover, retrospective reports from spontaneous reporting systems are less likely to be representative of the general population and can be biased toward the reporting of more severe cases.

V. Rationale for pooling data across studies

This dissertation aims to overcome the sample size limitations of individual studies in assessing the risk of adverse pregnancy outcomes after exposure to artemisinins in pregnancy through pooling data across multiple prospective studies. Large sample sizes

from well-designed studies are needed, with proper assessment of both the timing of the exposure to the antimalarial and of all potential outcomes. Safety outcomes are difficult to measure reliably in individual studies, as clinical trials are primarily designed to assess efficacy endpoints and are not powered to investigate rare safety endpoints (23). The limited power to detect rare adverse events that is characteristic of an individual trial can be overcome by pooling data. This can be accomplished by either a traditional systematic review and meta-analysis or through individual-level pooled data from multiple studies.

The Malaria in Pregnancy Consortium (MiPc) is a multi-year program led by the University of Liverpool School of Tropical Medicine (LSTM) and involving 47 partner organizations, including the University of Washington, to improve the control of malaria in pregnancy. Included in its mission is the evaluation of the risk of antimalarial drugs used in all trimesters of pregnancy for treatment and prevention. The MiPc Centralized Safety Database (CSD) stores data from eight major clinical trials (three treatment trials and five prevention trials). The Assessment of the Safety of Antimalarial Dug Use during early Pregnancy (ASAP) cohort study is based in select MiP Consortium sites and is coordinated by the University of Washington. The data obtained in the ASAP observational study allow for the assessment of the safety of the inadvertent or intentional use of antimalarials during the first trimester of pregnancy (24). Collectively, these studies provide the largest and most comprehensive dataset for assessing the safety of antimalarial drugs in pregnancy. This project leverages these two techniques to estimate the risk of adverse pregnancy outcomes after receipt of artemisinins during pregnancy,

namely a systematic review and meta-analysis, and a individual level pooled analysis using data from recently completed randomized-controlled trials and a cohort study.

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Chapter 1: The safety of artemisinin derivatives for the treatment of malaria in the 2nd or 3rd trimester of pregnancy: A systematic review and meta-analysis

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Abstract

Given the high morbidity for mother and fetus associated with malaria in pregnancy, safe and efficacious drugs are needed for treatment. Artemisinin derivatives are the most effective antimalarials, but are associated with teratogenic and embryotoxic effects in animal models when used in early pregnancy. However, several organ systems are still under development later in pregnancy. We conducted a systematic review and meta-analysis of the occurrence of adverse pregnancy outcomes among women treated with artemisinins monotherapy or as artemisinin-based combination therapy during the 2nd or 3rd trimesters relative to pregnant women who received non-artemisinin antimalarials or none at all. Pooled odds ratio (POR) were calculated using Mantel-Haenszel fixed effects model with a 0.5 continuity correction for zero events. Eligible studies were identified through Medline, Embase, and the Malaria in Pregnancy Consortium Library.

Twenty studies (11 cohort studies and 9 randomized controlled trials) contributed to the analysis, with 3,707 women receiving an artemisinin, 1,951 a non-artemisinin antimalarial, and 13,714 no antimalarial. The PORs (95% confidence interval (CI)) for stillbirth, fetal loss, and congenital anomalies when comparing artemisinin versus quinine were 0.49 (95% CI 0.24-0.97, I^2=0%, 3 studies); 0.58 (95% CI 0.31-1.16, I^2=0%, 6 studies); and 1.00 (95% CI 0.27-3.75, I^2=0%, 3 studies), respectively. The PORs comparing artemisinin users to pregnant women who received no antimalarial were 1.13 (95% CI 0.77-1.66, I^2=86.7%, 3 studies); 1.10 (95% CI 0.79-1.54, I^2=0%, 4 studies); and 0.79 (95% CI 0.37-1.67, I^2=0%, 3 studies) for miscarriage, stillbirth and congenital anomalies respectively.

Treatment with artemisinin in 2nd and 3rd trimester was not associated with increased risks of congenital malformations or miscarriage and may be was associated with a reduced risk of stillbirths compared to quinine. This study updates the reviews conducted by the WHO in 2002 and 2006 and supports the current WHO malaria treatment guidelines malaria in pregnancy.

Introduction

Given the high morbidity and mortality associated with malaria in pregnancy, safe and effective drugs are needed for treatment and prevention (1). However, there is limited information on the safety profile of most antimalarials when used during pregnancy, in part because few clinical trials enroll pregnant women, and there are few examples of systematic approaches to pregnancy pharmacovigilance (2,3).

The World Health Organization (WHO) currently recommends the use of artemisinin-based combination therapy (ACT) for the treatment of uncomplicated malaria in adults, children and in pregnant women in the 2nd or 3rd trimester (4). Seven days of quinine with clindamycin is recommended for uncomplicated malaria in the first trimester of pregnancy (5). The reports from relatively small observational studies and randomized controlled trials (RCTs) have not identified an increased risk of miscarriage or stillbirth after receipt of artemisinins during pregnancy, compared to women receiving non-artemisinin antimalarials or to pregnant women who did not receive any antimalarials (6–12).

The WHO recommends the use of ACTs in the first trimester if this is the only treatment immediately available, or if treatment with 7-day quinine plus clindamycin fails or if there is uncertainty about adherence to a 7-day treatment (13). Studies of rats, rabbits, and primates have reported the artemisinin class of antimalarial drugs to be associated with embryotoxicity and teratogenic effects (2). These animal studies suggest that the etiologically relevant time period for exposure in humans is within the first trimester.

Several organs are still under development in the 2nd trimester and therefore sensitive to teratogens, including the uterus (18 weeks), brain (until birth), eyes (24-36 weeks), and ears (18 weeks) (14), although animal data did not observe these types of anomalies after artemisinin exposure (15). Nevertheless, animal data do not always mirror the effects observed in humans.

We conducted a systematic review and meta-analysis examining the risk of adverse pregnancy outcomes associated with 2nd or 3rd trimester use of an artemisinin, compared with the experience of pregnant women treated with other antimalarial therapies and pregnant women without malaria and therefore no exposure to an antimalarial.

Methods

This analysis was completed in accordance with the PRISMA guidelines (see Supplemental Materials for the protocol, detailed search criteria, and PRISMA checklist) (16). The safety outcomes assessed were miscarriage (<28 weeks gestation), stillbirth (>/= 28 weeks gestation), fetal loss (composite of miscarriage or stillbirth), and congenital anomalies.

Search Strategy

We conducted an electronic search of Medline, Embase, and the Malaria in Pregnancy Library as of January 12, 2015, and the Medline search was updated on June 15, 2015, using the Patient, Intervention, Comparator, Outcome, Timing and Setting (PICOTS)

framework (Supplement Figure 1) (17,18). We also searched 'gray literature' databases, conference abstracts and manually reviewed reference lists of selected publications.

Inclusion/exclusion criteria

Studies fulfilling the following criteria were eligible for inclusion: prospective cohort studies or RCTs enrolling women of child bearing age (WOCBA) or pregnant women of any gestational age who received an artemisinin in the 2nd or 3rd trimester either as a monotherapy or as an ACT for the treatment of *P. falciparum*, *P. vivax* or mixed infections, and with information available on fetal loss and/or congenital anomalies, and information on timing of exposure in pregnancy (e.g. 2nd or 3rd trimester). Study exclusion criteria were case-control studies and cross-sectional surveys, studies not involving artemisinins treatment, studies reporting only 1st trimester artemisinin exposures, and studies that did not report pregnancy outcomes. In studies that reported exposures in both first trimester and 2nd or 3rd trimester, the pregnancies with first trimester exposures were excluded from the analysis.

Data Extraction

Two reviewers (SK and AE) independently screened titles and abstracts of all citations to identify potentially eligible studies (first screen). The second screen consisted of full-text review of studies selected by either reviewer in the first screen with agreement required for final study eligibility and inclusion in the systematic review. Any disagreements on study inclusion were resolved by discussions between the reviewers. The two reviewers independently extracted data using a standardized form and resolved any discrepancies

by consensus. The primary data abstracted included number, type, and timing of antimalarial drug exposures; patient characteristics including age, parity, parasitemia level, and HIV prevalence; and pregnancy outcomes including the number of miscarriages, stillbirths, and congenital anomalies reported by exposure. In addition we created a composite outcome of "any fetal loss" which combined any miscarriage or stillbirth for studies that did not clearly delineate the number of exposures in the 2nd and 3rd trimester.

Bias assessment

To assess the possibility of bias we applied the Cochrane Collaboration's tool to score RCT studies as having high, low, or unclear risk of bias (19). The Newcastle Ottawa scale was used to evaluate cohort studies for selection bias, comparability, and assessment of the outcome (20).

Exposure groups

The main exposure group of interest consisted of pregnant women who received artemisinin treatment in the 2nd or 3rd trimester. The comparison groups consisted of the following other exposure groups 1) treatment with oral quinine in the 2nd or 3rd trimester, 2) treatment with SP (for case-management) in the 2nd or 3rd trimester, 3) treatment with 'other antimalarials' (any non-artemisinin) in the 2nd or 3rd trimester, 4) women who received IPT-SP for prevention of malaria, and 5) pregnancies which did not receive an antimalarial drug in the 2nd or 3rd trimester (i.e. because they did not have clinical malaria).

Outcome definitions

Miscarriage was defined as pregnancy loss of <28 weeks of gestation and stillbirth as pregnancy loss of ≥28 weeks gestation. This cut-off was chosen because the majority of the studies were conducted in resource-limited settings without access to neonatal intensive care. Fetal loss was defined as miscarriage or stillbirth. Studies that reported miscarriages but did not report the number of exposures occurring before 28 weeks of gestation or studies that reported miscarriages and stillbirths using different definitions, were included in the analyses of any fetal loss.

We defined congenital malformation as any major or minor anomaly. We included all reported congenital anomalies in the analysis because of inconsistencies in the definition of major congenital malformations. We grouped congenital anomalies by organ system according to classifications developed by the Antiretroviral Pregnancy Registry (21).

Statistical Methods

Comparing risk estimates between exposure groups: To compare the risk between exposure groups, crude pooled odds ratios (PORs) and pooled risk differences (PRDs) were obtained from the reported count data. Our primary measure of association was a POR, but for analyses in which studies reported zero outcomes in both comparison arms, we conducted PRD models to allow all data to contribute to the model. We used a 0.5 continuity correction with a Mantel-Haenszel fixed effects model to correct for zero event cells (22). As sensitivity analysis, we used random effects model with DerSimonian-

Laird weighting and Peto Method with a 0.5 continuity correction, and Mantel-Haenzel fixed effects with continuity corrections of 0.01 and 0.69 (22). Where sample size permitted, stratification of the POR by geography and bias assessment did not change the results.

As per the Cochrane Collaboration guidelines, I^2 values >75% were considered indicative of considerable heterogeneity (23). We conducted stratified analyses by study type, geographic location, and comparison drug exposures, as well as bias level where possible (i.e. low, moderate, high; Supplement Table 3).

Results

A total of 944 articles or reports were identified. After removal of duplications and review of titles, a total of 309 abstracts were reviewed and 20 studies (11 cohort studies and 9 RCTs, 10 from Africa and 10 from Asia) met the inclusion criteria and were included in at least one of the analyses (Figure 1). Six studies did not include comparisons with other non-artemisinins and were used only descriptively and not included in the pooled ORs, with the remaining 14 studies providing data for the pooled ORs. Twelve studies did not report exposures by second and third trimester, and therefore were excluded from the analysis of miscarriage. The search did not identify any studies of artemisinins used for intermittent preventive treatment (IPT) of malaria during pregnancy that provided necessary data on pregnancy outcomes.

A total of 3,646 women in these 20 studies received an artemisinin during the 2nd or 3rd trimester of pregnancy. The most common exposures were: artemether-lumefantrine (AL) (N=2,000), dihydroartemisinin piperaquine (DP) (N=328), monotherapy with any artemisinin derivative including artemether and artesunate (N=225), artesunate sulfadoxine-pyrimethamine (AS-SP) (N=197), artesunate-mefloquine (AS-MQ) (N=94), artesunate-amodiaquine (AS-AQ) (N=83), and artesunate atovaquone-proguanil AAP (N=39). One study reported 412 women receiving artesunate or artemether alone or in combination with MQ, clindamycin, AP, artesunate iv, or AL but specific numbers were not reported by drug due to multiple treatments per patient (24). There were 1,951 women who received other antimalarials in the 2nd or 3rd trimester including: chlorproguanil-dapsone (CD) (N=81); chloroquine-SP (N=24); mefloquine monotherapy (N=36); quinine (N=661); SP (for case-management) (N=456); SP-AQ (N=80), and SP-azithromycin (AZM) (N=47), and for prevention IPTp-SP (N=566). In these 20 studies, an additional 13,714 pregnant women reported no exposure to antimalarials and no recorded illness malaria. Two studies with a no antimalarial comparison group were conducted in sub-Saharan Africa where the risk of malaria is high (7,25). The majority (n=8194) of the women who were not exposed to an antimalarial were from one site in Thailand with a low risk of malaria where all patients were screened weekly for malaria, and therefore no IPTp-SP was provided (12).

Ten studies reported data on presence of fever at enrollment, and 12 studies reported enrollment parasitemia (Supplement Table 1). The mean gestational age at enrollment ranged from 21 weeks to 38 weeks across the studies. Only three studies reported data on

HIV status, preventing stratification by HIV status (8,26,27). Further details about the characteristics of included women can be found in Supplement Table 1.

Miscarriage

Eight studies reported data on late miscarriages after receipt of an artemisinin in the second trimester, but only four studies reported comparisons with pregnant women who did not receive artemisinins and there were no studies with a quinine arm. A total of 34 miscarriages were reported among 939 women given artemisinins, including data from 5 studies reporting no miscarriages among 304 exposures.

Comparison to SP for treatment:

Only one study compared use of artemisinins to use of SP, with zero miscarriages among 169 ACTs exposures and 5 miscarriages among 205 SP exposures.

Comparison to no antimalarial drug exposure:

Three studies compared artemisinin users to women who did not receive an antimalarial drug. There were 34 miscarriages among 725 women who received an artemisinin, and 1009 miscarriages among 9264 women who received no antimalarials (POR=1.13, 95% CI 0.77-1.66, I^2=86.7%, 3 studies) (Figure 2).

Stillbirth

Sixteen studies reported a total of 83 stillbirths among 3,595 women who received an artemisinin in the 2nd or 3rd trimester.

Comparison to Quinine:

In studies with a quinine comparison arm, there were 14 stillbirths among 603 women who received an artemisinin and 20 stillbirths among 461 women who received quinine (POR 0.49, 95% CI 0.24-0.97, I^2=0%, 3 studies) (Figure 3).

Comparison to SP treatment:

In the two studies comparing use of artemisinins to use of SP for case-management, there were 13 stillbirths among 511 women who received an artemisinin, and 14 stillbirths among 516 women who received SP (POR 0.93, 95% CI 0.43-2.01, I^2=56%, 2 studies).

Comparison to other antimalarials for treatment:

Receipt of an artemisinin was not associated with a clear increase in the risk of stillbirth compared to receipt of SP combinations (POR 3.47, 95% CI 0.46-26.03 I^2=100%, 2 studies); these results were based on only two studies with considerable evidence of heterogeneity. Receipt of artemisinin compared to mefloquine and/or quinine for the treatment of malaria was not associated with an increased risk of stillbirth OR 1.03 (95% CI 0.21-4.96).

Comparison to IPT-SP:

Only one study compared women who received artemisinins for treatment to women who only received IPT-SP. There was no increased risk of stillbirth after receipt of an artemisinin compared to women who received only IPT-SP, OR=0.84 (95% CI 0.22-3.29).

Comparison to no antimalarial drug exposure:

In the studies that compared use of an artemisinin to no exposure to antimalarials, there were 60 stillbirths among 2131 women who received an artemisinin, and 277 among

12,622 women who received no antimalarials (POR 1.10, 95% CI 0.79-1.54, I^2=0%, 4 studies).

Any Fetal Loss

In twelve studies that did not specify if exposures occurred in the 2nd or 3rd trimester, there were 38 fetal losses among 1505 women who received an artemisinin in the 2 or 3rd trimester.

Comparison to Quinine:

In the 6 studies with a quinine arm, there were 19 fetal loses among 731 women who received artemisinins, and there were 24 fetal losses among 559 quinine exposures (POR= 0.58, 95% CI 0.31-1.06, I^2=0%, 6 studies) (Figure 4).

Comparison to SP for treatment:

In the two studies with an SP arm, there were 9 fetal losses among 121 women who received an artemisinin and there were zero fetal losses among 66 women who received *SP for treatment:*

(POR 5.86, 95% CI 0.73-46.88, I^2=0%, 2 studies).

Comparison to other non-artemisinin antimalarials for treatment:

Receipt of artemisinins in the 2nd or 3rd trimester was not associated with an increased risk of fetal loss compared to SP combinations or other antimalarials (POR 1.71, 95% CI 0.57-5.07, I^2=0%, 3 studies, and POR 1.70 95% CI 0.70-4.15, I^2=0%, 2 studies, respectively).

Comparison to IPT-SP:

Only one study compared artemisinin to IPT-SP. There was no increased risk of fetal loss among women who received an artemisinin for treatment compared to women who only received IPT-SP (OR=0.84, 95% CI 0.22-3.29).

Comparison to no antimalarial drug exposure:

Based on the results of a single study, there was no altered risk of fetal loss among women who received an artemisinin compared to women who received no antimalarial drugs (OR=0.93, 95% CI 0.50-1.74).

Congenital Anomalies

Thirteen studies reported a total of 55 congenital anomalies among 2,807 exposures to artemisinins in the 2nd or 3rd trimesters.

Comparison to quinine:

There were 4 congenital anomalies among infants born to 242 women who received an artemisinin and 4 congenital anomalies among 239 women who received quinine (POR=1.00, 95% CI 0.27-3.75, I^2=0%, 3 studies) (Figure 5).

Comparison to SP for treatment:

In the two studies comparing artemisinins to SP, there were 32 congenital anomalies among 532 women who received an artemisinin and 21 congenital anomalies among 472 women who received SP (POR=1.37, 95% CI 0.78-2.39, I^2=70.3%, 2 studies).

Comparison to no antimalarial drug exposure:

In studies with women who were not exposed to antimalarials in pregnancy, there were 12 congenital anomalies among 1745 women who received an artemisinin and 64

congenital anomalies among 4857 women who did not receive antimalarials in pregnancy (POR 0.79, 95% CI 0.37-1.67, I^2=0%, 3 studies).

Across the 13 studies reporting data on congenital anomalies, the most common congenital anomaly reported was umbilical hernia; 21 among infants exposed in utero to ACT during the 2 or 3rd trimester and an additional 12 umbilical hernias reported among 1,113 infants whose mother used other antimalarials during pregnancy. There were three central nervous system (CNS) defects among infants exposed to ACTs (anencephaly, hydrocephaly, and hemimegaloencephaly), but no CNS defects were reported among infants exposed to other antimalarials. One heart defect was reported among artemisinin exposed infants (acyanotic heart disease), and none among infants exposed to other antimalarials or no antimalarials in the 2 or 3rd trimester of pregnancy (Table 2). Studies did not routinely report congenital anomalies stratified by major and minor anomalies; therefore, it was not possible to conduct an analyses of the risk of any major anomaly. Furthermore, small numbers of events precluded the analysis of congenital anomalies stratified by organ system.

Sensitivity Analyses

In order to assess the robustness of our findings for our POR models we conducted sensitivity analyses using different correction factors for zero cells (0.69 and 0.01), and two different pooling techniques, Peto method and random effects models, given that there is a lack of consensus in the literature on the most appropriate method for modeling rare outcomes (22). Sensitivity analyses using different correction factors for zero cells

(0.69 and 0.01), and two different pooling techniques, Peto method and random effects models, did not change the overall interpretation of the analyses, but the results were most sensitive to changes in the correction factor and not the modeling technique (Supplementary Table 2).

Discussion

There was no indication that the risk of late miscarriage was higher in artemisinin recipients compared to women who received no antimalarials in the 2nd trimester or to SP for treatment, although the latter comparison included only a single study. There were fewer studies that compared the risk of late miscarriage, but in 3 studies that compared the risk against women who received no antimalarials in the 2nd trimester, and in 1 study against women who received SP for treatment, there was no evidence for an increased risk. The risk of stillbirths in women treated with artemisinins in the 2nd or 3rd trimester was lower compared to those treated with oral quinine during the same gestational period. Additionally, there was no increased risk of stillbirth after receipt of an artemisinin compared to receiving other antimalarials for treatment, IPT-SP only, or receiving no antimalarials. No increased risk was observed for fetal loss or congenital abnormalities when compared to quinine, SP, other antimalarials, IPT-SP, or to women who did not receive any antimalarials.

This meta-analysis of over 3500 artemisinin exposures builds upon the existing literature by including monotherapy and multiple artemisinins combinations therapies and using multiple comparison groups. Previous WHO guidelines in 2002 and 2006 were developed

based on the findings for 607 and 1000 2nd or 3rd trimester exposure respectively (28,29). Specifically, the results support the findings of a previous meta-analyses of the safety of the artemether-lumefantrine (AL) used in pregnancy which showed that AL was not associated with an increased risk of adverse pregnancy outcomes compared to quinine and SP for treatment (30). Additionally these results complement recent findings from an updated analysis of first trimester malaria on the Thailand/Burma border which found no difference in the risk of miscarriage between women treated with an artemisinin compared to women treated with quinine (31).

This study has several limitations. The findings from the 11 cohort studies may be confounded by indication due to the non-random administration of treatment. Of the nine studies that randomized patients to a treatment, five compared an artemisinin to quinine, so confounding is unlikely to be an important concern for this comparison. Confounding due to malaria infection is of particular concern in the studies comparing ACTs for treatment to no antimalarials, because these studies did not take into account the effect of malaria on poor pregnancy outcomes. Despite this limitation, we found equivalent risk of adverse pregnancy outcomes for women who received artemisinins compared to women who received other therapies and no antimalarials. Additionally because of small sample sizes, we were unable to stratify our analyses by potential effect modifiers such as geography (Asia vs. Africa), which may account for the observed heterogeneity of our results given the different epidemiology of malaria in the two regions and only 3 studies reported any data on HIV infection. Included studies had limited ability to detect an effect on miscarriages because of their recruitment strategies: recruiting women from

ANCs can lead to underestimates of the occurrence of miscarriage because women often present late in the 2nd trimester, and women who have an early miscarriage will not present at all, limiting the interpretation of the findings. Additionally, there may be further misclassification of outcome due to limitations of the gestational age assessment. Finally, there may be heterogeneity in the rigor by which studies assessed outcomes such as congenital anomalies. Although each study contributing to the analysis was assessed for bias using either the Cochrane Collaboration Tool or the Newcastle Ottawa Scale, these tools may not fully capture all sources of bias in the assessment of safety outcomes.

This analysis was limited to artemisinin use for case-management only. Recently a prevention study which randomized pregnant women to IPT with DP was published (32). As more studies of ACT used for IPT with pregnancy outcomes become available, additional meta-analyses of safety outcomes may be warranted, especially if they enroll women from first-trimester.

Despite identifying over 3500 women who received artemisinins in the 2nd or 3rd trimester, the sample size was too limited to allow the evaluation of the risks of specific congenital anomalies. Also, in most studies the ascertainment of anomalies was based only on surface exams conducted at birth which would not detect congenital anomalies of internal organs or congenital anomalies of the brain leading to functional deficits. According to the Centers for Disease Control and Prevention, major congenital anomalies in the U.S. are detected in 5% of children by age 5, but only 60% of these anomalies are detected at birth (33). The studies included in the analyses reported congenital anomalies

(major and minor) among 1% of births or less. This low proportion is likely due to the fact that birth defects such as cardiac defects and those related to brain development would not be detected by surface exams, and therefore this study cannot rule out or estimate the risk of these specific anomalies. Given that the brain is still developing through the 2nd and 3rd trimester, it would be important to assess exposed infants later in infancy or childhood to rule out an increased risk of these types of defects. In the review, only three studies followed infants up to one year after birth (10,11,34).

The results of this study build upon the limited data to-date on the safety of ACT used in pregnancy. These data suggest that the risk of miscarriage and congenital anomalies is similar among women treated with artemisinins in 2nd or 3rd trimester of pregnancy and women treated with quinine or other non-artemisinin antimalarials. Furthermore, the risk of stillbirth was lower compared to quinine recipients, possibly reflecting a higher efficacy of artemisinins against infection (35). Though these results were based on few exposures across a limited number of studies, they support the current WHO guidelines recommending the use of ACTs for treatment of malaria in the 2nd or 3rd trimester of pregnancy.

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Chapter 1 Tables

Table 1a: Description of cohort studies identified in the systematic review and included in the meta-analysis

Study	Location and time period	Study Population	ACT Exposures	Comparator Exposures	2-3rd Trimester Outcomes
Adam, 2004 (36)	Sudan October 1997-February 2001	Pregnant women who presented with symptoms of *P. falciparum* malaria and had confirmed malaria parasites who were treated with quinine and returned to the hospital with recurrent malaria symptoms and parasite detected within three weeks. Mean age 27.1 years	Art im=28 1st trim=1/28 2nd trim=12/28 3rd trim=15/28	N/A	**Miscarriage**=0/12 **Stillbirth**=0/27 **CA**=0/27
Adam, 2006 (37)	Sudan September 2004-March 2005	Pregnant women with uncomplicated *P. falciparum* malaria. Mean age 29.4 (s.d. 4.3) years	AS+SP =32 2nd trim=10/32 3rd trim=22/32	N/A	**Miscarriage**=0/10 **Stillbirth**=0/32 **CA**=0/32
Deen, 2001 (25)	Gambia March, 2000	All women of reproductive age (15-44 years) residing in the 42 study villages. Villages were part of a mass drug administration campaign. Pregnant women exposed to Mass drug	AS+SP=287 1st trim=77/287 2nd trim=90/287 3rd trim=28/287	Placebo=40 No exp.=132	**Miscarriage:** AS-SP=0/90 Comm. +Placebo=2/132 **Stillbirth:** AS-SP=11/287 Comm. +Placebo=

		administration were followed.			6/172 **CA:** AS+SP=6/287 Comm. +Placebo= 2/172
Manyando, 2010 (8)	Zambia October 2004-July 2008	Pregnant women attending antenatal clinic who were grouped based on the drug used to treat their last episode of malaria. Mean age NR.	AL=495 1st trim=159 2nd trim=169 3rd trim=162	SP=506 1st trim=125 2nd trim=205 3rd trim=176	**Miscarriage:** AL=0/169 SP=5/205 **Stillbirth:** AL=9/473 SP=13/478 **CA:** AL=29/449 SP=18/444
McGready, 2001 (12)	Thailand 1986-2001	Pregnant women who had microscopy confirmed *P. falciparum* or mixed *P. falciparum* and *P. vivax* infections. Mean age 24.8 (s.d. 6.4) years	Artesunate or artemether alone or in combination with MQ, C AP, or artesunate iv, or AL N=461 1st trim=40 2nd trim=201 3rd trim=211	No exp.=8154	**Miscarriage:** Artemisinin= 20/201 Comm.=1003/8154 **Stillbirth:** Artemisinin=7/386 Comm.= 114/7058 **CA:** Artemisinin=3/386 Comm.=56/3707
*McGready, 1999 (38)	Thailand 1991-1996	Pregnant women in camps for refugees with uncomplicated, multi-drug resistant *P. falciparum* malaria.	AS=61 2-3rd trim=61 *study reports outcomes for 78 exposures	Q=72 2-3rd trim=72 MQ =36 2-3rd trim=36	**Stillbirth:** AS=2/78 MQ or Q=8/322 **Any Fetal Loss:** AS=3/78 Q+MQ=16/322 **CA:**

					AS=0/73 MQ or Q=4/322
Mosha, 2014(39)	Tanzania April-September 2012	2-3rd trimester pregnant and non-pregnant women with diagnosed uncomplicated malaria. *P. falciparum* detected by microscopy and hemoglobin level >/= 7g/dl. Median age 25, range 18-41 years	AL=55 2nd trim=17 3rd trim=16 Not pregnant AL N=22	N/A	**Miscarriage**: 0/17 **Stillbirth**: 0/33 **CA**: 0/33
Rulisa, 2012(7)	Rwanda June 2007-July 2009	Pregnant women age 18+ treated with AL after diagnosis of simple *P. falciparum* malaria based on blood smear or clinical symptoms. Controls were pregnant women with no malaria. Age range 16-48.	AL=1072 2nd trim=434 3rd trim=542	No exp.=978	**Miscarriage:** AL 14/434 No antimalarial=4/978 **Stillbirth:** AL=31/1072 No antimalarial=23/978 **CA:** AL=3/1072 No antimalarial-3/978
Poespoprodjo, 2014(40)	Indonesia April 2004-June 2009	All pregnant women and newborn infants admitted to maternity ward screened for malaria.	DP=336 1st trim=8 2-3rd trim=328 DP+ivArt=77 1st trim=10 2-3rd trim=67	Quinine=347 Oral Q 1st trim=38 iv Q 1st trim=50 2-3rd trim=259 CQ+SP=24 2-3rd trim=24 No exp.=4408	**Stillbirth:** DP=9/328 DP+ivART=2/67 CQ+SP=0/24 Q=16/259 Community=134/4408
Wang, 1989(41)	China	Pregnant women with malaria with typical	Artemisinin in oil=2	N/A	**Miscarriage:** Art oil=0/2

	1976-1980	symptoms and signs; Plasmodium found in thick blood smear with a density over 500 mm^3 and antimalarial had not been administered or with a known grade III chloroquine resistance. Mean age 25.8 (s.d. 4.1) years	2^{nd} trim=2 Artemether=4 2^{nd} trim=4		Art=0/4 **Stillbirth:** Art oil=0/2 Art=0/4 **CA:** Art oil=0/2 Art=0/4
^Nakelembe, 2012(42)	Uganda and Burkina Faso	HIV negative women in the 2nd or 3rd trimester who were screened for malaria during normal IPT schedule and given AL if positive or SP if negative and followed through pregnancy	AL=287 2-3^{rd} trim=287	IPT with SP=566 2-3^{rd} trim=566	**Stillbirth:** AL=3/287 IPT SP only= 7/566

*Pregnancy outcomes were reported for 78 artemisinin exposures, and 322 combined MQ and Q exposures. These ACT exposures are also included in the McGready 2001 cohort study with different comparison group (no antimalarial drug exposures).
^ Data provided by the authors
Trim trimester; ART im: artemether intramuscular; AS: artesunate; SP: sulfadoxine pyrimethamine; AL: artemether lumefantrine; CA: congenital anomal; MQ: mefloquine; C: Clindamycin; CD: chlorproguanal-dapsone; iv: intravenous; AP: atovaquone proquanil; Q: quinine; AS7: artesunate 7 days; CQ: chloroquine; AQ: amodiaquine; DP: dihydroartemisinin-piperaquine; IPT: intermittent preventative therapy; MDA: Mass drug administration; ANC: antenatal care; No exp: no exposure to antimalarials for treatment.

Table 1b: Description of RCT studies identified in the systematic review and included in the meta-analysis

Study	Location and time period	Study Population	ACT Exposures	Comparison Exposures	2-3rd Trimester Outcomes
Bounyasong, 2001(43)	Thailand January 1995-December 1998	Pregnant women with *P. falciparum*, not more than 4% parasitized red cells, gestational age at least 28 weeks estimated by ultrasound.	AS+MQ=28 2-3rd trim=28	Q=29 2-3rd trim=29	**Any Fetal Loss:** AS-MQ= 0/28 Q= 0/29 **CA:** AS-MQ=0/28 Q= 0/29
Kalilani, 2007(26)	Malawi September 2003-September 2004	Pregnant women (EGA 14-26 weeks) between 15 and 49 years old, with peripheral *P. falciparum* parasitemia; method of measuring gestational age not described. Median age 20 (range 17-24).	AS+SP=47 2-3rd trim=47/47	SP=47 2-3rd trim=47 SP+AZM=47 2-3rd trim=47	**Stillbirth:** AS-SP= 4/38 SP= 1/38 SP+AZM= 0/42 **Any fetal loss:** AS-SP=4/38 SP=0/38 SP+AZM=4/42 **CA:** AS-SP=0/38 SP= 0/38 SP-AZM=0/42
McGready, 2000(11)	Thailand October 1995-July 1997	Pregnant women in 2nd or 3rd trimester, estimated by fundal height seen at ANC who had microscopy confirmed uncomplicated *P. falciparum* infection	AS-MQ=66 2-3rd trim=66	Q=42 2-3rd trim=42	**Any Fetal Loss:** AS-MQ=2/66 Q =0/42 **CA:** AS-MQ=0/66 Q=0/42
McGready, 2001(44)	Thailand	Pregnant women in 2nd or 3rd trimester estimated by fundal	AS7 N=64 2-3rd trim=64	Q+C N=65 2-3rd trim=65	**Stillbirth**: AS7=1/64

	October 1997-January 2000	height seen at ANCs who had microscopy confirmed *P. falciparum* infections. Age range 15-41 years			Q+C=1/65 **Any Fetal Loss:** Art=1/64 Q+C=1/65 **CA:** Art=0/64 Q+C=1/65
McGready, 2005(45)	Thailand December 2001-July 2003	Pregnant women with first episode of *P. falciparum* or mixed infection (*P. vivax*), 14-31 weeks gestation estimated by ultrasound or fundal height, Ht >= 20%. Mean age 26 (s.d. 7) years	AAP N=39 2-3rd trim=39	Q N=42 2-3rd trim=42	**Any Fetal Loss:** AAP=0/34 Q=0/38 **CA:** AAP=2/34 Q=1/38
McGready, 2008(10)	Thailand April 2005-August 2006	Patients with acute *P. falciparum* malaria in 2nd or 3rd trimester estimated by ultrasound. Originally only allowed 2nd infection in pregnancy (already failed quinine), but later widened to allow first infections in pregnancy. Age range 14-44 Years	AL N=125 2-3rd trim=125 AS7 N=128 2-3rd trim=128		**Stillbirth:** AL=1/117 AS7= 1/120 **Any Fetal Loss:** AL=1/117 AS7=2/120 **CA:** AL=3/117 AS7=4/120
Mutabingwa, 2009(27)	Tanzania January 2004-September 2006	Pregnancy with either a positive blood smear for *P. falciparum* with at least 800 parasites/µL in an asymptomatic woman or any of the following symptoms within 2 days prior to consultation: history of fever, headache, vomiting, chills/rigors	AS-AQ=83 2-3rd trim=83	SP=28 2-3rd trim=28 SP-AQ=80 2-3rd trim=80 CD=81 2-3rd trim=81	**Any Fetal Loss:** AS-AQ=4/83 SP-AQ=1/80 SP=0/28 CD=1/81 **CA:*** AS+AQ=3/83

		and/or any of the following signs: temperature ≥37.5 & <39.5 °C, Hb ≥7 and <9 g/dl) with *P. falciparum* parasitemia at any density. All cases were between 14-34 weeks gestation defined by presence of fetal heartbeat. Median age 21 years			SP= 3/28 CD= 7/81 SP+AQ=8/80
Piola, 2010(6)	Uganda October 2006-May 2009	Women with viable pregnancy with an estimated gestation of ≥13 weeks determined by ultrasound or LMP and malaria infection detected by microscopy (*P. falciparum* mixed or monoinfection). SP may have been used for prevention before entry to the study and some inadvertently when entered.	AL=152 2-3rd trim=152	Q=152 2-3rd trim=152	**Stillbirth:** AL=2/144 Q=3/137 **Any fetal loss:** AL=5/144 Q=7/137 **CA:** AL=3/144 Q=2/137
Sowunmi, 1998(46)	Nigeria January 1994-March 1997	All patients referred to University College Hospital with persistent *P. falciparum* parasitemia and acute uncomplicated malaria after failure of supervised therapy with standard regimen of chloroquine or after a single dose of SP or both CQ and SP. Oral fluid intolerance, no history of allergy to known antimalarial drugs, 2nd or 3rd trimester determined by ultrasound	Art+MQ=23 2-3rd trim=23 Art im=22 2-3rd trim=22		**Stillbirth:** Art+MQ=0/23 Art im= 0/22 **Any fetal loss:** Art+MQ=0/23 Art im= 0/22 **CA:** Art+MQ=0/23 Art im= 0/22

*Only minor CA
Trim: trimester; ART im: artemether intramuscular; AS: artesunate; AZM: azithromycin; SP: sulfadoxine pyrimethamine; AL: artemether lumefantrine; CA: congenital anomaly; C: Clindamycin; MQ: mefloquine; CD: chlorproguanal-dapsone; iv: intravenous; AP: atovaquone proquanil; Q: quinine; AS7: artesunate 7 days; CQ: chloroquine; AQ: amodiaquine; DP: dihydroartemisinin-piperaquine; IPT: intermittent preventative therapy

Table 2: Reported congenital anomalies grouped by organ system and stratified by antimalarial drug exposure during 2-3rd trimester of pregnancy

Group and Malformation	No antimalarial exposure	ACT	Other antimalarial
Central Nervous System (CNS)			
Anencephaly		1	
Spina bifida	1		
Hydrocephalus		1	
Unspecified neurologic disorder	1		
Hemimegaloencephaly		1	
Face and Neck (FACE)			
Midline cyst on nose§		1	1
Unspecified anomaly of ear			1
Unspecified anomaly of eye		1	
Nose-small§		1	
Aglossia		1	
Obstructive Heart Defects—Left Sided (CV-LT)			
Acyanotic heart disease		1	
Respiratory System (RES)			
Thoracic asymmetry	1		
Female Genitalia (G-FEMALE)			
Small labia§		1	
Male Genitalia (G-MALE)			
Undescended testicles		2	
Hernia inguinal			1
Limb Reduction/Addition Defects (LIMB)			
Other and unspecified polydactyly		5	6
Hyperextensibility of joint§		1	
Specified or unspecified reduction defect of unspecified limb		1	
Other Musculoskeletal Defects (MS-O)			
Umbilical hernia§		21	12
Human tail		1	
Unspecified malformation of left foot		1	
Skin and Skin Derivatives (SKIN)			
Jaundice§	1		
Hyperpigmentation§		1	
Dermal Cyst§			1
Lanugo§		1	

Haemangioma	1		
Unspecified anomaly of skin		2	
Chromosome Anomaly (CHROM)			
Trisomy 18		1	
Trisomy 21		1	
Other Organs and Organ Systems (OTHER)			
Amniotic banding		1	
Alagille's (genetic)		1	
Unspecified anomaly	56	6	23
Total malformations	61	55	45

* No cleft lip and/or palate, conotruncal heart defects, obstructive heart defects—right sided, other heart defects, other circulatory system, upper gastrointestinal system, lower gastrointestinal system, or renal and urinary system defects were reported.
§Denotes reported congenital anomalies that do not meet the inclusion criteria for the Antiretroviral Pregnancy Registry's Organ Classification System.

Chapter 1 Figures:

Figure 1: PRISMA flow diagram for search results June 15, 2015

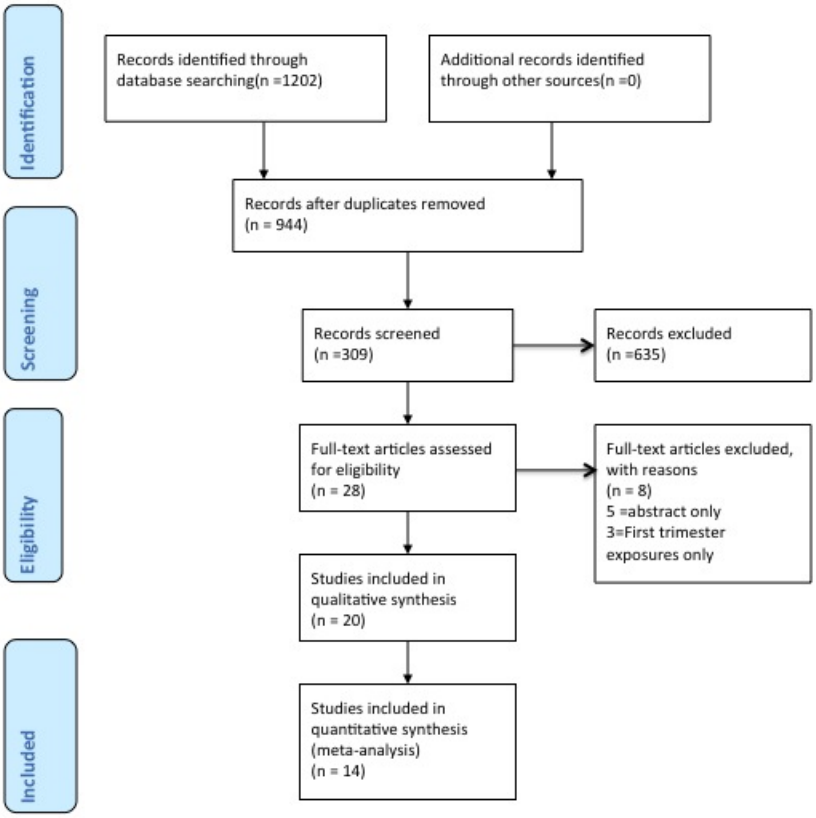
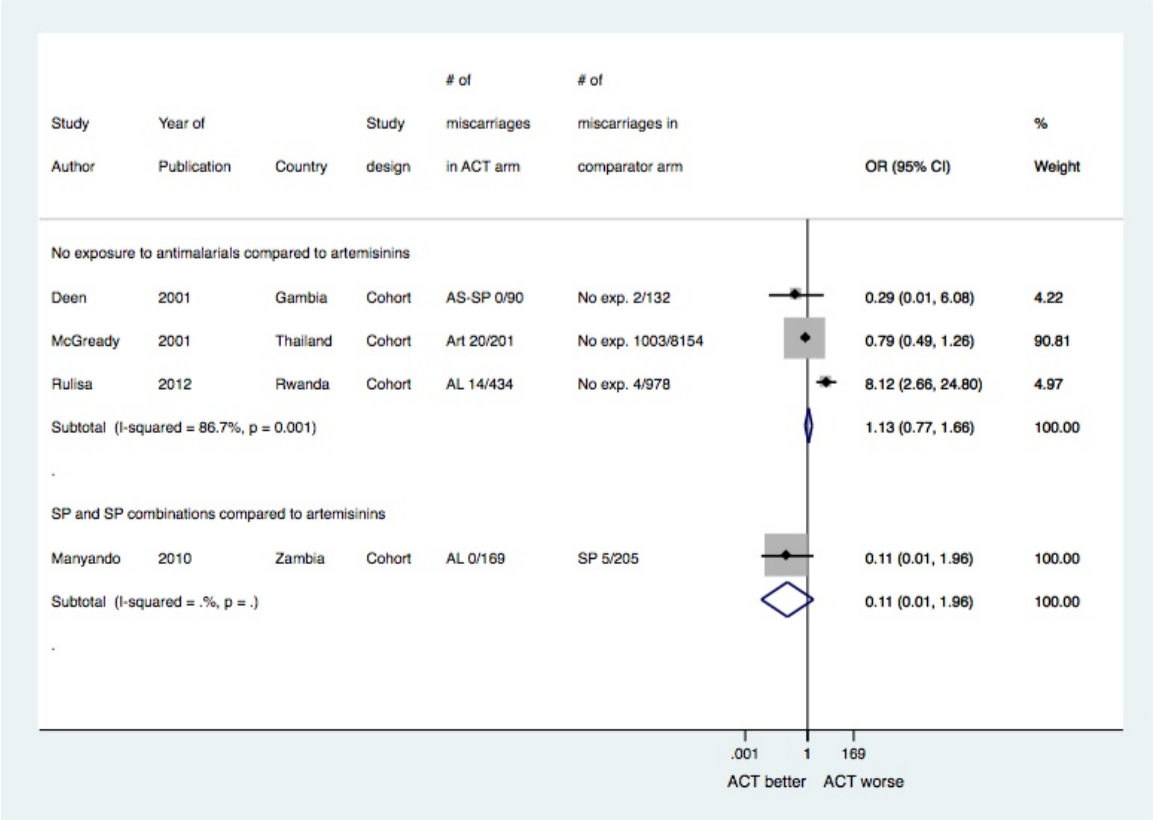
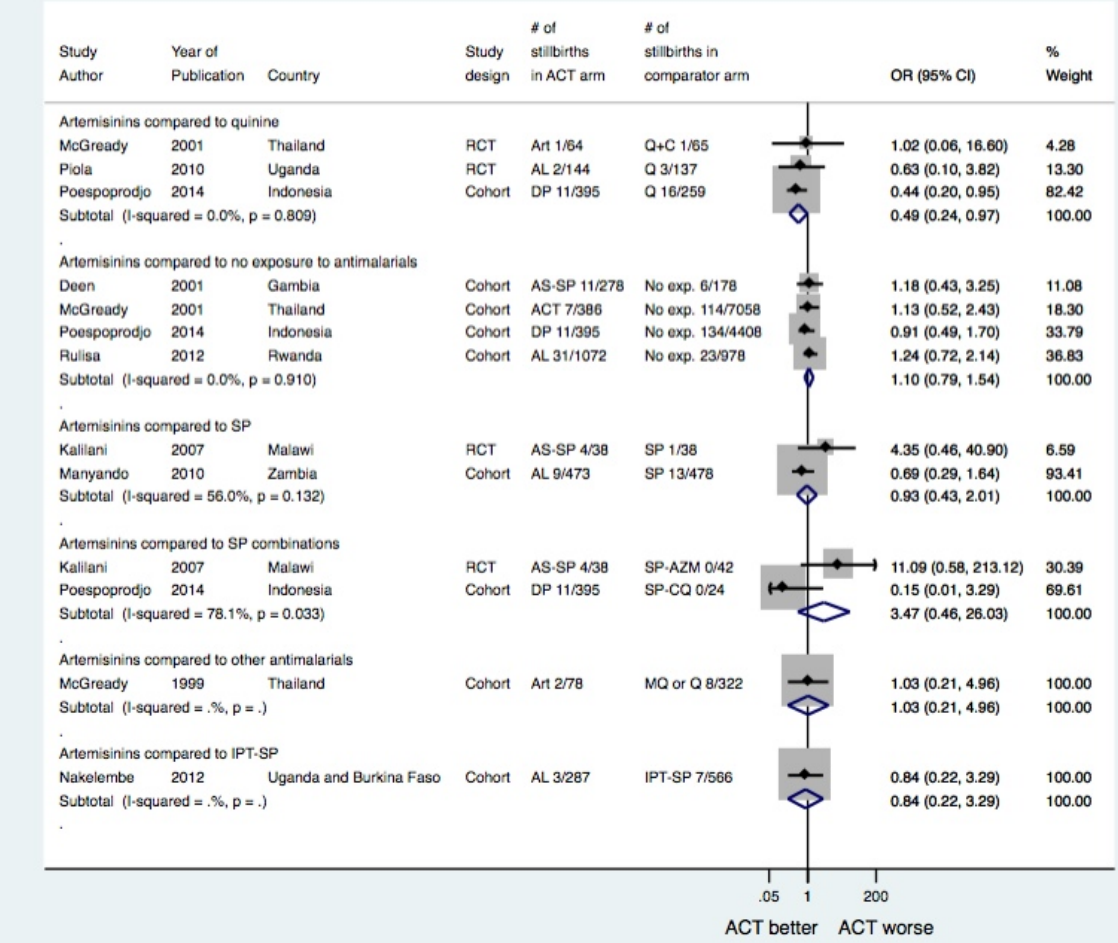


Figure 2: Pooled odds ratio for miscarriage after 2nd trimester exposures to artemisinins stratified by comparison group



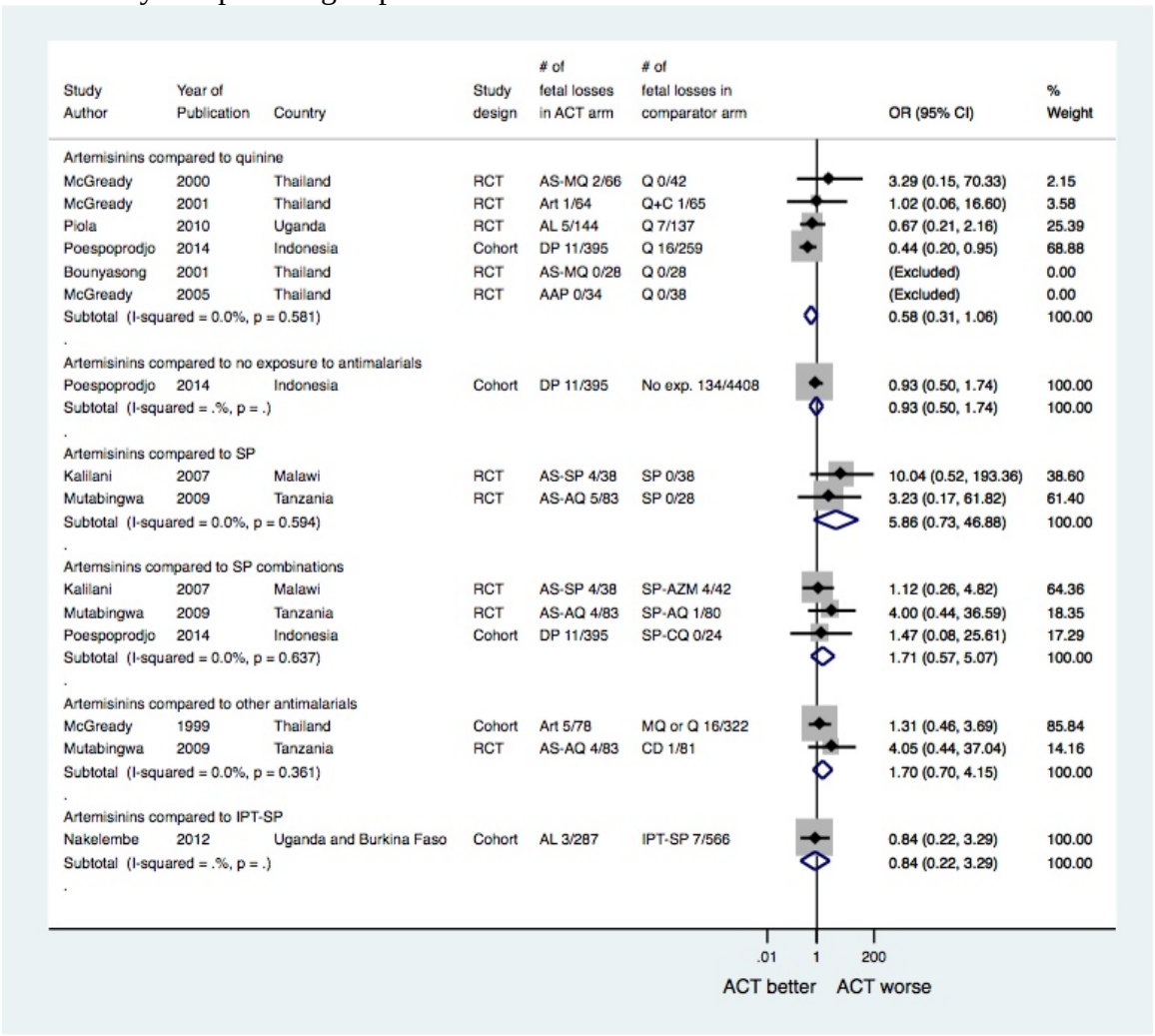
*McGready 2001 reported multiple types of artemisinin exposures that were combined for this analysis (12)
ART artemisinins, AL artemether-lumefantrine, AS-SP artesunate sulfadoxine pyrimethamine, SP sulfadoxine pyrimethamine, No exp. No exposure to antimalarials

Figure 3: Pooled odds ratio for stillbirth after 2-3rd trimester exposures to artemisinins compared to other drugs



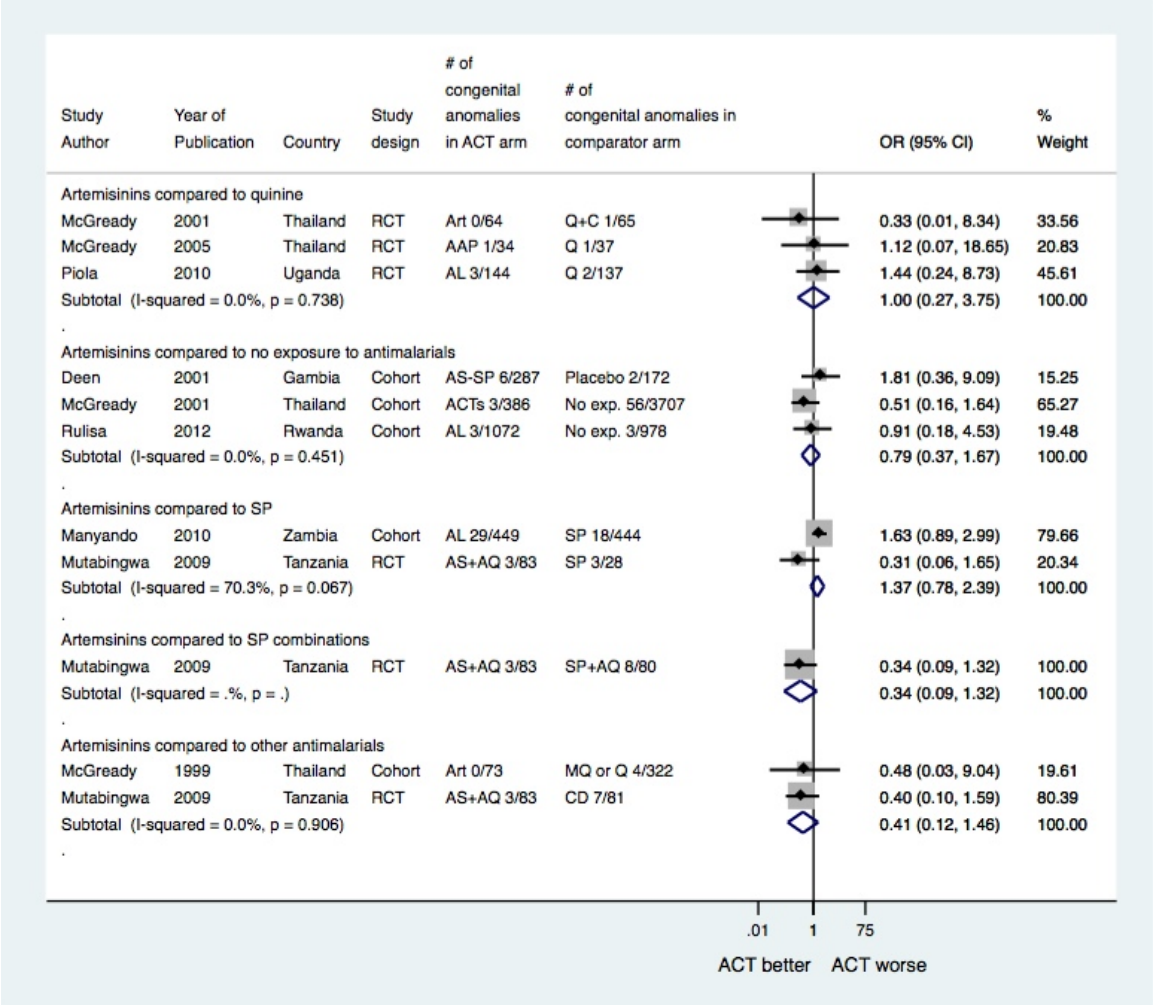
*McGready 2001 reported multiple types of artemisinin exposures that were combined for this analysis and reported as ACT (12)
^McGready 1999 MQ or Q exposures include patients given MQ, Q, or both (33)
ART: artesunate, AL: artemether-lumefantrine, AAP: artesunate atovaquone proguanil, AS-AQ: artesunate-amodiaquine, Q: quinine, Q+C: quinine+clindamycin, SP: sulfadoxine pyrimethamine, AQ: amodiaquine, SP+CQ: sulfadoxine pyrimethamine chloroquine, MQ: mefloquine, SP-AZM: sulfadoxine pyrimethamine azithromycin, CD: chlorproguanal-dapsone, IPT: intermittent preventative treatment, RCT: randomized controlled trial, ACT: artemisinin combination therapy, No exp. no exposure to antimalarials.

Figure 4: Pooled Odds ratio for fetal loss after 2-3rd trimester exposures to artemisinins stratified by comparison group



*McGready 2001 reported multiple types of artemisinin exposures that were combined for this analysis and reported as ACTs (12)
^McGready 1999 MQ or Q exposures include patients given MQ, Q, or both (33)
ART: artesunate, AS-MQ: artesunate mefloquine, AL: artemether-lumefantrine, DP: dihydroartemisinin-piperaquine, AAP: artesunate atovaquone proguanil, AS-SP: artesunate sulfadoxine pyrimethamine, AS-AQ: artesunate-amodiaquine, Q: quinine, Q+C: quinine+clindamycin, SP: sulfadoxine pyrimethamine, AQ: amodiaquine, SP+CQ: sulfadoxine pyrimethamine chloroquine, MQ: mefloquine, SP-AZM: sulfadoxine pyrimethamine azithromycin, CD: chlorproguanal-dapsone, IPT: intermittent preventative treatment, RCT: randomized controlled trial, ACT: artemisinin combination therapy, No exp. No exposure to antimalarials.

Figure 5: Pooled odds ratio for congenital anomalies after 2-3rd trimester exposures to artemisinins stratified by comparison group



*McGready 2001 reported multiple types of artemisinin exposures that were combined for this analysis and reported as ACTs (12)
ART: artesunate, AL: artemether-lumefantrine, AAP: artesunate- atovaquone proguanil, AS-AQ: artesunate-amodiaquine, Q: quinine, Q+C: quinine+clindamycin, SP: sulfadoxine pyrimethamine, AQ: amodiaquine, SP+AQ: sulfadoxine pyrimethamine amodiaquine, MQ: mefloquine, CD: chlorproguanal-dapsone, RCT: randomized controlled trial, ACT: artemisinin combination therapy, No exp. No exposure to antimalarials

Chapter 1 Supplementary Materials

Methods search:
A multi-concept Boolean search strategy was developed with the assistance of a librarian using keywords and MeSH terms with no restriction for time of publication or language. The search terms for Medline consisted of: (Pregnant women OR pregnan* AND malaria) AND (Artemisinin* OR “Artemisinin Combination Therapy” OR ACT OR artemether OR artesunate OR dihydroartemisinin OR treatment) AND (Pregnancy complication [mh] OR safety OR “serious adverse event” OR miscarriage OR stillbirth OR “pregnancy loss” OR “spontaneous abortion” OR “birth defect” OR congenital abnormalities OR “congenital malformations” OR “congenital anomalies”) AND Clinical trials OR trials OR cohort study [mh] OR prospective [tw]. Search terms for the other databases are included below:

EMBASE Search: January 13, 2015: 295 Articles
'pregnant woman' AND malaria AND (artemisinin* OR 'artemisinin combination therapy' OR act OR artemether OR artesunate OR dihydroartemisinin) AND [embase]/lim NOT [medline]/lim AND 'human'/de

Malaria in Pregnancy Library: January 13, 2015: 384 Articles
Artemisinin OR artemether OR artesunate OR dihydroartemisinin

Chapter 1 Supplementary Figures

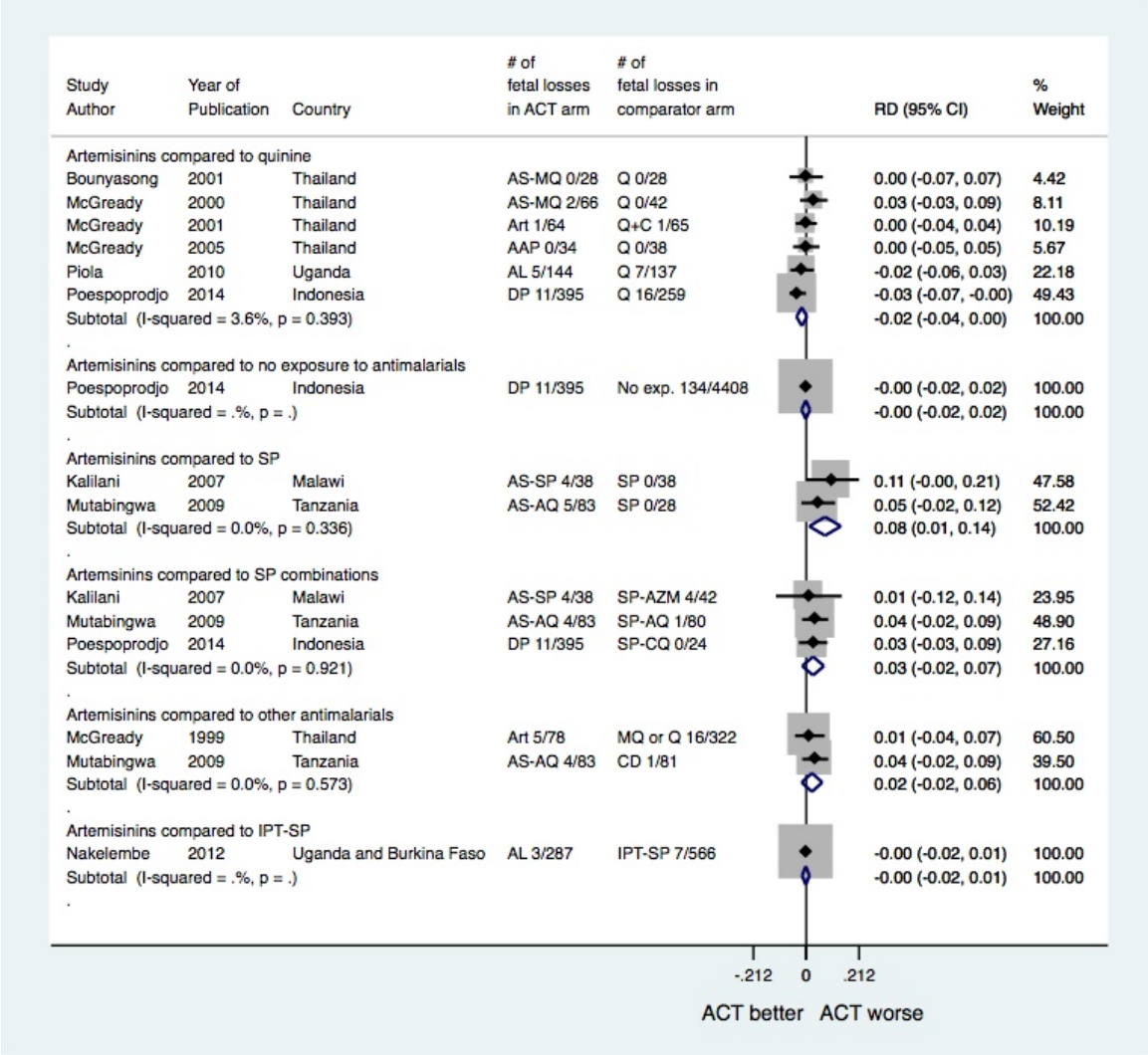
Supplementary Figure 1: PICOTS Framework of the systematic search

Components	Characteristics
Population	Pregnant women with malaria -Subgroup analysis: o By trimester o Severe malaria and uncomplicated malaria o Age o Parity o Geography
Intervention	Exposed to an artemisinin during pregnancy either as a monotherapy or in combination with another drug.
Control	Non-artemisinin based treatment for malaria (including quinine, SP, CQ, and MQ), or placebo
Outcomes	Adverse pregnancy outcomes as measured at birth: 1. Miscarriage –defined as pregnancy loss <28 weeks 2. Stillbirth –defined as pregnancy loss >/= 28 weeks 3. Congenital Anomalies –including major and minor 4. Fetal loss if gestational age at time of loss cannot be obtained
Timing	No time limits will be placed on the search
Setting	Any prospective cohort study or randomized trial which enrolled pregnant women with malaria. No restriction for language will be made

Supplementary Figure 2: PubMed Search Strategy, Search Date June 15, 2015

	Framework	Search terms	Number of articles
P	**Population**	(Pregnant women OR pregnan* AND malaria)	**P: 4340**
I	**Intervention**	AND (Artemisinin* OR "Artemisinin Combination Therapy" OR ACT OR artemether OR artesunate OR dihydroartemisinin OR treatment)	**I: 9020942** **P + I: 2665**
C	**Control**	-	**P+I+C: 2665**
O	**Outcome**	AND (Pregnancy complication [mh] OR safety OR "serious adverse event" OR miscarriage OR stillbirth OR "pregnancy loss" OR "spontaneous abortion" OR "birth defect" OR congenital abnormalities OR "congenital malformations" OR "congenital anomalies")	**O: 1261727** **P + I +C+ O: 1631**
T	**Timing**	-	**P+I+C+O+T: 1631**
S	**Setting**	AND Clinical trials OR trials OR cohort study [mh] OR prospective [tw]	**P+I+O+C+T+S: 516**

Supplementary Figure 3: Pooled Risk Difference for fetal loss after 2-3rd trimester exposures to artemisinins, stratified by comparison group



*McGready 2001 reported multiple types of artemisinin exposures were combined for this analysis and reported as artemisinins (12)
^McGready MQ or Q exposures include patients given MQ, Q, or both
ART: artesunate, AS-MQ: artesunate mefloquine, AL: artemether-lumefantrine, DP: dihydroartemisinin-piperaquine, AAP: artesunate atovaquone proguanil, AS-SP: artesunate sulfadoxine pyrimethamine, AS-AQ: artesunate-amodiaquine, CD: Chlorproguanil-dapsone; Q: quinine, Q+C: quinine+clindamycin, SP: sulfadoxine pyrimethamine, AQ: amodiaquine, SP+CQ: sulfadoxine pyrimethamine chloroquine, MQ: mefloquine, SP-AZM: sulfadoxine pyrimethamine azithromycin, Comm: community controls, IPT: intermittent preventative treatment, ACT: artemisinin combination therapy

Chapter 1 Supplementary Tables

Supplementary Table 1: Clinical presentation of pregnant women upon enrollment from included studies

First Author	Gestational Age at Enrollment in weeks	HIV prevalence (Country Prevalence)	Fever (>38 °C)	Parasitemia
Adam 2004 (36)	Artemether:27 (s.d. 7.3)	NR (Moderate)	NR	8468 (525-34500)**
Adam 2006 (37)	29.7 (range 16-36)	NR (Moderate)	Mean 38.1 (range 37.5-40)[φ]	7762 (3800-15250) [§]
Deen 2001 (25)	NR	NR (Low)	NR	NR
Manyando 2010 (8)	NR	ACT: 30% tested No exp.: 38% tested ~20% positive (High)	NR	NR
McGready, 2001 (24)	Artemisinins 24.6 (range 3-40)	NR (low)	17%	NR
McGready 1999 (38)	NR	NR (Low)	AS: 16.0% Q: 4.4% MQ: 5.0%	AS: 814 (16-130385)** Q: 1078 (4-53719) MQ: 1249 (53-43257)
Mosha 2014 (39)	AL: 27 (range 14-37)	NR (High)	AL: 37.1 (range 36-39)[φ]	25280 (560-198,080)^
Rulisa 2012 (7)	AL: 25.8 (95%CI 25.3-26.4), No drug: 28.5 (95%CI 28-29)	NR (Moderate)	NR	NR
Poespoprodjo 2014 (40)	DP: 38 (24-44) DP+iv ART:39 (20-43) Q: 39 (34-40) CQ+SP: 38 (21-41) No exp.: 39 (19-44)	NR (Low)	DP: 10.7% DP + iv ART: 9% Oral Q: 28% CQ+SP: 33% No exp.: 5.2%	NR

Wang 1989 (41)	NR	NR (Low)	NR	NR
Nakelembe 2012 (42)	NR	All HIV negative (High)	NR	NR
Bounyasong 2001 (43)	AS-MQ: 26.7 weeks Q: 26.4 weeks	NR (Moderate)	NR	AS-MQ: 1330* Q: 1313
Kalilani 2007 (26)	AS-SP: 22 (IQR 20-23) SP: 22 (IQR 20-24) SP+AZM:24 (IQR 20-24)	AS-SP: 7/33 SP: 9/26 SP+AZM: 10/28 (High)	NR	AS-SP: 685.6 (120-4259)** SP:963 (180-22499) SP+AZM: 1183 (150-22499)
McGready 2000 (11)	AS-MQ: 24 (range 12-40) Q: 24 (15-38)	NR (low)	AS-MQ: 15.2 Q: 20.4%	AS-MQ: 11651 (32-241127)** Q: 19086 (79-149386)
McGready 2001 (44)	AS7: 28 (range 16-40) Q+C26 (range 16-40)	NR (low)	AS7: 14.1% Q+CD: 21.8%	AS7: 9822 (16-93019)** Q+CD: 11098 (16-109724)
McGready 2005 (45)	AAP: 21 (s.d 5.3) Q: 21 (s.d. 4.5)	NR (low)	AAP: 59% Q: 73.8%	AAP: 2596 (33-123027)** Q: 2083 (33-109648)
McGready 2008 (10)	AL: 23.7 (s.d. 6.8) Artesunate: 24.8 (s.d. 7.6)	NR (low)	AL: 57.6% AS7: 58.6%	AL: 3548 (48-158489)** AS7: 3162 (65-457-088)
Mutabingwa 2009 (27)	AS-AQ: 6 months (IQR 5-8 months) SP: 7 months (IQR 6-8) SP-AQ: 7 (IQR 6-8) CD: 6 months (IQR 5-8)	SP: 0/27 CD: 1/80 SP+AQ: 1/82 AS+AQ: 0/79 (High)	NR	SP: 184 (55-535)* CD: 106 (23-650) SP+AQ: 25 (51-578) AS+AQ: 181 (62-628)
Piola 2010 (6)	AL: 22.3 (range 9-38) Q: 24.7 (range 10-39)	NR (High)	AL: 23% Q: 20%	AL: 1418 (IQR 4727)[§] Q: 1995(IQR 9771)
Sowunmi 1998 (46)	NR	NR (Moderate)	AL: 38.1+/-0.2[ϕ] A+MQ: 38.1 +/-0.1	AL: 29719 (1674-250138)** A+MQ: 28044 (2001-199992)

HIV prevalence low (<1%), moderate (1-5%), high (>5%) from the UNAIDS 2013 report, except for studies along the Thai/Burma border which were estimated using McGready et al. 2015 (47).

^Parasitemia (counts/µL)

*Median parasite count (per 200 WBC)

** Geometric mean density (range)

[§]Geometric mean density (IQR)

[φ]Mean temperature (°C)
Abbreviations: NR not reported, IQR interquartile range, AS-MQ artesunate mefloquine, AL artemether-lumefantrine, DP dihydroartemisinin-piperaquine, AAP artesunate-Atovaquone/Proguanil, AS-SP artesunate sulfadoxine pyrimethamine, AS-AQ artesunate-amodiaquine, CD: chlorproguanal-dapsone, Q quinine, Q+C quinine+clindamycin, SP sulfadoxine pyrimethamine, AQ amodiaquine, SP+CQ sulfadoxine pyrimethamine chloroquine, MQ mefloquine, SP-AZM sulfadoxine pyrimethamine azithromycin, No exp. No exposure, IPT intermittent preventative treatment, RCT randomized controlled trial, ACT artemisinin combination therapy, sd standard deviation

Supplementary Table 2: Sensitivity analyses of pooled odds ratios using different modeling techniques and correction factors

	M-H fixed with 0.5 corr. POR (95%CI)	D-L Random Effects with 0.5 corr. POR (95%CI)	Peto with 0.5 corr. POR (95%CI)	M-H fixed with 0.01 corr. POR (95%CI)	M-H fixed with 0.69 corr. POR (95%CI)
2nd trimester exposures and risk of miscarriage					
ACT vs. No exp.	1.13 (0.77, 1.66) I^2+86.7%, 3 studies	1.59 (0.23, 10.33)	1.13 (0.76-1.66)	1.13 (0.77, 1.66)	1.13 (0.77, 1.66)
2 or 3rd trimester exposures and risk of congenital anomalies					
ACT vs. Quinine	1.00 (0.27, 3.75) I^2=0%, 3 studies	1.04 (0.26, 4.10)	1.00 (0.25, 4.03)	1.00 (0.25, 4.05)	1.00 (0.27, 3.65)
ACT vs. No exp.	0.79 (0.37, 1.67) I^2=0%, 3 studies	0.82 (0.36, 1.85)	0.81 (0.41, 1.61)	0.79 (0.37, 1.67)	0.79 (0.37, 1.67)
ACT vs. SP	1.37 (0.76, 2.39) I^2=70.3%, 2 studies	0.86 (0.18, 4.18)	1.37 (0.76, 2.41)	1.37 (0.78, 2.39)	1.37 (0.78, 2.39)
2 or 3rd trimester exposures and risk of stillbirth					
ACT vs. Quinine	0.49 (0.24, 0.97) I^2=0%, 3 studies	0.48 (0.24, 0.97)	0.48 (0.24, 0.96)	0.49 (0.24, 0.97)	0.49 (0.24, 0.97)
ACT vs. No exp.	1.10 (0.79, 1.54) I^2=0%, 4 studies	1.10 (0.79, 1.54)	1.10 (0.79, 1.54)	1.10 (0.79, 1.54)	1.10 (0.79, 1.54)
ACT vs. SP	0.93 (0.43, 2.01) I^2=56.0%, 2 studies	1.29 (0.23, 7.11)	0.93 (0.43, 2.01)	0.93 (0.43, 2.01)	0.93 (0.43, 2.01)
ACT vs. SP combinations	3.47 (0.46-26.03) I^2=78.1%, 2 studies	1.32 (0.01, 27.05)	8.39 (1.20, 58.79)	155.19 (0.0, 7.66e+07)	2.62 (0.45, 15.02)
2 or 3rd trimester exposures and risk of fetal loss					
ACT vs. Quinine	0.58 (0.31, 1.08) I^2=0%, 6 studies	0.56 (0.30, 1.04)	0.57 (0.31, 1.06)	0.58 (0.32, 1.08)	0.58 (0.32, 1.08)
ACT vs. SP	5.86 (0.73, 46.88) I^2=0% 2 studies	5.69 (0.70, 45.97)	5.91 (1.31, 26.64)	2.60 (0.0, 256e+08)	4.43 (0.73, 26.96)
ACT vs. SP combinations	1.71 (0.57, 5.07) I^2=0%, 3 studies	1.62 (0.53, 4.96)	1.88 (0.67, 5.27)	2.04 (0.65, 6.42)	1.61 (0.55, 4.67)

M-H Mantel Haenszel, D-L DerSimonian-Laird, ACT Artemisinin combination therapy, Corr continuity correction factor, SP sulfadoxine pyrimethamine, No exp. No exposure
*Random effects with DerSimonian-Laird models take into account the heterogeneity in the intervention effects of the studies being pooled. Peto Method uses an observed compared to expected model to create a "Peto OR," but should be used cautiously when there are large differences in sizes of the comparison groups. Continuity correction factors can have a strong effect on studies based on their sample sizes, therefore we conducted sensitivity analyses using different correction factors.

Supplementary Table 3: Newcastle Ottawa Scale Assessing Bias in Cohort Studies

Study	Selection (★★★★)	Comparability (★★)	Outcome (★★★)	Bias
Adam 2004	★★		★★	Moderate
Adam 2006	★★		★★	Moderate
Dean 2001	★★★★	★	★★	Low
Manyando 2010	★★★	★	★★★	Low
McGready 2001	★★★		★★★	Moderate
McGready 1999	★★		★★★	Moderate
Mosha 2014	★★		★	High
Rulisa 2012	★★★★	★	★★★	Low
Poespoprodjo 2014	★★★	★	★★	Moderate
Wang 1989	★★★		★★★	Moderate
Nakelembe 2012	★★★★		★★	Moderate

The maximum number of stars for selection, comparability and outcome is 4, 2, and 3 respectively. Scores of 0-3, 4-6, and 7-9 were rated as high, moderate and low bias respectively.

Supplementary Table 4: Cochrane Bias Assessment of Randomized Controlled Trials

Study	Random Sequence Generation	Allocation Concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Overall threat of bias
Bounyasong, 2001	?	High	High	High	?	High
Kalilani, 2007	Low	Low	High	High	?	Moderate
McGready, 2000	?	Low	High	High	?	Moderate
McGready, 2001	?	Low	High	High	?	High
McGready, 2005	Low	Low	High	High	?	Moderate
McGready, 2008	?	Low	High	High	Low	Moderate
Mutabingwa, 2009	Low	Low	?	High	Low	Low
Piola, 2010	Low	low	Low	?	High	Low
Sowunmi, 1998	?	?	High	High	Low	Moderate

Chapter 2: An assessment of the risk of congenital anomalies following maternal receipt of antimalarial medicines

Abstract

Artemisinin-based combination therapies (ACT) are the most effective antimalarials, but are associated with teratogenic and embryotoxic effects in animal models when administered in early pregnancy. To assess the risk of congenital malformations after receipt of an ACT during pregnancy, we pooled data from five randomized-controlled trials (RCT) and one multi-site observational cohort study from the Malaria in Pregnancy Consortium (MIPc). We included all live born, singleton infants of mothers who participated in the studies. Newborns were assessed for congenital anomalies using a newborn surface examination manual produced by the MiPc. Congenital anomalies were grouped by organ system using a system developed by the Antiretrovirals in Pregnancy Registry (APR). We calculated prevalence risk ratios (PRR) comparing ACT exposed infants to ACT unexposed infants. A total of 16,164 live born singleton infants were included in the analysis. Of these, 5,981 (37.0%) had a confirmed ACT exposure during pregnancy; 162 were exposed to ACTs during the first trimester. Overall, 428 (2.6%) of infants had at least one congenital anomaly, of which 114 (0.7%) infants had an APR-confirmed, not genetically or chromosomally linked, anomaly. There was no increased risk of an APR defined congenital anomaly associated with receipt of an ACT during pregnancy compared to no such use (PRR 0.91, 95%CI 0.52-1.58). The PRR for congenital anomalies among the infants of women who received an ACT in the 1st, 2nd, and 3rd trimester compared to infants with no exposure to an ACT was 1.54 (95% CI 0.55-4.34), 0.79 (95%CI 0.47-1.34) and 1.08 (95%CI 0.57-2.07), respectively. Evidence from this study supports the current WHO guidelines that call

for the use of ACTs in 2nd and 3rd trimester to treat malaria. The findings from the analysis of the first trimester exposures are inconclusive due to the small number of malformations observed. Additional pharmacovigilance is recommended to obtain more reassurance of safety.

Introduction

Given the high morbidity and mortality associated with malaria in pregnancy, safe and effective drugs are needed for treatment and prevention (1). However, there is limited information on the safety profile of most antimalarials when used during pregnancy, in part because few clinical trials enroll pregnant women, and there are few examples of systematic approaches to the conduct of pharmacovigilance during pregnancy, especially in malaria endemic regions of the world (2,3). Artemisinin-based combination therapy (ACT) is the most effective treatment for malaria. The World Health Organization (WHO) currently recommends the use of ACT for the treatment of uncomplicated malaria in adults, children and in pregnant women in the 2nd or 3rd trimester. Seven days of quinine with clindamycin is recommended for uncomplicated malaria in the first trimester of pregnancy. The WHO recommends the use of ACTs in the first trimester only if the life of the woman is in danger, it is the only treatment immediately available, if treatment with 7-day quinine plus clindamycin fails, or uncertainty of compliance with a 7-day treatment (4).

The WHO does not currently recommend ACT as the first line treatment for malaria in the first trimester owing to the lack of sufficient human safety data and because studies of rats, rabbits, and primates have reported the artemisinin class of antimalarial drugs to be associated with embryotoxicity and teratogenic effects (2). These animal studies suggest that the etiologically relevant time period in women would be between 4 and 10 weeks post conception (5). Several organs are still under development in the 2nd trimester and therefore potentially sensitive to teratogens, including the uterus (18 weeks), brain (until birth), eyes (24-36 weeks), and ears (18

weeks) (6), although studies in experimental animals have not observed these types of anomalies following artemisinin exposure (7).

Despite the findings from animal studies, the reports from relatively small observational studies and randomized controlled trials (RCTs) have not identified an increased risk of congenital anomalies after receipt of artemisinins during pregnancy, compared to women receiving non-artemisinin antimalarials or to pregnant women who did not receive any antimalarials for treatment (8–14). Major structural and genetic congenital anomalies are rare outcomes occurring in approximately 3% of live births in the United States (15). By pooling data from RCTs and from a prospective cohort study of pregnant women who received ACTs in which the presence of congenital anomalies at birth was systematically ascertained, we were able to assess the risk of congenital malformations after receipt of an ACT during pregnancy.

Methods

Data Sources

We pooled data from five randomized-controlled trials (RCT) (16–20) and one multi-site cohort study (21) from the Malaria in Pregnancy Consortium (MIPc). MiPc is led by the Liverpool School of Tropical Medicine (LSTM) in the United Kingdom and was funded by the Bill & Melinda Gates Foundation to lead research on better treatment and prevention options for pregnant women at risk for malaria (www.mip-consortium.org). Four of the trials randomly assigned eligible pregnant women in the 2nd trimester to different prevention strategies for malaria. One trial randomly assigned eligible pregnant women with malaria in the 2nd or 3rd trimester to different ACTs for treatment. Per protocol, each trial reported all serious adverse

events (SAE) to a centralized safety database housed at LSTM. By definition, congenital anomalies are defined as an SAE; therefore, all congenital anomalies were recorded using the same reporting mechanism. Upon completion, each trial sent their closed database to the LSTM to be combined with the safety data to build a combined safety database for analyses. The cohort study, the Assessment of the Safety of Artemisinins in Pregnancy (ASAP), enrolled pregnant women of all gestational ages, including enrolling pregnant women in the first trimester. This cohort study identified pregnant women either at antenatal clinic visits, or for one site, in the community (22).

MiPc Studies (Major Activities (MA)) Included in the Analysis:

MA1: Safe and Efficacious Artemisinin-based Combination Treatments for African Pregnant Women with Malaria (PREGACT): This trial is an open-label, non-inferiority, four arm trial comparing the efficacy and safety of four different ACT combinations including dihydroartemisinin-piperaquine (DP), artesunate-mefloquine (AS-MQ), artesunate-amodiaquine (AS-AQ), and artemether-lumefantrine (AL) in four countries: Burkina Faso, Ghana, Malawi, and Zambia. The study enrolled pregnant women with malaria, gestational age 16-37 weeks with no other major illnesses that effect pregnancy (19). ClinicalTrials.gov Identifier: NCT00852423.

MA3: Efficacy of Intermittent Screening and Treatment (IST) or Intermittent Preventative Treatment (IPT) with Dihydroartemisinin-Piperaquine, versus IPT with Sulfadoxine-Pyrimethamine for the Control of Malaria in Pregnancy in Kenya (STOP MiP Kenya): This trial is a three arm prevention study comparing IPT-DP and IST-DP to the standard of care IPT-SP in Western Kenya. The study enrolled HIV negative pregnant women, gestational age 16-32 weeks

inclusive with no prior history of IPT in the current pregnancy (18). ClinicalTrials.gov Identifier: NCT01669941.

MA5: Intermittent Preventive Treatment versus Scheduled Intermittent Screening and Treatment of Malaria in Pregancy (IPT_IST): This is a two arm, open label, non-inferiority trial comparing intermittent screening and treatment (IST) with AL to the standard of care, IPT-SP for the prevention of malaria in pregnancy in four West African countries: Burkina Faso, Gambia, Ghana, and Mali. The study enrolled primagravid or secondigravid, HIV negative pregnant women, gestational age 16-30 weeks (20). ClinicalTrials.gov Identifier: NCT0184213.

MA6: Scheduled Intermittent Screening and Treatment in Pregnancy (ISTp) versus Intermittent Preventive Treatment with Sulphadoxine-Pyrimethamine (IPTp-SP) in Women Protected by Insecticide Treated Nets (ITNs) for the Control of Malaria in Pregnancy in Malawi: a Randomized Controlled Trial. This is a two arm, open label trial to compare IST with DP to the standard of care, IPT-SP, for the prevention of malaria in pregnancy. The study enrolled HIV negative pregnant women gestational age 16-28 weeks inclusive with no history of IPT use during the current pregnancy (16). ISRCTN registry number: ISRCTN69800930.

MA7: Intermittent Preventive Treatment with Azithromycin-Containing Regimens in Pregnant Women in Papua New Guinea (IPTp in PNG). This is single blinded study comparing IPT with SP+azithromycin to IPT with SP+chloroquine for the prevention of malaria in pregnancy. The study enrolled pregnant women gestational age 14-26 weeks with no known chronic illnesses (17). ClinicalTrials.gov Identifier: NCT01136850.

ASAP Cohort Study: The Assessment of Safety of Antimalarial drug use in early Pregnancy (ASAP): This is a three-site, prospective cohort study of early pregnancy exposures to ACT in Kenya, Burkina Faso, and Mozambique. The study conducted active surveillance of exposure to ACT during pregnancy, including the first trimester of pregnancy. Pregnant women were enrolled in the community and during antenatal clinic (ANC) visits and followed through pregnancy (21).

Inclusion Criteria

For this analysis, we included all live born, singleton infants born to mothers who participated in one of the five trials or who were enrolled in the ASAP cohort study. We excluded stillbirths, miscarriages, and multiple gestations due to incomplete assessment for congenital anomalies.

Assessment of Exposure

In the trials, pregnant women were randomly assigned to receive one of four specific ACTs for treatment of malaria, ACTs for prevention, IPT-SP or an IPT-SP combination for prevention. In prevention trials, pregnant women were treated for any malaria infections that occurred during the trial with ACTs due to the failure of prophylaxis. Pregnant women also self-reported to study personnel their antimalarial drug use, including ACTs that occurred before enrollment in the trial but during pregnancy. In the cohort study, antimalarial drug exposures were abstracted from pharmacy dispensing data, clinical registers, and were also self-reported. For the purposes of analysis, we classified randomly assigned ACT exposures (for treatment or prevention) and ACT given for treatment during enrollment in the prevention trials as “confirmed” ACT exposures in

the trials. In the trials, all exposures that were self-reported before enrollment in the trials were classified as "unconfirmed" exposures. Of note, all ACT exposures from MA7, a prevention trial comparing two different SP combinations, were classified as unconfirmed exposures because they were self-reported. For the cohort study, we classified confirmed ACT exposures as any exposure recorded in at least two of three possible sources: pharmacy data, clinical registers, or self report; ACT exposures which were only recorded in one source were classified as unconfirmed in accordance with the approach used in the ASAP study (21). In order to assess the timing of exposure to ACTs, the studies used different methods for accessing gestational age including ultrasound, fundal height, and last menstrual period (LMP) at enrollment and Ballard scores at birth. In one trial, MA3, after post hoc calculation using the corrected gestational age based on the Ballard score at birth, 21 women received an ACT in the first trimester (<14 weeks).

Assessment of Outcome

For all sites, a nurse or other healthcare provider assessed newborns for congenital anomalies guided by a newborn surface examination manual produced by the MiPc. In the trials, all congenital anomalies were reported to the centralized safety database as severe adverse events (SAE). In addition in one site, ASAP-Kenya, a physician reviewed the majority of anomalies reported by nurses during the surface exam either by photo review or in person up to one year after the birth of the infant. For the ASAP cohort study, anomalies that were confirmed by a physician and had photos were further reviewed by the an expert panel established by the WHO pilot Pregnancy Registry Project (23). To ensure consistency across all participating studies for this analysis, all written descriptions of reported congenital anomalies were reviewed and coded

using MedDRA by two members of the study team (SK, CP). A pediatrician (JG) reviewed the final MedDRA codes and description of the anomaly to verify the presence of a congenital anomaly. For the purposes of analysis, we defined a case as an infant with at least one congenital anomaly detected during the surface exam by the nurse, or in the exception of ASAP-Kenya, confirmed by a physician and met the study team's consensus definition (JG, SK, and CP). The study team's definition excluded Mongolian spots and inverted nipples. The definition also excluded cases of molding or other malformations of the sutures, as they were likely due to the birthing process and not a true congenital anomaly. In addition, we excluded two cases of cerebral palsy and one case of Erbs palsy because these were likely due to birth trauma and are not congenital anomalies.

For analytical purposes three definitions of congenital anomalies with increasing specificity were created. First, we created a category of any congenital anomaly that met the study team's consensus definition, which included infants with minor congenital anomalies including skin tags and dimples, supernumerary nipples, and umbilical hernias. For the second more specific definition, we grouped the anomalies by organ system using a classification system developed by the Antiretroviral Pregnancy Registry (APR) (24). This classification system excludes minor congenital anomalies such as umbilical hernias, skin tags and dimples, and supernumerary nipples unless combined with another congenital anomaly. Third, we created a congenital anomaly definition that used the APR classifications but further excluded chromosomal anomalies (e.g., trisomy 21) because they are not caused by exposures during pregnancy and also excluded postaxial polydactyly because it is genetically linked in African populations (25). APR

confirmed, non-genetically or chromosomally linked congenital anomalies served as our primary outcome of interest.

Analytical Technique

We calculated the prevalence of congenital anomalies at birth among women who received ACTs and women had not received one of these drugs, stratified by study site of enrollment. We used a generalized linear model (GLM) with negative binomial family to calculate the prevalence ratio (PR) (with 95% confidence intervals) of congenital anomalies among women with confirmed exposures to ACTs during pregnancy compared to women who did have any record of receiving an ACT during pregnancy. All analyses controlled for study site of enrollment or country of enrollment as appropriate. In order to assess the etiologically relevant time period for exposure to ACTs, analyses were stratified by trimester, controlling for the site of enrollment. In addition, we calculated the PRR for individual ACT combinations compared to no ACT and the risk of congenital anomalies.

Sensitivity analyses

To investigate the robustness of our findings to the differences in detection of congenital anomalies by study site, we conducted sensitivity analyses in which, one at a time, each study site was excluded from the analyses. Additionally, we conducted analyses that applied less stringent criteria to the outcome by including the 23 anomalies not reviewed by physicians in the ASAP-Kenya site because it was determined post-hoc that ACT exposed infants were more likely to not be reviewed by a physician. Finally because anomalies from the ASAP study were

further reviewed by a WHO panel, we conducted an analysis using the WHO definition of congenital anomalies.

Results:

Demographics

We obtained data on a total of 17,929 pregnancies, of which 16,164 were included in the analysis after excluding 1,765 (9.8%) stillbirths, miscarriages, twin pregnancies, and pregnancies not prospectively followed. Detailed demographics (e.g. marital status, education level) were not available in all studies, limiting the number of variables that could be assessed as potential confounders. Of the 16,164 pregnancies, 5,981 (37.0%) had a confirmed ACT exposure at some time during pregnancy, with AL (n=2,790) and DP (n=1,837) being the most commonly used ACTs. An additional 508 mothers self-reported un-confirmed ACT exposures, bringing the total to 6,489 (40.1%) pregnancies with either confirmed and/or unconfirmed ACT exposure in utero. Of the first confirmed ACT exposures, 2.7%, 68.9% and 28.4% occurred first in the first, second and third trimester respectively. The first trimester exposures (n=162) were primarily (n=77, 47.5%) from one site, ASAP-Kenya. Altogether, the three ASAP sites represented 141 (87.0%) of the confirmed first trimester ACT exposures.

Overall, 428 (2.6%) of infants had at least one congenital anomaly as reported by healthcare workers, primarily midwifes. In ASAP-Kenya, a physician reviewed 212 cases and confirmed 145 of the 235 suspected congenital anomaly cases. For the remaining 193 cases reported by other studies, a study team (CP, SK, JG) reviewed the case descriptions and confirmed 190 as having at least one major or minor congenital anomaly meeting the study team's definition,

bringing the total number of confirmed congenital anomalies to 322 across all studies. After applying the APR definition, which excludes minor congenital anomalies such as sacral dimples that occur in isolation, 206 (1.3%) of infants were classified by the study team (SK, JG, CP) to have had congenital anomaly. After removing genetically linked and chromosomal anomalies, there were 114 (0.7%) infants with 'APR defined, non-genetically or chromosomally linked' anomaly. Because of the small numbers of congenital anomalies, analysis by organ system was not possible (Table 2).

The prevalence of APR defined, non-genetically or chromosomally linked congenital anomalies ranged from 1.6% in ASAP-Kenya to 0.1% in MA5-Gambia. The differences in reported prevalence between countries, studies and study sites were statistically significant ($p<0.001$). Consequently, all analyses were adjusted for study-site, or if necessary country of enrollment (Table 3).

ACT exposure during pregnancy:

At some time during pregnancy: The site-adjusted prevalence of congenital anomalies was similar between ACT exposed and unexposed infants regardless of the congenital anomaly definition used: PPR 0.98 (0.71-1.35), 0.75 (0.49-1.16), and 0.87 (0.49-1.55) for any congenital anomaly, APR defined, and APR defined with no genetic or chromosomal congenital anomalies, respectively (Table 4). Exclusion of HIV positive and pregnancies with unknown HIV status did not materially change the results (Table 4b).

Further analysis by type of ACT also showed no association between confirmed AL or DP exposures anytime during pregnancy compared to no ACT exposure and APR defined non-genetically or chromosomally linked congenital anomalies controlling for country of enrollment (PRR 1.13, 95%CI 0.68-1.87, Table 5a; and PRR 0.56, 95%CI 0.23-1.36, Table 5b, respectively).

1st trimester ACT exposures: The risk of APR defined non-genetically or chromosomally linked congenital anomalies among the infants of women who received a confirmed ACT in the first trimester was not significantly higher at p value of 0.05 than non-exposed infants of women who never received an ACT in pregnancy, (PRR 1.54, 95% CI 0.55-4.35) (Table 6). To examine the influence of individual studies on the primary analysis, we excluded each study, one at a time. The point estimates varied to a modest degree with the exclusion of specific studies, fluctuating from PRR 1.35 (95%CI 0.41-4.44) with the exclusion of MA3 to PRR 1.79 (95%CI 0.43-7.50) with the exclusion of ASAP-Kenya (Table 7a).

As a sensitivity analysis, because in ASAP-Kenya the physicians were more likely to not review ACT exposed infants than ACT unexposed infants based on a post-hoc analysis (2/16 (12.5%) vs. 7/119 (5.9%) respectively), we included anomalies not reviewed by the physician but reported by a nurse and met our study team's consensus definition. We also analyzed the data using only the nurses' diagnosis for all sites. After inclusion of these 9 additional infants with congenital anomalies, the risk of congenital anomalies among the infants of women who received a confirmed ACT in the first trimester was higher than among infants of women who never received an ACT in pregnancy, but this result was not statistically significant at p = .05

(PRR of 2.06 (95%CI 0.87-4.87) (Table 7b)). When using the same diagnostic criteria across all studies (review by a healthcare worker at birth only and met our study team's consensus definition), the results were similar (PRR 1.92 (0.93-4.04)) (Table 7c). The WHO panel excluded 24 anomalies that met the APR definition and included 1 congenital anomaly that did not meet the APR definition. Using the WHO definition, the risk of congenital anomalies among the infants of women who received a confirmed ACT in the first trimester was higher than among infants of women who never received an ACT in pregnancy, but this result was not statistically significant at p =.05 (PRR 2.91 (95%CI 0.85-9.99)) (Table 7d). In the analysis restricted to HIV-negative women, the risk of congenital anomalies among the infants of women who received a confirmed ACT in the first trimester was higher than among infants of women who never received an ACT in pregnancy, but this result was not statistically significant at p=.05 (PRR 1.66 (95%CI 0.58-4.72)) (Table 7e).

There were four cases among the 162 exposed infants that met the most specific analytical definition for congenital anomalies, making analysis of anomalies within specific organ systems impossible. This included one case of bilateral clubfoot and three infants with multiple minor congenital anomalies (Table 8). In the sensitivity analyses, when including the 9 nurse's suspected congenital anomalies that were not reviewed by the physician but met the study team's consensus definition, there was one additional musculoskeletal anomaly, a clubfoot, and a suspected bent limb (i.e. limb malformation that was non-specific) among the ACT exposed infants. No congenital heart defects were reported among exposed infants, but this study was unable to a fully access cardiovascular anomalies because most assessments were based on physical exams at birth only.

2nd and 3rd trimester ACT exposures:

There was no increased risk of congenital anomalies among infants first exposed to an ACT in the 2nd trimester compared to infants who were never exposed PRR 0.79 (95%CI 0.47-1.34) (Table 9), and no increased risk associated with exposure to an ACT in the third trimester compared to the infants who were never exposed to an ACT (PRR 1.08, 95%CI 0.57-2.07) (Table 10).

Other medication use:

Data on drug use in pregnancy, including prescription and non-prescription medicines, were provided by MA1, MA5, and the ASAP cohort study. Of the 10,475 women with self-reported drug use data, all but 21 women reported using at least one drug during pregnancy. Pregnant women who reported using antiretrovirals, bronchodilators, cough medicine and antiemetic drugs were more likely to have an infant with any congenital anomaly than women who did not report using these drugs (5.1% vs. 2.0%), (2% vs. 0.4%), (3.9% vs. 0.7%), (5.5% vs. 2.8%) respectively. The use of antibiotics and antifungal medications was similar between women who did and did not have an infant with a congenital anomaly, and women who reported using multivitamins and antiparasitic medications had a lower prevalence of infants with congenital anomalies. No angiotensin converting enzyme inhibitors, warfarin, sedatives, or anti-epileptic drugs were reported by women with an infant with a congenital anomaly (Table 11a). Pregnant women with a confirmed ACT exposure were more likely to report using acetaminophen and multivitamins, including folic acid, than women with no confirmed ACT exposure (39.0% vs. 17.6%), (66.5% vs. 6.2%), respectively and less likely to use ARV (0.6% vs. 2.4%), (Table 11b).

Assessment of ascertainment bias:

To investigate the potential for ascertainment bias due to nurses being aware of pregnant women receiving ACTs when assessing infants for congenital anomalies, we compared the prevalence of the small number of genetically linked anomalies (n=77), which do not result from exposure to a teratogen among infants exposed to an ACT and infants who were not exposed to an ACT in pregnancy. Infants exposed to a confirmed ACT at any time during pregnancy were 36% less likely to have a genetically linked congenital anomaly than infants who were not exposed to an ACT in pregnancy, but this result was not statistically significant (PRR of 0.64, 95%CI 0.31-1.30) (Table 12).

Discussion:

This study represents the largest analysis of the risk of congenital anomalies among infants exposed to ACTs at anytime during pregnancy, including 5,981 confirmed ACT exposures of which 162 were in the first trimester. Our study found no evidence of a statistically significant increased risk of congenital anomalies among infants exposed at some time in pregnancy to ACTs. Upon stratification by trimester of exposure, there was no increased risk for infants exposed in 2nd or 3rd trimester compared to infants who were never exposed to an ACT during pregnancy. These results are in accord with those from multiple randomized clinical trials and observational studies of pregnant women who received ACTs in the 2nd and 3rd trimester in both sub-Saharan Africa and Asia (9,10,26–28). In addition, these results support the findings from a recently completed meta-analysis of the safety of artemisinins in pregnancy (Kovacs unpublished data).

The results from the first trimester-exposed infants are inconclusive. The number of congenital anomalies was quite small, and so even though a modest excess in the point estimate was observed among ACT-exposed infants (PRR 1.54), the result was not statistically significant at the p=0.05 level. The point estimate increased to a PRR of 2.06 (95%CI 0.87-4.87) in a sensitivity analysis to address potential selection bias for the physician review and to a PRR of 2.91 (95%CI 0.85-9.99) using the WHO definition, but chance is a plausible explanation for these results as well. Reassuringly, among the four congenital malformations in the first trimester ACT-exposed infants, there was no specific signal in terms of type of anomaly or location within the body. Our findings mirror the results of a cohort study along the Thai-Myanmar border. In their study, there were two infants with congenital anomalies among 109 ACT exposed infants and 233 infants with congenital anomalies among 18,803 infants whose mothers never had malaria in the first trimester (RR 1.48 95%CI 0.37-5.88) (calculated post hoc using data from Table 2 in Moore et. al) (29).

Animal studies report that artemisinin exposures lead to reductions in embryonic erythroblasts resulting in embryolethality and malformations (30). Studies of artemisinin exposures in rats and monkeys found congenital anomalies of the heart and long bones (5,31). The animal studies led researchers to hypothesize that exposure to ACTs in the first trimester could increase the risk of cardiovascular and skeletal anomalies. Among the 1st-trimester ACT exposed cases in the present study, there was one musculoskeletal anomaly, a case of clubfoot, which is considered to be multifactorial in origin. In the sensitivity analyses, when including the 9 suspected congenital anomalies that were not reviewed by the physician, there was one additional musculoskeletal

anomaly, (a unilateral clubfoot), and a limb malformation that was non-specific in infants with confirmed 1st trimester ACT exposure. Idiopathic clubfoot is multifactorial in origin, with vascular deficiencies, environmental factors, in utero positioning, abnormal muscle insertions, and genetic factors playing a role (32). We observed no congenital heart defects among the exposed infants, but infants were diagnosed only using surface exams, limiting our ability to fully assess the presence of cardiovascular defects.

This study has several limitations. Our findings cannot distinguish between congenital anomalies associated with ACT use and congenital anomalies associated with fever. In large meta-analyses, self-report of fever during pregnancy was associated with an increased risk of congenital anomalies, including neural tube defects, heart defects and oral clefts (33,34). In the included studies, ACTs were given for both prevention and treatment; therefore, not all ACT exposed women necessarily had a fever at the time of exposure. Furthermore, women who were unexposed to an ACT may have had a fever during pregnancy due to another cause. Second, we could not control for other potential confounders such as diseases and medication use during pregnancy that could cause a congenital anomaly. In addition, our sample size was too small to adequately assess the risk of congenital anomalies as a whole after receipt of an ACT in the first trimester, and too small to assess the risk of specific congenital anomalies after ACT exposure in the 2nd and 3rd trimester. Finally, there were insufficient data to compare receipt of ACT in the first trimester to receipt of quinine, which could have controlled for the potential adverse effects of first trimester malaria on pregnancy outcomes.

Despite using the same manual, there were clear differences in ascertainment of congenital anomalies by site that could be due to differences in training or regional differences in the epidemiology of congenital anomalies. Of note, nurses at the ASAP-Kenya site were asked to report any unusual findings from the newborn physical examination that would need to be assessed by the study pediatrician to determine the presence of congenital anomaly. Therefore, the threshold for reporting a possible anomaly in this study was much lower compared to the other studies resulting in 11% of infants having a reported congenital anomaly in ASAP-Kenya compared to 2% in entire cohort. Additionally within the ASAP-Kenya, the nurses reviewing the infants were not blinded to the exposure status of the women, as they collected information on medical history during pregnancy, potentially leading to over diagnosis among ACT exposed cases. Reassuringly, an assessment of this potential bias revealed that nurses generally reported genetically linked anomalies similarly between the ACT exposed and ACT unexposed infants within the study sites (Table 11).

Evidence from this study supports the current WHO guidelines which call for the use of ACTs in 2nd and 3rd trimester to treat malaria (35). The findings from this analysis of first trimester exposures to ACTs can neither rule out an increased risk of congenital anomalies nor provide clear evidence of harm. ACTs are more effective and have a better tolerability profile in pregnant women than quinine, the only antimalarial recommended for treatment of malaria in the first trimester (36). Quinine must be take three times a day for seven days compared to a three day treatment for ACT, potentially resulting in lower adherence (37). Furthermore, recent studies have shown that use of ACTs in the first trimester was not associated with an increased risk of miscarriage compared to quinine (29,22). There are limited data available on first trimester

exposure to ACTs with complete assessment of subsequent congenital anomalies. Additional post-marketing surveillance, including follow-up of first-trimester exposed infants, to obtain more reassurance is recommended.

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Chapter 2 Tables

Table 1a: Descriptive statistics for all live born singleton infants stratified by study of enrollment

	MA1 N=3125	MA3 N=1324	MA5 N=4802	MA6 N=1710	MA7 N=2655	ASAP-K N=1192	ASAP-M N=673	ASAP-B N=683	Total N=16164
Study Type	RCT-treatment	RCT-prevention	RCT-prevention	RCT-prevention	RCT-prevention	Cohort	Cohort	Cohort	
Country(s)	Burkina Faso, Ghana, Malawi, Zambia	Kenya	Burkina Faso, Gambia, Ghana, Mali	Malawi	Papua New Guinea	Kenya	Mozambique	Burkina Faso	
Study Exposures	AS-AQ, DP, AL, AS-MQ	IPT-DP, IPT-SP, IST-DP	IPT-SP, IST-AL	IPT-SP, IST-DP	SP-AZM, SP-CQ	N/A	N/A	N/A	
Maternal Characteristics									
Maternal age [mean, sd]	23.0 (5.8)	23.6 (5.8)	20.4 (3.3)	22.6 (5.1)	24.4 (5.4)	25.8 (6.7)	24.2 (6.2)	27.1 (6.6)	22.9 (5.5)
EGA at enrollment [mean, range]	24.6 (13-37)	22.8 (4.7-35.8)	20.6 (range 12-30)	20.9 (range 13.7-29.9)	21.1 (range 6-28)	17.7 (range 0.3-41.7)	21.3 (range 7-37)	23.9 (range 5.7-39.3)	21.6 (range 0.3-41.7)
Primagravida	893 (28.6)	425 (32.1)	2608 (54.3)	0 (0)	1329 (50.1)	568 (60.0)	165 (33.7)	107 (18.9)	6095 (39.0)
Married		999 (75.5)	4342 (91.0)			924 (78.4)		674 (98.7)	6962 (80.7)
Vag. Delivery	2895 (93.0)			1641 (96.4)	1891 (93.0)	1152 (98.4)		677 (99.1)	8256 (94.9)
Deliver at health facility	2695 (86.5)			1479 (86.9)		834 (70.0)	613 (91.5)	601 (88.0)	6222 (84.5)
Infant Characteristics									
Birth weight [mean, sd]	2877 (447)	N/A	2835 (449)	2930 (431)	2945 (476)	3259 (570)	3091 (426)	2856 (442)	2917 (474)
Male	1591 (51.1)		2422 (50.5)	858 (50.4)		577 (49.3)	312 (47.0)		5760 (50.4)
EGA Delivery [mean, range]	38.6 (23-46)	39.0 (20-46)	37.9 (22-46)	38.2 (27-45)	38.1 (19-46)	39.1 (28-45)	38.9 (17-46)	38.6 (29-45)	38.3 (17-46)
NND	122 (3.9)	22 (1.7)	98 (2.0)	23 (1.4)	29 (1.1)	21 (1.8)	N/A	N/A	315 (2.0)
PNND	N/A	1 (0.1)	12 (0.3)	N/A	N/A	N/A	N/A	N/A	13 (0.08)
Congenital Anomalies									
Reported	48 (1.5)	35 (2.6)	31 (0.6)	17 (1.0)	17 (0.6)	235 (19.7)	11 (1.6)	34 (5.0)	428 (2.6)
Verified	46 (1.5)	34 (2.6)	31 (0.7)	17 (1.0)	17 (0.6)	131 (11.0)	11 (1.6)	34 (5.0)	321 (2.0)
APR	43 (1.4)	26 (2.0)	31 (0.7)	16 (0.9)	17 (0.6)	48 (4.0)	11 (1.6)	14 (2.1)	206 (1.3)
APR no genetic	23 (0.8)	18 (1.4)	17 (0.4)	5 (0.3)	16 (0.6)	20 (1.7)	6 (0.9)	9 (1.3)	114 (0.7)

MA major activity; RCT randomized controlled trial; DP dihydroartemisinin-piperaquine; AS-MQ artesunate-mefloquine; AS-AQ artesunate-amodiaquine; AL artemether-lumefantrine; IPT intermittent preventative treatment; IST intermittent screen and treat; SP Sulfadoxine-Pyrimethamine; AZM azithromycin; CQ chloroquine; EGA estimated gestational age; Vag. Vaginal; sd standard deviation; NND neonatal death; PNND post-neonatal death; APR antiretroviral pregnancy registry;

Table 1b: Prevalence of ACT exposure for live born, singleton infants stratified by study of enrollment

	MA1 N=3125	MA3 N=1324	MA5 N=4802	MA6 N=1710	MA7 N=2655	ASAP-K N=1192	ASAP-M N=673	ASAP-B N=683	Total N=16164
Exposures									
Conf. ACT	**3125 (100)**	**830 (62.7)**	**1262 (26.3)**	**448 (26.2)**	**0 (0)**	**240 (20.1)**	**35 (5.2)**	**41 (6.0)**	**5981 (37.0)**
1st trimester (<14 weeks)	1 (0.03)	21 (2.5)	0 (0)	0 (0)	N/A	77 (32.1)	24 (68.6)	40 (97.6)	162 (2.7)
2nd trimester (14-28)	2163 (69.2)	528 (63.6)	1031 (81.7)	265 (81.5)	N/A	98 (40)	4 (11.4)	1 (2.4)	4090 (68.4)
3rd trimester (>=28)	949 (30.7)	280 (33.7)	226 (17.9)	82 (18.3)	N/A	65 (27.1)	0 (0)	0 (0)	1612 (27.0)
Unknown trimester	2 (0.1)	1 (0.1)	5 (0.4)	1 (0.2)	N/A	N/A	7 (20.0)	0 (0)	16 (0.3)
Conf. + Unconf. ACT	**3125 (100)**	**877 (66.2)**	**1307 (27.2)**	**448 (26.2)**	**87 (3.3)**	**569 (47.7)**	**35 (5.2)**	**41 (6.0)**	**6489 (40.1)**
1st trimester (<14 weeks)	11 (0.4)	76 (8.7)	0 (0)	0 (0)	4 (4.6)	300 (52.7)	24 (68.6)	40 (97.6)	455 (7.0)
2nd trimester (14-28)	2163 (69.2)	546 (62.3)	1041 (79.7)	365 (81.5)	69 (79.3)	173 (30.4)	4 (11.4)	1 (2.4)	4361 (66.9)
3rd trimester (>=28)	949 (30.4)	248 (28.3)	261 (20.0)	82 (18.3)	13 (14.9)	96 (16.9)	0 (0)	0 (0)	1649 (25.5)
Unknown trimester	3 (0.1)	7 (0.8)	5 (0.4)	1 (0.2)	1 (1.2)	N/A	7 (20.0)	0 (0)	24 (0.5)
Specific confirmed antimalarial exposures									
AL	928 (29.7)	324 (24.5)	1262 (26.3)	0 (0)	0 (0)	240 (20.1)	35 (5.2)	1 (0.2)	2790 (17.2)
AS-AQ	789 (25.3)	0 (0)	0 (0)	1 (0)	2 (0)	0 (0)	0 (0)	40 (5.9)	832 (5.1)
DHA-PPQ	763 (24.4)	626 (47.3)	0 (0)	448 (26.2)	0 (0)	0 (0)	0 (0)	0 (0)	1837 (11.4)
AS-MQ	766 (24.5)	0 (0)	1 (0)	2 (0)	3 (0)	0 (0)	5 (0)	6 (0)	782 (4.8)
Quinine Oral	845 (27.0)	25 (1.9)	0 (0)	1 (0)	2 (0)	79 (6.5)	5 (0.7)	162 (23.7)	1116 (6.9)
IPT									
IPT-SP	678 (21.7)	437 (33.0)	2404 (50.1)	837 (49.0)	2642 (99.5)	786 (64.8)	289 (42.9)	464 (67.9)	8537 (52.8)
IPT-SP only	0 (0)	302 (22.8)	2383 (49.6)	836 (48.9)	2642 (99.5)	388 (32.0)	270 (40.1)	373 (54.6)	7194 (44.4)
No IPT or antimalarial	0 (0)	185 (14.0)	1157 (24.1)	426 (24.9)	13 (0.5)	220 (18.2)	349 (51.9)	129 (18.9)	2479 (15.3)

MA major activity; Conf. confirmed; Unconf. unconfirmed; ACT artemisinin-based combination therapy; DP dihydroartemisinin-piperaquine; AS-MQ artesunate-mefloquine; AS-AQ artesunate-amodiaquine; AL artemether-lumefantrine; IPT intermittent preventative treatment; SP Sulfadoxine-Pyrimethamine;

Table 2: Infant's congenital anomalies grouped by APR organ system and stratified by *any* ACT exposure during pregnancy (prevalence per 10,000 live births)

	ACT Exp.	Prev.	No Exp.	Prev.	Total	Prev.
Any Confirmed	**140**	**215.75**	**182**	**188.11**	**322**	**199.21**
APR defined CA	**93**	**143.32**	**113**	**116.80**	**206**	**127.44**
APR defined (not genetically linked)	**50**	**77.05**	**64**	**66.15**	**114**	**70.53**
Chromosomal*	**0**	**0.00**	**1**	**1.03**	**1**	**0.62**
Trisomy 21		0.00	1	1.03	1	0.62
Cleft lip/cleft palate	**2**	**3.08**	**2**	**2.07**	**4**	**2.47**
Cleft lip	1	1.54		0.00	1	0.62
Cleft lip and palate	1	1.54	2	2.07	3	1.86
Central Nervous System	**4**	**6.16**	**3**	**3.10**	**7**	**4.33**
Meningocele	1	1.54		0.00	1	0.62
Spina bifida	3	4.62	2	2.07	5	3.09
Exomphalus		0.00	1	1.03	1	0.62
Cardiovascular	**2**	**3.08**	**2**	**2.07**	**4**	**2.47**
Pulmonary artery atresia		0.00	1	1.03	1	0.62
Atrial septal defect	1	1.54		0.00	1	0.62
Congenital heart disease NOS	1	1.54		0.00	1	0.62
Cardiomegaly		0.00	2	2.07	2	1.24
Face/Neck	5	**7.71**	12	**12.40**	**17**	**10.52**
Anomaly of external ear		0.00	2	2.07	2	1.24
Absence of ear		0.00	1	1.03	1	0.62
Congenital nose malformation		0.00	1	1.03	1	0.62
Cataract congenital		0.00	1	1.03	1	0.62
Choanal stenosis	1	1.54		0.00	1	0.62
Congenital skull malformation NOS	1	1.54		0.00	1	0.62
Cystic hygroma		0.00	1	1.03	1	0.62
Micrognathia	1	1.54		0.00	1	0.62
Microphthalmos		0.00	1	1.03	1	0.62
Congenital Ptosis	1	1.54		0.00	1	0.62
Unspecified anomaly of the eye		0.00	3	3.10	3	1.86
Other unspecified anomaly of the hard palate		0.00	1	1.03	1	0.62
Unspecified anomaly of the face		0.00	1	1.03	1	0.62
Strabismus congenital	1	1.54		0.00	1	0.62
Genital-Female	**0**		**3**		**3**	
Incomplete vagina			1		1	
Genital malformation NOS			2		2	
Genital-Male	2		8		**10**	
Undescended testis			5		5	
Hypospadias			1		1	
Phimosis			2		2	

Genital malformation NOS	2				2	
Gastrointestinal malformation	1	**1.54**	1	**1.03**	**2**	**1.24**
Gastrointestinal malformation		0.00	1	1.03	1	0.62
Biliary atresia	1	1.54		0.00	1	0.62
Limb Reduction/Addition	**49**	**75.51**	**48**	**49.61**	**97**	**60.01**
Excluding Genetic Polydactyly	**13**	**20.03**	**7**	**7.24**	**20**	**12.37**
Achondroplasia	1	1.54		0.00	1	0.62
Brachydactyly	1	1.54		0.00	1	0.62
Limb malformation	2	3.08		0.00	2	1.24
Limb reduction	1	1.54	1	1.03	2	1.24
Oligodactyly	1	1.54		0.00	1	0.62
Polydactyly NOS*	5	7.71	5	5.17	10	6.19
Polydactyly of fingers*	35	53.94	39	40.31	74	45.78
Polydactyly of toes	2	3.08	3	3.10	5	3.09
Malformation of lower limb	1	1.54		0.00	1	0.62
Musculoskeletal	**7**	**10.79**	**17**	**17.57**	**24**	**14.85**
Club foot	4	6.16	10	10.34	14	8.66
Congenital hand malformation		0.00	3	3.10	3	1.86
Gastroschisis		0.00	1	1.03	1	0.62
Pectus carinatum		0.00	1	1.03	1	0.62
Syndactyly	2	3.08		0.00	2	1.24
Toes webbed	1	1.54	1	1.03	2	1.24
Unspecified deformity of forearm		0.00	1	1.03	1	0.62
Other	**0**	**0.00**	**1**	**1.03**	**1**	**0.62**
Congenital anomaly NOS		0.00	1	1.03	1	0.62
Renal	**0**	**0.00**	**1**	**1.03**	**1**	**0.62**
Congenital ureteric anomaly		0.00	1	1.03	1	0.62
Respiratory	**3**	**4.62**	**0**	**0.00**	**3**	**1.86**
Congenital tracheomalacia	2	3.08		0.00	2	1.24
Laryngomalacia	1	1.54		0.00	1	0.62
Skin	**0**	**0.00**	**1**	**1.03**	**1**	**0.62**
Congenital skin disorder		0.00	1	1.03	1	0.62
Syndrome	**0**	**0.00**	**1**	**1.03**	**1**	**0.62**
Prune belly syndrome		0.00	1	1.03	1	0.62
Multiple Major	**6**	**9.25**	**2**	**2.07**	**8**	**4.95**
Multiple Minor	**5**	**7.71**	**3**	**3.10**	**8**	**4.95**
Minor CA *	**54**	**83.22**	**75**	**77.52**	**129**	**79.81**
Chin depression	1	1.54		0.00	1	0.62
Clitoromegaly		0.00	1	1.03	1	0.62
Congenital umbilical hernia	24	36.99	34	35.14	58	35.88
Nipple supernumerary	0	0.00	2	2.07	2	1.24
Preauricular cyst	16	24.66	21	21.71	37	22.89
Preauricular skin tag	5	7.71	3	3.10	8	4.95

Sacral dimple congenital	5	7.71	13	13.44	18	11.14
Skin tag	3	4.62		0.00	3	1.86
Clinodactyly		0.00	1	1.03	1	0.62

All prevalence estimates are unadjusted per 10,000 live births. Infant sex was not provided by all participating trials, therefore the true denominator for gender specific congenital anomalies is not known, and prevalence estimates were not made.

*Denotes congenital anomalies that do not meet the definition of APR defined not genetically or chromosomally linked congenital anomalies and were excluded from the primary analyses.

Table 3: Bivariate analysis for APR defined congenital anomalies not determined to be genetically or chromosomally linked

	APR non genetically linked N (%)	No congenital anomaly N (%)	X^2 p value
Age (yrs.)			0.006
20-35	69 (0.7)	9890 (99.3)	
<20	32 (0.6)	5357 (99.4)	
>=35	13 (1.6)	799 (98.4)	
Study			<0.001
MA1	23 (0.7)	3102 (99.3)	
MA3	18 (1.4)	1306 (98.6)	
MA5	17 (0.4)	4785 (99.6)	
MA6	5 (0.3)	1705 (99.7)	
MA7	16 (0.6)	2639 (99.4)	
ASAP-Kenya	20 (1.7)	1172 (98.3)	
ASAP-Burkina Faso	9 (1.3)	674 (98.7)	
ASAP-Mozambique	6 (0.9)	667 (99.1)	
Country			<0.001
Burkina Faso	29 (1.0)	2811 (99.0)	
Gambia	1 (0.1)	1050 (99.9)	
Ghana	6 (0.3)	1917 (99.7)	
Kenya	38 (1.5)	2478 (98.5)	
Malawi	8 (0.3)	2501 (99.7)	
Mali	2 (0.2)	1200 (99.8)	
Mozambique	6 (0.9)	667 (99.1)	
PNG	16 (0.6)	2639 (99.4)	
Zambia	8 (1.0)	787 (99.0)	
Study Site			<0.001
MA1 Burkina Faso	10 (1.2)	814 (98.8)	
MA1 Ghana	2 (0.3)	705 (99.7)	
MA1 Malawi	3 (0.4)	796 (99.6)	
MA1 Zambia	8 (1.0)	787 (99.0)	
MA3 Kenya	18 (1.4)	1306 (98.6)	
MA5 Burkina Faso	10 (0.8)	1323 (99.3)	
MA5 Gambia	1 (0.1)	1050 (99.9)	
MA5 Ghana	4 (0.3)	1212 (99.7)	
MA5 Mali	2 (0.2)	1200 (99.8)	
MA6 Malawi	5 (0.3)	1705 (99.7)	
MA7 Papau New Guinea	16 (0.6)	2639 (99.4)	
ASAP Burkina Faso	9 (1.3)	674 (98.7)	

ASAP Kenya	20 (1.7)	1172 (98.3)	
ASAP Mozambique	6 (0.9)	667 (99.1)	
Gravidity			0.773
Primagravida	33 (0.5)	6062 (99.5)	
Multigravida	77 (0.8)	9447 (99.2)	
HIV*			0.826
Positive	4 (1.00)	392 (99.0)	
Negative	18 (1.4)	1273 (98.6)	
Unknown	2 (1.8)	111 (98.3)	

*HIV positive women were only enrolled in the ASAP-Kenya and ASAP-Mozambique sites

Table 4a: Prevalence of congenital anomalies per 100 women among infants with confirmed ACT exposure at anytime during pregnancy compared to no exposure to ACT (confirmed or unconfirmed) stratified by study site

	Verified		APR		APR not genetically linked	
Study Site	**ACT exp. N (%)**	**Non-exp. N (%)**	**ACT exp. N (%)**	**Non-exp. N (%)**	**ACT exp. N (%)**	**Non-exp. N (%)**
MA1 Burkina Faso	19 (2.3)	N/A	19 (2.3)	N/A	10 (1.2)	N/A
MA1 Ghana	7 (1.0)	N/A	6 (0.9)	N/A	2 (0.3)	N/A
MA1 Malawi	5 (0.6)	N/A	5 (0.6)	N/A	3 (0.4)	N/A
MA1 Zambia	15 (1.9)	N/A	13 (1.6)	N/A	8 (1.0)	N/A
MA3 Kenya	19 (2.3)	14 (3.1)	13 (1.6)	12 (2.7)	9 (1.1)	8 (1.8)
MA5 Burkina Faso	6 (1.3)	8 (0.9)	6 (1.3)	8 (0.9)	6 (1.3)	4 (0.5)
MA5 Gambia	0 (0)	2 (0.2)	0 (0)	2 (0.2)	0 (0)	1 (0.1)
MA5 Ghana	2 (0.4)	3 (0.4)	2 (0.4)	3 (0.4)	2 (0.4)	2 (0.3)
MA5 Mali	1 (0.4)	9 (1.0)	1 (0.4)	9 (1.0)	0 (0)	2 (0.2)
MA6 Malawi	4 (0.9)	13 (1.0)	3 (0.7)	13 (1.0)	0 (0)	5 (0.4)
MA7 Papua New Guinea	N/A	17 (0.7)	N/A	17 (0.7)	N/A	16 (0.6)
ASAP Burkina Faso	2 (4.9)	32 (5.0)	1 (2.4)	13 (2.0)	1 (2.4)	8 (1.3)
ASAP Kenya	29 (12.1)	74 (11.9)	7 (2.9)	26 (4.2)	4 (1.7)	12 (1.9)
ASAP Mozambique	1 (2.9)	10 (1.6)	1 (2.9)	10 (1.6)	0 (0)	6 (0.9)
Site Adj. Prev. per 100 live births	3.23 (1.92-4.56)	3.03 (2.58-3.49)	1.58 (0.51-2.65)	1.58 (0.13-1.90)	0.79 (0.14-1.44)	0.85 (0.61-1.08)
Site Adj. PRR	**0.95 (0.70-1.28)**	**Referent**	**0.76 (0.50-1.16)**	**Referent**	**0.91 (0.52-1.58)**	**Referent**

Table 4b: Prevalence of congenital anomalies among confirmed ACT exposed at any time in pregnancy compared to no exposure (confirmed or unconfirmed) stratified by study site, all trimesters HIV negative women only

	Verified		APR		APR not genetically linked	
Study Site	**ACT exp. N (%)**	**Non-exp. N (%)**	**ACT exp. N (%)**	**Non-exp. N (%)**	**ACT exp. N (%)**	**Non-exp. N (%)**
MA1 Burkina Faso	19 (2.3)	N/A	19 (2.3)	N/A	10 (1.2)	N/A
MA1 Ghana	7 (1.0)	N/A	6 (0.9)	N/A	2 (0.3)	N/A
MA1 Malawi	5 (0.6)	N/A	5 (0.6)	N/A	3 (0.4)	N/A
MA1 Zambia	15 (1.9)	N/A	13 (1.6)	N/A	8 (1.0)	N/A
MA3 Kenya	19 (2.3)	14 (3.1)	13 (1.6)	12 (2.7)	9 (1.1)	8 (1.8)
MA5 Burkina Faso	6 (1.3)	8 (0.9)	6 (1.3)	8 (0.9)	6 (1.3)	4 (0.5)
MA5 Gambia	0 (0)	2 (0.2)	0 (0)	2 (0.2)	0 (0)	1 (0.1)
MA5 Ghana	2 (0.4)	3 (0.4)	2 (0.4)	3 (0.4)	2 (0.4)	2 (0.3)
MA5 Mali	1 (0.4)	9 (1.0)	1 (0.4)	9 (1.0)	0 (0)	2 (0.2)
MA6 Malawi	4 (0.9)	13 (1.0)	3 (0.7)	13 (1.0)	0 (0)	5 (0.4)
MA7 Papua New Guinea	N/A	17 (0.7)	N/A	17 (0.7)	N/A	16 (0.6)
ASAP Burkina Faso	2 (4.9)	32 (5.0)	1 (2.4)	13 (2.1)	1 (2.4)	8 (1.3)
ASAP Kenya	26 (13.0)	53 (11.9)	6 (3.0)	21 (4.7)	3 (1.5)	10 (2.2)
ASAP Mozambique	1 (4.2)	8 (1.8)	1 (4.2)	8 (1.8)	0 (0)	4 (0.9)
Site Adj. Prev. per 100 live births	3.65 (1.98-5.31)	3.08 (2.56-3.60)	1.83 (0.41-3.25)	1.69 (0.13-2.07)	0.81 (0.08-1.54)	0.89 (0.61-1.16)
Site Adj. PRR	**0.98 (0.71-1.35)**	**Referent**	**0.75 (0.49-1.16)**	**Referent**	**0.87 (0.49-1.55)**	**Referent**

Table 5a: Association between confirmed exposure to AL at anytime during pregnancy compared to no confirmed ACT exposure and congenital anomalies, all trimesters

	Verified		APR Defined		APR not genetically linked	
	AL N (%)	**No ACT N (%)**	**AL N (%)**	**No ACT N (%)**	**AL N (%)**	**No ACT N (%)**
Burkina Faso	15 (2.0)	40 (2.7)	15 (2.0)	21 (1.4)	11 (1.5)	12 (0.8)
Gambia	0 (0)	2 (0.2)	0 (0)	2 (0.2)	0 (0)	1 (0.1)
Ghana	2 (0.4)	3 (0.4)	2 (0.4)	3 (0.4)	2 (0.4)	2 (0.3)
Kenya	34 (6.0)	88 (8.2)	12 (2.1)	38 (3.6)	8 (1.4)	20 (1.9)
Malawi	4 (1.1)	13 (1.0)	4 (1.1)	13 (1.0)	2 (0.5)	5 (0.4)
Mali	1 (0.4)	9 (1.0)	1 (0.4)	9 (1.0)	0 (0)	2 (0.2)
Mozambique	1 (2.9)	10 (1.6)	1 (2.9)	10 (1.6)	0 (0)	6 (0.9)
PNG	N/A	17 (0.6)	N/A	17 (0.6)	N/A	16 (0.6)
Zambia	7 (2.6)	N/A	6 (2.2)	N/A	3 (1.1)	N/A
Adj. Prev	2.11 (0.94-3.29)	1.79 (1.48-2.09)	1.60 (0.45-2.75)	1.23 (0.96-1.50)	0.56 (0.23-0.91)	0.67 (0.47-0.87)
Adj. PRR	**0.77 (0.57-1.04)**	**Referent**	**0.89 (0.61-1.32)**	**Referent**	**1.13 (0.68-1.86)**	**Referent**

*Adjusted for country of enrollment to allow MA1 data to be included

Table 5b: Association between confirmed exposure to DP at anytime in pregnancy and congenital anomalies compared to no confirmed ACT exposure

	Verified		APR Defined		APR not genetically linked	
	DP N (%)	**No ACT N (%)**	**DP N (%)**	**No ACT N (%)**	**DP N (%)**	**No ACT N (%)**
Ghana	0 (0)	N/A	0 (0)	N/A	0 (0)	N/A
Kenya	16 (2.6)	14 (3.1)	10 (1.6)	12 (2.7)	7 (1.1)	8 (1.8)
Malawi	5 (0.7)	13 (1.0)	4 (0.6)	13 (1.0)	1 (0.1)	5 (0.4)
Zambia	4 (1.6)	N/A	3 (1.2)	N/A	2 (0.8)	N/A
Adj. Prev	1.33 (0.61-2.05)	1.96 (1.18-2.75)	0.95 (0.33-1.57)	1.77 (1.03-2.50)	0.59 (0.09-1.08)	0.78 (0.57-0.99)
Adj. PRR	0.77 (0.43-1.37)	Referent	0.58 (0.30-1.13)	Referent	0.56 (0.23-1.36)	Referent

*Adjusted for country of enrollment, only MA1, MA3, and MA6 reported confirmed DP exposures and were included in the analysis.

Table 6: Association between confirmed first trimester exposures to an ACT and the risk of congenital anomalies compared to no ACT exposure (confirmed or unconfirmed) during pregnancy

	Verified		APR Defined		APR not genetically linked	
	1st trim ACT N (%)	No ACT N (%)	1st trim ACT N (%)	No ACT N (%)	1st trim ACT N (%)	No ACT N (%)
MA3	1 (4.8)	14 (3.1)	1 (4.8)	12 (2.7)	1 (4.8)	8 (1.8)
ASAP-Kenya	10 (13.0)	74 (11.9)	3 (3.9)	26 (4.2)	2 (2.6)	12 (1.9)
ASAP-Burkina Faso	2 (5.0)	32 (5.0)	1 (2.5)	13 (2.0)	1 (2.5)	8 (1.3)
ASAP-Mozambique	1 (4.2)	10 (1.6)	1 (4.2)	10 (1.6)	0 (0)	6 (0.94)
Adj. Prev	7.07 (3.04-11.09)	5.72 (4.77-6.67)	3.70 (.36-7.04)	2.59 (1.94-3.24)	2.00 (0.00-4.00)	1.41 (0.93-1.89)
Adj. PRR	**1.14 (0.67-1.93)**	**Referent**	**1.22 (0.53-2.79)**	**Referent**	**1.54 (0.55-4.34)**	**Referent**

*Adjusted for study-site of enrollment

Table 7a: Sensitivity analysis of confirmed first trimester exposures to ACT and congenital anomalies compared to no ACT exposure

	Verified PRR (95%CI)	APR defined PRR (95%CI)	APR not genetically linked PRR (95%CI)
No MA3	1.12 (0.65-1.93)	1.14 (0.46-2.84)	1.35 (0.41-4.44)
No ASAP-Burkina Faso	1.16 (0.66-2.06)	1.21 (0.49-3.01)	1.43 (0.43-4.73)
No ASAP- Kenya	1.31 (1.49-3.52)	1.68 (0.53-5.41)	1.79 (0.43-7.50)
No ASAP-Mozambique	1.09 (0.63-1.89)	1.09 (0.44-2.71)	1.70 (0.60-4.82)

*All PRR were adjusted for study site of enrollment

Table 7b: Sensitivity analysis of association between confirmed first trimester exposures to an ACT and the risk of congenital anomalies compared to no ACT exposure (confirmed or unconfirmed) during pregnancy including the physician not reviewed cases from ASAP-Kenya

	Verified		APR Defined		APR not genetically linked	
	1st trim ACT N (%)	No ACT N (%)	1st trim ACT N (%)	No ACT N (%)	1st trim ACT N (%)	No ACT N (%)
MA3	1 (4.8)	14 (3.1)	1 (4.8)	12 (2.7)	1 (4.8)	8 (1.8)
ASAP-Kenya	12 (15.6)	84 (13.5)	5 (6.5)	28 (4.5)	4 (5.2)	14 (2.3)
ASAP-Burkina Faso	2 (5.0)	32 (5.0)	1 (2.5)	13 (2.0)	1 (2.5)	8 (1.3)
ASAP-Mozambique	1 (4.2)	10 (1.6)	1 (4.2)	10 (1.6)	0 (0)	6 (0.94)
Adj. Prev	7.84 (3.71-11.96)	6.20 (5.21-7.18)	4.47 (.98-7.96)	2.68(2.02-3.35)	2.76 (0.51-5.01)	1.51 (1.00-2.01)
Adj. PRR	**1.17 (0.72-1.91)**	**Referent**	**1.52 (0.73-3.13)**	**Referent**	**2.06 (0.87-4.87)**	**Referent**

*Adjusted for study site of enrollment

Table 7c: Sensitivity analysis of association between confirmed first trimester exposures to an ACT and the risk of congenital anomalies compared to no ACT exposure (confirmed or unconfirmed) during pregnancy using the nurses diagnosis for all sites

	Verified		APR Defined		APR not genetically linked	
	1st trim ACT N (%)	**No ACT N (%)**	**1st trim ACT N (%)**	**No ACT N (%)**	**1st trim ACT N (%)**	**No ACT N (%)**
MA3	1 (4.8)	14 (3.1)	1 (4.8)	12 (2.7)	1 (4.8)	8 (1.8)
ASAP-Kenya	14 (18.2)	94 (15.1)	7 (9.1)	29 (4.7)	6 (7.8)	23 (3.7)
ASAP-Burkina Faso	2 (5.0)	32 (5.0)	1 (2.5)	14 (2.2)	1 (1.3)	13 (2.0)
ASAP-Mozambique	1 (4.2)	10 (1.6)	1 (4.2)	10 (1.6)	0 (0)	5 (0.94)
Adj. Prev	8.60 (4.38-12.82)	6.67 (5.66-7.69)	5.23 (1.61-8.86)	2.78 (2.10-3.45)	3.53 (1.06-5.99)	1.98 (1.40-2.55)
Adj. PRR	**1.20 (0.76-1.90)**	**Referent**	**1.79 (0.93-3.45)**	**Referent**	**1.92 (0.93-4.04)**	**Referent**

*Adjusted for study site of enrollment

Table 7d: Sensitivity analysis of association between confirmed first trimester exposures to an ACT and the risk of congenital anomalies compared to no ACT exposure (confirmed or unconfirmed) during pregnancy using the WHO definition of congenital anomalies for the ASAP study sites

	Verified Congenital Anomaly		WHO Defined		WHO defined not genetically linked	
	1st trim ACT N (%)	**No ACT N (%)**	**1st trim ACT N (%)**	**No ACT N (%)**	**1st trim ACT N (%)**	**No ACT N (%)**
MA3	1 (4.8)	14 (3.1)	1 (4.8)	12 (2.7)	1 (4.8)	8 (1.8)
ASAP-Kenya	10 (13.0)	74 (11.9)	2 (2.6)	17 (2.3)	1 (1.30	3 (0.5)
ASAP-Burkina Faso	2 (5.0)	32 (5.0)	1 (2.5)	8 (1.3)	1 (2.5)	3 (0.5)
ASAP-Mozambique	1 (4.2)	10 (1.6)	1 (4.2)	6 (0.9)	0 (0)	2 (0.3)
Adj. Prev.	7.07 (3.04-11.09)	5.72 (4.77-6.67)	3.32 (0.06-6.57)	1.75 (1.2-2.28)	1.61 (0-3.46)	0.57 (0.28-0.86)
Adj. PRR	**1.14 (0.67-1.93)**	**Referent**	**1.49 (0.59-3.75)**	**Referent**	**2.91 (0.85-9.99)**	**Referent**

*Adjusted for study site of enrollment

Table 7e: Sensitivity analysis of association between confirmed first trimester exposures to an ACT and the risk of congenital anomalies compared to no ACT exposure (confirmed or unconfirmed) during pregnancy, HIV negative patients only

	Verified		APR Defined		APR not genetically linked	
	1st trim ACT N (%)	**No ACT N (%)**	**1st trim ACT N (%)**	**No ACT N (%)**	**1st trim ACT N (%)**	**No ACT N (%)**
MA3	1 (4.8)	14 (3.1)	1 (4.8)	12 (2.7)	1 (4.8)	8 (1.8)
ASAP-Kenya	10 (15.4)	53 (11.9)	3 (4.6)	21 (4.7)	2 (3.1)	10 (2.2)
ASAP-Burkina Faso	2 (5.7)	32 (5.1)	1 (2.5)	13 (2.1)	1 (2.9)	9 (1.3)
ASAP-Mozambique	1 (6.7)	8 (1.8)	1 (6.7)	8 (1.8)	0 (0)	4 (0.9)
Adj. Prev	8.77 (3.38-14.16)	5.82 (4.73-6.91)	4.81 (.82-9.53)	2.83 (2.05-3.60)	2.23 (0.00-4.47)	1.50 (0.93-2.06)
Adj. PRR	**1.31 (0.77-2.23)**	**Referent**	**1.31 (0.57-3.03)**	**Referent**	**1.66 (0.58-4.72)**	**Referent**

*Adjusted for study site of enrollment

Table 8: Specific verified congenital anomalies stratified by confirmed first trimester ACT exposure and no ACT exposure (confirmed or unconfirmed) in pregnancy (prevalence per 10,000 live births).

	ACT exp.	Prev.	No exp.	Prev.	Total	Prev.
Any Confirmed	**14**	**864.20**	**130**	**553.19**	**144**	**573.25**
APR defined CA	**6**	**370.37**	**61**	**259.57**	**67**	**266.72**
APR defined (not genetically linked)	**4**	**246.91**	**34**	**144.68**	**38**	**151.27**
Cleft lip/cleft palate	**0**	**0.00**	**1**	**4.26**	**1**	**3.98**
Cleft lip and palate	0	0.00	1	4.26	1	3.98
Cardio Vascular	**0**	**0.00**	**2**	**8.51**	**2**	**7.96**
Cardiomegaly		0.00	2	8.51	2	7.96
Face/Neck	**0**	**0.00**	**9**	**38.30**	**9**	**35.83**
Anomaly of external ear		0.00	2	8.51	2	7.96
Cataract congenital		0.00	1	4.26	1	3.98
Microphthalmos		0.00	1	4.26	1	3.98
Congenital skin disorder		0.00	1	4.26	1	3.98
Unspecified anomaly of eye		0.00	3	12.77	3	11.94
Other unspecified anomaly of the palate		0.00	1	4.26	1	3.98
Genital-Female	**0**		**3**		**3**	
Incomplete vagina			1		1	
Genital malformation NOS			2		2	
Genital-Male	**0**		**7**		**7**	
Undescended testis			5		5	
Phimosis			2		2	
Lower GI	**0**	**0.00**	**1**	**4.26**	**1**	**3.98**
Gastrointestinal malformation		0.00	1	4.26	1	3.98
Limb Reduction/Addition	**2**	**123.46**	**22**	**93.62**	**24**	**95.54**
Excluding Genetic Polydactyly	0	0.00	0	0.00	0	0.00
Polydactyly NOS		0.00	5	21.28	5	19.90
Polydactyly of fingers	2	123.46	17	72.34	19	75.64
Muscular-Skeletal	**1**	**61.73**	**4**	**17.02**	**5**	**19.90**
Club foot	1	61.73	3	12.77	4	15.92
Unspecified deformity of forearm		0.00	1	4.26	1	3.98
Other	**0**	**0.00**	**1**	**4.26**	**1**	**3.98**
Congenital anomaly NOS		0.00	1	4.26	1	3.98
Renal	**0**	**0.00**	**1**	**4.26**	**1**	**3.98**
Congenital ureteric anomaly		0.00	1	4.26	1	3.98
Skin	**0**	**0.00**	**1**	**4.26**	**1**	**3.98**
Congenital skin disorder		0.00	1	4.26	1	3.98
Multiple Major	**0**	**0.00**	**1**	**4.26**	**1**	**3.98**
Multiple Minor	**3**	**185.19**	**2**	**8.51**	**5**	**19.90**
Minor CA	**8**	**493.83**	**75**	**319.15**	**83**	**330.41**

Cliteromegaly	0	0.00	1	4.26	1	3.98
Clinodactyly	0	0.00	1	4.26	1	3.98
Congenital umbilical hernia	2	123.46	34	144.68	36	143.31
Nipple supernumerary	0	0.00	2	8.51	2	7.96
Preauricular cyst	3	185.19	21	89.36	24	95.54
Preauricular skin tag	2	123.46	3	12.77	5	19.90
Sacral dimple congenital	0	0.00	13	55.32	13	51.75
Skin tag	1	61.73	0	0.00	1	3.98

*Note seven infants with congenital anomalies were reported by nurses by were not reviewed by physicians from ASAP-Kenya, and therefore excluded from the table. This included the following unconfirmed cases: one additional clubfoot and one limb malformation NOS among infants exposed to an ACT in the first trimester, and one infant with clubfoot, one infant with undescended testis, and three infants with minor congenital anomalies among infants with no ACT exposure during pregnancy.

Table 9: Association between confirmed second trimester exposures an ACT and the risk of congenital anomalies compared to no ACT exposure during pregnancy

	Verified Congenital Anomaly		**APR Defined**		**APR not genetically linked**	
	2nd trim ACT N (%)	**No ACT N (%)**	**2nd trim ACT N (%)**	**No ACT N (%)**	**2nd trim ACT N (%)**	**No ACT N (%)**
Burkina Faso	18 (1.9)	40 (2.7)	18 (1.9)	21 (1.4)	12 (1.2)	12 (0.8)
Gambia	0 (0)	2 (0.2)	0 (0)	2 (0.2)	0 (0)	1 (0.1)
Ghana	3 (0.4)	3 (0.4)	3 (0.4)	3 (0.4)	2 (0.3)	2 (0.3)
Kenya	24 (3.8)	88 (8.2)	9 (1.4)	38 (3.6)	4 (0.6)	20 (1.9)
Malawi	9 (0.9)	13 (1.0)	8 (0.8)	13 (1.0)	3 (0.3)	5 (0.4)
Mali	1 (0.5)	9 (1.0)	1 (0.5)	9 (1.0)	0 (0)	2 (0.2)
Mozambique	0 (0)	10 (1.6)	0 (0)	10 (1.6)	0 (0)	6 (0.9)
PNG	N/A	17 (0.6)	N/A	17 (0.6)	N/A	16 (0.6)
Zambia	6 (1.2)	N/A	5 (1.0)	N/A	4 (0.8)	N/A
Adj. Prev	0.99 (0.65-1.32)	1.79 (1.48-2.09)	0.66 (0.37-0.96)	1.22 (0.96-1.50)	0.36 (0.15-0.57)	0.67 (0.47-0.87)
Adj. PRR	**0.59 (0.44-0.81)**	Referent	**0.74 (0.51-1.09)**	**Referent**	**0.79 (0.47-1.34)**	**Referent**

*Adjusts for country of enrollment, not site of enrollment in order for MA1 to be informative

Table 10: Association between confirmed third trimester exposures an ACT and the risk of congenital anomalies compared to no ACT exposure during pregnancy

	Verified		APR Defined		APR not genetically linked	
	3rd trim ACT N (%)	**No ACT N (%)**	**3rd trim ACT N (%)**	**No ACT N (%)**	**3rd trim ACT N (%)**	**No ACT N (%)**
Burkina Faso	7 (2.2)	40 (2.7)	7 (2.2)	21 (1.4)	4 (1.2)	12 (0.8)
Gambia	0 (0)	2 (0.2)	0 (0)	2 (0.2)	0 (0)	1 (0.1)
Ghana	6 (1.6)	3 (0.4)	5 (1.3)	3 (0.4)	2 (0.5)	2 (0.3)
Kenya	13 (3.8)	88 (8.2)	7 (2.0)	38 (3.6)	6 (1.7)	20 (1.9)
Malawi	0 (0)	13 (1.0)	0 (0)	13 (1.0)	0 (0)	5 (0.4)
Mali	0 (0)	9 (1.0)	0 (0)	9 (1.0)	0 (0)	2 (0.2)
Mozambique	N/A	10 (1.6)	N/A	10 (1.6)	N/A	6 (0.9)
PNG	N/A	17 (0.6)	N/A	17 (0.6)	N/A	16 (0.6)
Zambia	9 (3.0)	N/A	8 (2.6)	N/A	4 (1.3)	N/A
Adj. Prev.	1.62 (0.98-2.27)	1.79 (1.48-2.09)	1.26 (0.68-1.85)	1.23 (0.96-1.50)	0.72 (0.28-1.15)	0.67 (0.47-0.87)
Adj. PRR	**0.64 (0.42-0.96)**	**Referent**	**0.90 (0.55-1.48)**	**Referent**	**1.08 (0.57-2.07)**	**Referent**

*Adjusts for country of enrollment, not site of enrollment in order for MA1 to be informative

Table 11a: Other drugs taken during pregnancy based on self-report by the pregnant mother

	Verified Congenital Anomaly N (%)	No Verified Congenital Anomaly N (%)
Any Non-antimalarial	252 (99.2)	10202 (99.8)
Antibiotics	54 (21.3)	1960 (19.2)
ARVs	13 (5.1)	201 (2.0)
Acetaminophen	34 (13.9)	2786 (27.3)
Multivitamin/Iron/Folic acid	50 (19.7)	3453 (33.78)
Antihistamine	16 (6.3)	271 (2.7)
Antiparasitic	10 (3.9)	851 (8.3)
Antifungal	12 (4.7)	500 (4.9)
Antihypertensive	0 (0)	38 (0.4)
Analgesics (excluding acetaminophen)	2 (0.8)	98 (1.0)
Vaccine	3 (1.2)	114 (1.1)
Antimalarial	2 (0.8)	174 (1.7)
Bronchodilators	5 (2.0)	43 (0.4)
Benzodiazepine	0 (0)	2 (0.02)
Anti mucolytic	0 (0)	72 (0.7)
Narcotics	1 (0.4)	4 (0.04)
Cough medicine	10 (3.9)	76 (0.7)
Sedatives	0 (0)	22 (0.2)
Antiemetic	14 (5.5)	283 (2.8)
Ergometrine	0 (0)	10 (0.1)
Vasodilators	0 (0)	11 (0.1)
Steroids	0 (0)	14 (0.1)
Antiarrhythmic	0 (0)	1 (0.01)
Proton Pump Inhibitor	1 (0.4)	2 (0.02)
Phenobarbital	0 (0)	8 (0.08)
Diuretics	0 (0)	1 (0.01)
Buscopan	9 (3.5)	361 (3.5)
Antacids	4 (1.6)	151 (1.5)
Magnesium	0 (0)	59 (0.6)
Other	11 (4.3)	354 (3.5)

*Note this table is only based on the first 3 drugs reported by the pregnant woman. Women reported up to 46 drugs used during pregnancy in an open text format leading to difficulties coding the data. These data were only available from the ASAP sites, MA1 and MA5.

Table 11b: Other drugs taken during pregnancy based on self-reported by the pregnant mother stratified by ACT exposure status

	Confirmed ACT exposure N (%)	No Confirmed or Unconfirmed ACT N (%)
Any Non-antimalarial	4682 (99.5)	5398 (100)
Antibiotics	851 (19.6)	1060 (18.1)
ARVs	27 (0.6)	131 (2.4)
Acetaminophen	1832 (39.0)	950 (17.6)
Multivitamin/Iron/Folic acid	3129 (66.5)	336 (6.2)
Antihistamine	137 (2.9)	111 (2.1)
Antiparasitic	407 (8.7)	448 (8.3)
Antifungal	162 (3.4)	338 (6.3)
Antihypertensive	7 (0.2)	26 (0.5)
Analgesics (excluding acetaminophen)	51 (1.1)	49 (0.9)
Vaccine	117 (2.5)	0 (0)
Antimalarial	85 (1.8)	53 (1.0)
Bronchodilators	17 (0.4)	21 (0.4)
Benzodiazepine	1 (0.02)	1 (0.02)
Anti mucolytic	24 (0.5)	48 (0.9)
Narcotics	4 (0.1)	1 (0.02)
Cough medicine	43 (0.9)	30 (0.6)
Sedatives	16 (0.3)	6 (0.1)
Antiemetic	154 (3.3)	143 (2.7)
Ergometrine	7 (0.2)	3 (0.1)
Vasodilators	11 (0.2)	0 (0)
Steroids	7 (0.2)	6 (0.01)
Antiarrhythmic	1 (0.01)	0 (0)
Proton Pump Inhibitor	0 (0)	2 (0.04)
Phenobarbital	1 (0.02)	5 (0.1)
Diuretics	0 (0)	1 (0.02)
Buscopan	55 (1.2)	315 (5.8)
Antacids	30 (0.6)	119 (2.2)
Magnesium	37 (0.8)	20 (0.4)
Other	150 (3.2)	205 (3.8)

*Note this table is only based on the first 3 drugs reported by the pregnant woman. Women reported up to 46 drugs used during pregnancy in an open text format leading to difficulties coding the data. These data were only available from the ASAP sites, MA1 and MA5.

Table 12: Sensitivity analysis of association between confirmed ACT exposure and the risk of genetically linked congenital anomalies compared to no ACT exposure (confirmed or unconfirmed) during pregnancy

	Conf. ACT N (%)	**No ACT N (%)**	**1st Trim ACT N (%)**	**No ACT N (%)**
MA3	4 (0.5)	4 (0.9)	0 (0)	4 (0.9)
MA5–Burkina Faso	0 (0)	4 (0.5)		
MA5-Gambia	0 (0)	1 (0.1)		
MA5-Ghana	0 (0)	1 (0.1)		
MA5-Mali	1 (0.4)	7 (0.7)		
MA6	3 (0.7)	8 (0.6)		
ASAP-Kenya	2 (0.9)	7 (1.3)	1 (1.5)	7 (1.3)
ASAP-Burkina Faso	0 (0)	5 (0.8)	0 (0)	5 (0.8)
ASAP-Mozambique	1 (2.9)	4 (0.6)	1 (4.2)	4 (0.6)
Adj. Prev	0.75 (0-1.60)	0.60 (0.40-0.80)	1.76 (0-4.47)	0.90 (0.50-1.30)
Adj. PRR	**0.64 (0.31-1.30)**	**Referent**	**1.35 (0.31-5.80)**	**Referent**

*Adjusts for study-site of enrollment

Conclusion

The findings from this project provide substantial evidence of the safety of ACT use in the 2nd and 3rd trimester of pregnancy. The meta-analysis of data from 20 studies of 3707 ACT exposed women 2nd or 3rd trimester identified no increased risk for miscarriage, stillbirth or congenital anomalies regardless of the comparison group used, including no antimalarial drug exposure in pregnancy. The pooled analysis of the MiPc studies including 4090 and 1612 women exposed in the 2nd and 3rd trimester, respectively, found no increased risk of congenital anomalies following receipt of ACT compared to women who never received an ACT in pregnancy. Combined, these two studies provide data on 9526 women who received an ACT in the 2nd or 3rd trimester, far exceeding the 1000 exposures documented by the WHO when the guidelines for treatment of malaria in pregnancy using ACTs were proposed (1).

Despite leveraging all available data from the MiPc, we identified only 162 women with confirmed ACT exposures in the first trimester, limiting our power to assess the risk of congenital anomalies. Although the point estimates for the primary analysis and all subsequent sensitivity analyses were >1, none of the analyses reached statistical significance at a p value= .05. Our finding mirrors results from an observational cohort in Thailand which observed a RR of 1.48 (95%CI 0.37-5.88) associated with first trimester exposure (2). In contrast, a study of 164 women who received AL in the first trimester in Tanzania found no increased risk of congenital anomalies compared to women with no antimalarial exposure (3). Ultimately, our results regarding first trimester ACT exposure are inconclusive.

The findings of these two studies highlight the need for more high quality data on the pregnancy outcomes for women who received an ACT in early pregnancy. For ethical reasons, pregnant women are often excluded from clinical trials over concerns of exposing both the mother and fetus to an unknown teratogen (5). Therefore, data on the safety of drugs in pregnancy often are lacking, and patients and clinicians must rely on animal studies which may not directly translate to human populations (6). To alleviate this problem, the WHO has developed a protocol for implementing pregnancy exposure registries in LMIC which proposes to pool data into the WHO's pregnancy registry database (7). Pregnancy registries provide valuable prospectively-collected data on drug exposures and pregnancy outcomes in order to assess the safety of drugs in pregnancy. By prospectively collecting data, these systems eliminate the potential for recall bias, which plagues many case-control studies of drug exposures in pregnancy. These systems are not without major limitations, primarily the voluntary enrollment of patients. To mitigate this limitation, pregnancy registries must focus on enrolling all exposed women through opt-out procedures instead of classic opt-in enrolment. As LMIC countries adopt pregnancy registries and as drug developers establish pregnancy registries in conjunction with the introduction of new antimalarials, we will gain additional data on critical exposures such as ACT use in early pregnancy.

This study demonstrated the difficulty in diagnosing congenital anomalies in low-resource settings. Despite using the same training manual across all of the MiPc studies, the reported prevalence of congenital anomalies at birth differed greatly between studies and individual study sites. Furthermore, the current strategy of using surface exams to detect anomalies will miss congenital heart defects, a group of anomalies of particular concern. Although

electrocardiograms on all infants is burdensome in low-resource settings, pulse oximetry is simple, effective screening tool to detect congenital heart defects (8). Future assessments of the risk congenital anomalies at birth should consider adding pulse oximetry and/or electrocardiograms to the physical examinations, where practical. In addition, assessments at birth will also miss neurological disorders that only become apparent when child is older. For example, most children with Fetal Alcohol Syndrome are diagnosed as toddlers or older when neurological deficits are first observed (9). Future studies assessing the risk of congenital anomalies should consider following infants for longer periods of time in order to assess neurological development.

This project speaks to the necessity and difficulty of assessing the safety of antimalarial drugs in pregnancy. Future policy recommendations must both weigh the known benefits of ACT treatment for malaria with the uncertain risks in pregnancy. ACTs are more effective and have a better tolerability profile in pregnant women than quinine (10). Furthermore, recent studies have shown that use of ACTs in the first trimester was not associated with an increased risk of miscarriage compared to quinine (2,11). These clear benefits must be weighed against the not-yet-excluded possibility of an increased risk of congenital anomalies observed in this study. Despite pooling across multiple studies, there were limited data available on first trimester exposure to ACTs with complete assessment of subsequent congenital anomalies. If guidelines for the treatment of first trimester malaria are changed, post-marketing surveillance, including follow-up of exposed infants, will be needed.

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Appendix: Chapter 1 Protocol

The Safety of artemisinins in pregnancy for the treatment of malaria: A systematic review and meta-analysis

Systematic Review Protocol

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SK wrote the protocol with input from AVE, AS and FTK. SK developed the search terms. SK and AVE will review all abstracts independently, and AS will serve as the tiebreaker. SK and AVE will abstract all data independently. SK will conduct all analyses.

Guarantor:
Feiko ter Kuille on behalf of the Malaria in Pregnancy Consortium

Support:
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Introduction

Rationale:

This systematic review addresses a key problem in malaria control efforts - the safety of artemisinins in pregnancy. In 2007, an estimated 54.7 million pregnancies occurred in areas of stable *P. falciparum* malaria transmission and an additional 70.5 million occurred in areas of low transmission or areas with only *P. vivax* malaria (1,2). Malaria is responsible for up to 100,000 neonatal deaths due to being born small for gestational age (SGA) and 10,000 maternal deaths (3,4). Severe malaria may lead to abortion, stillbirth, prematurity and low birth weight (4). In addition, malaria is associated with maternal anemia which can lead to death if severe (4). Given the high morbidity and mortality associated with malaria in pregnancy, safe and efficacious drugs are needed for treatment and prevention, but because of ethical concerns, few studies enroll pregnant women (5,6).

Studies of rats and rabbits have found artemisinins to be embryotoxic and teratogenic (7). A review of all antimalarial drugs used in pregnancy noted artesunate is very toxic to rat and rabbit embryos, with fetal reabsorption in rats reported at low doses (28-223 mg/kg/day) given orally on days 9-14 of gestation (5). High rates of congenital anomalies, including bent and/or shortened long bones and treatment related heart defects, were observed in rat litters (5). Primate studies demonstrated similar embryo toxicity, with 55% and 100% embryo lethality for 12 and 30 mg/kg/d oral doses respectively (8). In addition, observations of three live embryos from the 30mg group noted reduction in blood cells in the vasculature and the cardiac chambers were distended with thin walls (8). The primary embryonic targets for artemisinins are the primitive erythroblasts proliferating during gestational periods of 10-14 days in the rat and 18-40 days in

the monkey. In humans this corresponds to a sensitive period from post conception day 21 to approximately post conception week 9 (8).

Despite the concerning data from animal studies limited human studies of artemisinins show no increased risk of adverse pregnancy outcomes including miscarriage, stillbirth or congenital anomalies. Five cohort studies and three randomized clinical trials have studied the safety of artemisinins in pregnancy (9–15) but all had small sample sizes and were not powered to examine safety outcomes. Of these eight studies, four reported exposures to artemisinins during the first trimester and four reported outcomes from the same cohort of women attending health centers in the refugee camps along the Thailand-Burma border. No study found a statistically significant increased rate of spontaneous abortions, stillbirths, neonatal deaths, prematurity, or congenital anomalies. The highest prevalence of congenital anomalies was observed in women exposed to AS during the first trimester (4.5%) but it was not statistically significantly higher than the prevalence of 1% observed in women not exposed to artemisinins during the first trimester (12). All eight studies were limited by small sample sizes to detect rare but clinically significant differences in the rates of adverse pregnancy studies. By pooling data from randomized controlled trials and prospective cohort studies through meta-analysis, we will increase the power and thoroughly assess the risk of adverse pregnancy outcomes associated with exposure to artemisinins during pregnancy.

Research question:

Are women exposed to artemisinins during pregnancy at increased risk of adverse pregnancy outcomes including miscarriage, stillbirth, and congenital anomalies compared to women exposed to quinine and other non-artemisinin based antimalarials?

Primary Objectives:

1. To estimate the pooled event rate for miscarriage, stillbirth, and congenital anomalies associated with exposure to artemisinins for treatment during pregnancy.
2. To estimate the pooled relative risk and risk difference for miscarriage, stillbirth, and congenital anomalies associated with exposure to artemisinins for treatment during pregnancy compared to women exposed to non-artemisinin based antimalarials.
3. To estimate the pooled relative risk and risk difference for miscarriage, stillbirth and congenital anomalies among women exposed to specific ACT combinations for treatment compared to each other (e.g. artesunate-mefloquine compared to artemether-lumefantrine) using direct and indirect comparisons from randomized controlled trials.

Methods

Eligibility Criteria:

Due to a limited number of randomized controlled trials including pregnant women, we will include both prospective cohort studies and trials. Our search will include pregnant women of any age with malaria regardless of severity. We will stratify our analyses by age groups, malaria severity (uncomplicated and severe), and parity (<2 and 2+). For details of the eligibility criteria, see the PICOTS Framework.

PICOTS Framework:

Components	Characteristics
Population	Pregnant women with malaria -Subgroup analysis: o By trimester o Severe malaria and uncomplicated malaria o Age o Parity o Geography
Intervention	Exposed to an artemisinin during pregnancy either as a monotherapy or in combination with another drug.
Control	Non-artemisinin based treatment for malaria (including quinine, SP, CQ, and MQ), or placebo
Outcomes	Adverse pregnancy outcomes as measured at birth: 1. Miscarriage –defined as pregnancy loss <28 weeks 2. Stillbirth –defined as pregnancy loss >/= 28 weeks 3. Congenital Anomalies –including major and minor
Timing	No time limits will be placed on the search
Setting	Any prospective cohort study or randomized trial which enrolled pregnant women with malaria. No restriction for language will be made

Search Strategy:

An electronic literature search applying the aforementioned PICOTS framework will be conducted using the following clinical databases: MEDLINE, EMBASE, and the Malaria in Pregnancy Consortium (MiPc) Library (16). A multi-concept Boolean search strategy will be applied using keywords and MeSH. We will additionally search 'gray literature' databases, conference abstracts, manually review reference lists of selected publications as well as records recommended by contacting experts so as to encompass a broad range of available literature. A librarian will be involved in this search and the strategy optimized for each database searched. We will merge citations from all individual databases into one citation software file. Duplicates will be removed and the last date of the search documented.

PubMed Search Strategy: Search Date June 2015, 2015

	Framework	Search terms	Number of articles
P	**Population**	(Pregnant women OR pregnan* AND malaria)	**P:** 4138
I	**Intervention**	AND (Artemisinin* OR "Artemisinin Combination Therapy" OR ACT OR artemether OR artesunate OR dihydroartemisinin OR treatment)	**I: 8616747** **P + I: 2519**
C	**Control**	-	 **C+P+I: 2519**
O	**Outcome**	AND (Pregnancy complication [mh] OR safety OR "serious adverse event" OR miscarriage OR stillbirth OR "pregnancy loss" OR "spontaneous abortion" OR "birth defect" OR congenital abnormalities OR "congenital malformations" OR "congenital anomalies")	**O: 1184241** **P + I +C+ O: 1535**
T	**Timing**	-	**P+I+O+C+T: 1535**
S	**Setting**	AND Clinical trials OR trials OR cohort study [mh] OR prospective [tw]	**P+I+O+C+T+S: 487**

EMBASE Search: January 13, 2015: 295 Articles

'pregnant woman' AND malaria AND (artemisinin* OR 'artemisinin combination therapy' OR act OR artemether OR artesunate OR dihydroartemisinin) AND [embase]/lim NOT [medline]/lim AND 'human'/de

Malaria in Pregnancy Library: January 13, 2015: 384 Articles

Artemisinin OR artemether OR artesunate OR dihydroartemisinin

Data Management:

Two independent reviewers will screen abstracts of all citations that meet study eligibility in the first screen as outlined above. The second screen will consist of any studies selected by either one these reviewers. In the second screen, two independent reviewers will screen abstracts and full texts and agree on final study eligibility. The final number of articles to be obtained will be agreed upon with any disagreements on citations being resolved by consensus or by contacting a third reviewer who will serve as the tie breaker. Articles considered eligible after full-text review by the two independent reviewers will be included in the final set of studies for inclusion and those ineligible excluded from the final analysis. A log of all studies excluded and reasons for exclusion after the 1st and 2nd screen will be kept to account for any differences in inferences made.

The two reviewers will independently extract data using a standardized preformed data extraction form, the data compared and any discrepancies will be resolved by consensus. The abstracted data will be entered into a database for analysis.

Data Items:

We will abstract data on the study population including age, parity, and severity of malaria, drug exposures, and pregnancy outcomes. For a complete list of variables to be abstracted, see the data abstraction tools.

Outcomes: We will abstract information on all adverse pregnancy outcomes including miscarriage (pregnancy loss before 28 weeks), stillbirth (pregnancy loss at or after 28 weeks), and congenital anomalies (major and minor). We will equally prioritize each of the three adverse pregnancy outcomes.

Quality Assessment:

We will assess the quality of the clinical trials using The Cochrane Collaboration's tool for assessing risk of bias. Five domains of bias tackling selection (sequence generation and allocation concealment), performance (blinding of participants and personnel; and other potential threats to validity), detection (blinding of outcome assessment; and other potential threats to validity), attrition (incomplete outcome data), and reporting (selective outcome reporting) bias will be assessed. We will assess bias in cohort studies using the Newcastle Ottawa scale that evaluates studies for selection bias, comparability, and assessment of the outcome.

Data Analysis

We will conduct meta-analyses to generate pooled estimates of the event rate and relative risk with 95% confidence intervals (CI) for miscarriage, stillbirth and congenital anomalies stratified by study type. Given that safety outcomes are rare outcomes, we may calculate pooled risk differences instead of pooled relative risks in order to account for zero cells. To fully characterize the risk, we will compare women exposed to any artemisinins to women exposed to SP, quinine, and chloroquine, and to no antimalarial drug (and therefore no malaria). We will use random effects models and measure the amount of heterogeneity present in the data evaluated by the I^2 values. Because the importance of the observed value of the I^2 value depends on both the magnitude and direction of the effect as well the strength of evidence of heterogeneity from statistical testing, we will roughly use the following categories of the I^2: 0% to 40%: might not be important; 30% to 60%: may represent moderate heterogeneity; 50% to 90%: may represent substantial heterogeneity; 75% to 100%: considerable heterogeneity. We will conduct stratified analyses by study type, geographic location, trimester of exposure, and study time period. We will conduct sensitivity analyses to assess the influence of study quality on the results.

Additionally we will assess publication bias through funnel plots. A two-tailed test p value of < 0.05 will be considered statistically significant.

As a secondary analysis, using only the randomized controlled trials, we will use meta-analyses to estimate the risk of stillbirth, miscarriage, and congenital anomalies using direct and indirect comparisons of different specific Artemisinin Combination Therapies (ACT) to each and other, and to non-artemisinin based therapies.

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Data Abstraction Tool:

SCREENING 1 (Abstract)	Study Screening ID A ___ ___ ___ ___ e.g.A0001
1	First author: _____ Publication Year: ___ ___ ___ ___
2	Study design? □RCT □ Prospective Cohort □ Other □Can't tell
3	Study population includes pregnant women? □Yes □No □Can't tell
4	Study population includes patients exposed to artemisinins? □ Yes □No □Can't tell
5	Reports on adverse pregnancy outcomes (miscarriage, stillbirth, congenital anomalies)? □Yes □No □Can't tell
Eligibility Criteria Check all that apply	
□ Study design is a clinical trial or prospective cohort □ Study participants include pregnant women □ Study participants exposed to artemisinins □ Reports adverse pregnancy outcomes (stillbirths, miscarriages, congenital anomalies) □ Can't tell any of the above	
Study Eligible? □ Yes □Can't tell Proceed to Study Screening 2 □No …… END HERE	
Form completed by ____(Initials)	Date ___ ___ ___ dd mmm yyyy

SCREENING 2 (Full Text Review)	Study Screening ID B ___ ___ ___ ___ e.g.B0001
1	First author: _____ Publication Year: ___ ___ ___ ___
2	Language: □English □Other: _____
3	Study design? □RCT □ Prospective Cohort □ Other □Can't tell
4	Study population includes pregnant women? □Yes □No

5	Study population exposed to an artemisinin? □Yes □No
	Study reports severe adverse event? □Yes □No

Eligibility Criteria Check all that apply

□ Full text in English
□ Study design is a clinical trial or prospective cohort
□ Study participants include pregnant women
□ Study participants exposed to an artemisinin
□ Study reports on severe adverse events

Study Eligible?
□Yes Proceed to DATA EXTRACTION SRF (final analysis n_i)
□No …… END HERE (exclude from final analysis n_e)

Form completed by ____(Initials) Date ____ ____ ____ (dd mmm yyyy)

C. DATA EXTRACTION SRF (Full Analysis) Study Screening ID

C ____ ____ ____ ____

e.g.C0001

1	First author: _____ Publication Year: ____ ____ ____ ____
2	Country/Countries: _Thailand_____; _____; _____
3	Malaria transmission intensity □ Low □ Moderate □ High
	Malaria parasites in circulation □ P. falciparum □ P. vivax □ XBoth
4	Study start: Month ____ ____ Year ____ ____ ____ ____ □Not documented
5	Study end: Month ____ ____ Year ____ ____ ____ ____ □Not documented
6	Study Design: □RCT □ Prospective cohort

6	Intervention 1: Artemisinin _____ Dose _____mg Days_____ Combination Drug_____ Dose_____ Days_____ Purpose: □ Treatment □ IPT □ IST Intervention 2: Artemisinin _____ Dose _____mg Days_____ Combination Drug_____ Dose_____ Days_____ Purpose: □ Treatment □ IPT □ IST Intervention 3: Artemisinin _____ Dose _____mg Days_____ Combination Drug_____ Dose_____ Days_____ Purpose: □ Treatment □ IPT □ IST
7	Control: Drug: _____ Dose_____ Days_____ Purpose: □ Treatment □ IPT □ IST
	Length of follow-up: _____ years months days (circle 1)

Study Population	
	Inclusion criteria:
	Exclusion criteria:
	Measurement of gestational age by: □ Ultrasound □ LMP □ Ballard Score □ Fundal height □ Not reported □ Other _____
1	Age in years: |__|__| . |__| □Mean □Median □Not documented Age SD |__|__| Age range _____
2	Age: Number <5: |__|__|__| Proportion |__|__|__|% □ NR Number 5-18: |__|__|__| Proportion |__|__|__|% □ NR Adults: |__|__|__| Proportion |__|__|__|% □ NR Pregnant women: |__|__|__| Proportion |__|__|__|% □ NR
3	Sex: Number male: |__|__|__| Proportion |__|__|__|% Number female:|__|__|__| Proportion |__|__|__|%

4 Patient factors by Intervention:

	Intervention 1: _____ N (%)	Intervention 2: _____ N (%)	Intervention 3: _____ N (%)	Control Arm _____ N (%)	Total N(%)
Age <5					
Age 5-18					
Adults (or age>18)					
Male					
Female					
Pregnant					
Mean Parasetemia					
Uncomplicated malaria					
Severe malaria					
Parity </=2					
Parity >2					
1st Trimester					
2nd Trimester					
3rd Trimester					

Primary outcomes of interest

1 How many total SAEs were reported ?_____

	Interv. 1: N (%)	Interv. 2 N (%) Art 1st tri	Interv. 3 N (%)	Control arm N (%)	Total N (%)
Any SAE					
Death					
Hospitalizatio					

ns					
Stillbirth					
Miscarriage					
All Congenital Anomaly					
CA1 _____					
CA2 _____					
CA3 _____					
CA4 _____					
CA5 _____					
Early Neonatal Death (<7 days)					

How many SAEs were reported after 1st trimester exposures?_____

	Interv. 1: N (%)	Interv. 2 N (%)	Interv. 3 N (%)	Control arm N (%)	Total N (%)
Any SAE					
Death					
Hospitalizations					
Stillbirth					
Miscarriage					
All Congenital Anomaly					
CA1 _____					
CA2 _____					
CA3 _____					
CA4 _____					
CA5 _____					
Early Neonatal Death (<7 days)					

Effect Estimates:

Exposure _____ Comparator _____

Stillbirth: _____ OR RR RD (circle 1)

_____ aOR aRR aRD (circle 1)

Variables adjusted for _____

Miscarriage: _____ OR RR RD (circle 1)

_____ aOR aRR aRD (circle 1)

Variables adjusted for _____

Congenital Anomalies: _____ OR RR RD(circle 1)

_____ aOR aRR aRD (circle 1)

Variables adjusted for _____

Comments:

Trial Quality: The Cochrane Collaboration's tool for assessing risk of bias.

1 SELECTION BIAS

	Description	Bias Judgment (Yes/No/unclear)
RANDOM SEQUENCE GENERATION		

	ALLOCATION CONCEALMENT			

2	PERFOMANCE BIAS			
		Description	Bias Judgment (Yes/No/unclear)	
	BLINDING OF PARTICIPANTS AND PERSONNEL			

3	DETECTION BIAS			
		Description	Bias Judgment (Yes/No/unclear)	
	BLINDING OF OUTCOME ASSESSMENT			

4	ATTRITION BIAS			
		Description	Bias Judgment (Yes/No/unclear)	
	INCOMPLETE OUTCOME DATA			

Form completed by _____(Initials) Date ____/____/____
dd mmm yyyy

Bias Assessment for Cohort Studies:

Assessment of quality of a cohort study – Newcastle Ottawa Scale	
Selection (tick one box in each section)	
1. Representativeness of the intervention cohort a) truly representative of the average, pregnant, community-dwelling resident ★ b) somewhat representative of the average, pregnant, community-dwelling resident ★ c) selected group of patients, e.g. only certain socio-economic groups/areas d) no description of the derivation of the cohort	☐ ☐ ☐ ☐
2. Selection of the non intervention cohort a) drawn from the same community as the intervention cohort ★ b) drawn from a different source c) no description of the derivation of the non intervention cohort	☐ ☐ ☐
3. Ascertainment of intervention a) secure record (eg health care record) ★ b) structured interview ★ c) written self report d) other / no description	☐ ☐ ☐ ☐
4. Demonstration that outcome of interest was not present at start of study a) yes ★ b) no	☐ ☐
Comparability (tick one or both boxes, as appropriate)	
1. Comparability of cohorts on the basis of the design or analysis a) study controls for age and parity ★ b) study controls for any additional factors (malaria severity, concomitant therapies) ★	☐ ☐
Outcome (tick one box in each section)	
1. Assessment of outcome a) independent blind assessment ★ b) record linkage ★ c) self report d) other / no description	☐ ☐ ☐ ☐
2. Was follow up long enough for outcomes to occur a) yes, if median duration of follow-up >= 6 month ★ b) no, if median duration of follow-up < 6 months	☐ ☐
3. Adequacy of follow up of cohorts a) complete follow up: all subjects accounted for ★ b) subjects lost to follow up unlikely to introduce bias: number lost <= 20%, ★ or description of those lost suggesting no different from those followed c) follow up rate < 80% (select an adequate %) and no description of those lost d) no statement	☐ ☐ ☐ ☐