The Performance of a Rapid Diagnostic Test in Detecting Malaria Infection in Pregnant Women and the Impact of Missed Infections

Williams, John E; Cairns, Matthew; Njie, Fanta; Laryea Quaye, Stephen; Awine, Timothy; Oduro, Abraham; Tagbor, Harry; Bojang, Kalifa; Magnussen, Pascal; Ter Kuile, Feiko O; Woukeu, Arouna; Milligan, Paul; Chandramohan, Daniel; Greenwood, Brian

Published in:
Clinical infectious diseases : an official publication of the Infectious Diseases Society of America

DOI:
10.1093/cid/civ1198

Publication date:
2016

Document version
Publisher's PDF, also known as Version of record

Document license:
CC BY-NC-ND

Citation for published version (APA):
Williams, J. E., Cairns, M., Njie, F., Laryea Quaye, S., Awine, T., Oduro, A., Tagbor, H., Bojang, K., Magnussen, P., Ter Kuile, F. O., Woukeu, A., Milligan, P., Chandramohan, D., & Greenwood, B. (2016). The Performance of a Rapid Diagnostic Test in Detecting Malaria Infection in Pregnant Women and the Impact of Missed Infections. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 62(7), 837-844. https://doi.org/10.1093/cid/civ1198
The Performance of a Rapid Diagnostic Test in Detecting Malaria Infection in Pregnant Women and the Impact of Missed Infections

John E. Williams,1 Matthew Cairns,2 Fanta Njie,3 Stephen Laryea Quaye,1 Timothy Awine,1 Abraham Oduro,1 Harry Tagbor,4 Kalifa Bojang,3 Pascal Magnussen,6 Feiko O. ter Kuile,6 Arouna Woukeu,4 Paul Milligan,2 Daniel Chandramohan,2 and Brian Greenwood6

1Navrongo Health Research Centre, Ghana; 2Faculties of Epidemiology and Population Health and Infectious & Tropical Diseases, London School of Hygiene & Tropical Medicine, United Kingdom; 3Medical Research Unit, Fajara, The Gambia; 4Department of Community Medicine, Kwame Nkrumah University of Sciences and Technology, Kumasi, Ghana; 5Department of Veterinary Disease Biology and Centre for Medical Parasitology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark; and 6Department of Clinical Sciences, Liverpool School of Tropical Medicine, United Kingdom

Background. Intermittent screening and treatment in pregnancy (ISTp) is a potential strategy for the control of malaria during pregnancy. However, the frequency and consequences of malaria infections missed by a rapid diagnostic test (RDT) for malaria are a concern.

Methods. Primigravidae and secundigravidae who participated in the ISTp arm of a noninferiority trial in 4 West African countries were screened with an HRP2/pLDH RDT on enrollment and, in Ghana, at subsequent antenatal clinic (ANC) visits. Blood samples were examined subsequently by microscopy and by a polymerase chain reaction (PCR) assay.

Results. The sensitivity of the RDT to detect peripheral blood infections confirmed by microscopy and/or PCR at enrollment ranged from 91% (95% confidence interval [CI], 88%, 94%) in Burkina Faso to 59% (95% CI, 48%, 70%) in The Gambia. In Ghana, RDT sensitivity was 89% (95% CI, 85%, 92%), 83% (95% CI, 76%, 90%) and 77% (95% CI, 67%, 86%) at enrollment, second and third ANC visits respectively but only 49% (95% CI, 31%, 66%) at delivery. Screening at enrollment detected 56% of all infections detected throughout pregnancy. Seventy-five RDT negative PCR or microscopy positive infections were detected in 540 women; these were not associated with maternal anemia, placental malaria, or low birth weight.

Conclusions. The sensitivity of an RDT to detect malaria in primigravidae and secundigravidae was high at enrollment in 3 of 4 countries and, in Ghana, at subsequent ANC visits. In Ghana, RDT negative malaria infections were not associated with adverse birth outcomes but missed infections were uncommon.

Keywords. malaria in pregnancy; rapid diagnostic test; intermittent screening and treatment; Ghana.

Intermittent preventive treatment of malaria in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) reduces the incidence of maternal anemia, low birth weight, and neonatal mortality [1, 2]. The World Health Organization now recommends administration of IPTp-SP at each antenatal clinic (ANC) visit after the first trimester [3]. However, in parts of eastern and southern Africa Plasmodium falciparum has become highly resistant to SP and IPTp-SP is losing some of its impact [4–11]. Currently, there is no recommended drug to replace SP for IPTp, as both mefloquine and azithromycin/chloroquine were not well tolerated when evaluated for this purpose [12, 13]. However, dihydro-artemisinin/piperaquine shows promise as an alternative to SP for IPTp [14].

Intermittent screening and treatment (ISTp) is being evaluated as a potential alternative to IPTp-SP that might be applicable in areas where P. falciparum has developed a high level of resistance to SP, or where malaria transmission has fallen to a level at which very few women will benefit from receiving IPTp. ISTp involves screening pregnant women at each ANC visit with a rapid diagnostic test for malaria (RDT) and treating those who test positive. A pilot study of ISTp in Ghana using SP or artesunate-amodiaquine [15], and a multicenter trial using artemether-lumefantrine (AL) conducted in 4 countries in West Africa where P. falciparum is still sensitive to SP [16], have shown that ISTp was noninferior to IPTp-SP in preventing maternal anemia, low birth weight, and placental malaria. However, RDTs used in ISTp may miss some low density infections, which would not be treated, and these missed infections could have an adverse impact on the outcome of pregnancy. Therefore, we have investigated the performance of an RDT in pregnant women given ISTp during a recent multicenter trial [16], characterized the factors associated with infections missed by an RDT, and investigated the consequences of these infections on the outcome of pregnancy.
METHODS

Blood samples were obtained during a large multicenter trial of ISTp vs IPTp-SP conducted in 4 countries in West Africa [16]. Only women randomized to the ISTp-AL group are included in the analyses described in this article. The key features of this trial are summarized in Supplementary Table 1.

Study Procedures

Women who consented to join the trial were randomized individually to 1 of 2 study arms (IPTp-SP or ISTp-AL). Women were prescribed daily ferrous sulfate (200 mg) and folic acid (0.4 mg) for the duration of their pregnancy and provided with a long-lasting insecticide treated bed net (LLIN) at enrollment.

At the initial ANC visit in all 4 countries and, in Ghana, at subsequent ANC visits and at delivery, women were screened with the First Response Malaria Rapid Diagnostic Combo Test. This RDT detects both parasite lactate dehydrogenase (pLDH) of P. falciparum, P. vivax, P. malariae and P. ovale, and Histidine-Rich Protein 2 (HRP2) of P. falciparum; it was used throughout the study according to the manufacturer’s instructions. Women who were RDT positive were treated with AL (UNICEF, Copenhagen) twice daily for 3 days, with the first dose given under observation. At each ANC visit, a finger prick blood sample was obtained for preparation of a blood film and filter paper blood spots for molecular analyses; blood films were examined later and did not affect a woman’s clinical management. Women who attended an ANC outside a scheduled visit with symptoms compatible with malaria were screened with an RDT if clinic staff considered malaria to be a possible cause of their symptoms, and those who tested positive were treated with AL. Hemoglobin (Hb) concentration was determined at a woman’s last ANC attendance, scheduled to occur between 36 and 40 weeks of gestation. Study women were encouraged to deliver in hospital where placental biopsies were obtained. Birth weight was recorded at delivery in women who delivered in hospital and within 7 days of delivery in women who delivered at home.

Three co-primary outcomes defined for the main trial were used to explore the possible consequences of infections missed by a RDT: (a) low birth weight, defined as measured birth weight of a live, singleton birth of <2500 g recorded within 7 days of delivery, (b) Hb concentration measured at the last ANC visit, and (c) active malaria infection of the placenta defined as the presence of malaria parasites on histological examination of the placenta, with or without malaria pigment or inflammatory cells. Measured birth weight was also investigated as a key secondary outcome.

Laboratory Methods

Hemoglobin concentrations were measured using an Hb 301 Hemocue analyser (HemoCue, Anglom, Sweden). Thick blood smears were examined by 2 microscopists and discrepancies resolved by a third microscopist using a standardized algorithm [17]. Parasite density was estimated by counting the number of parasites per white blood cell, assuming a white cell count of 8000 per μL. One hundred thick blood film fields were read before a slide was considered negative. Nested polymerase chain reaction (PCR) assays were conducted at the MRC Unit, The Gambia. Following DNA extraction from filter paper samples, a first stage PCR assay was carried out using the primers rPLU5 + rPLU6, which detect P. falciparum, P. vivax, P. ovale, and P. malariae. A second stage reaction on positive samples was then conducted using the species-specific primers Plasmo-1 + Plasmo-2, for detection of P. falciparum. A third stage assay was conducted on samples that were genus specific but P. falciparum negative using additional species specific primers (Supplementary Methods). Quality control of the PCR assay at Duke University, North Carolina, showed that its limit of sensitivity was approximately 10 parasites per microliter. Only samples that were positive for P. falciparum are included in this study. Examination of placental biopsies was conducted as described in the Supplementary Methods.

Evaluation of RDT Performance

Stata version 13 (StataCorp, College Station, Texas) was used for all analyses. A woman was considered positive for P. falciparum infection if either the blood smear or PCR assay indicated P. falciparum and negative if both tests were negative. Geometric mean parasite density was determined at baseline based on microscopy. The sensitivity and specificity of the RDT against the combined PCR/blood smear gold standard was calculated for each ANC contact at which ISTp-AL was administered and at delivery. The diagnostic ability of RDT, microscopy, PCR, and PCR combined with microscopy to detect active malaria infection of the placenta was investigated. Infection status at baseline and at subsequent ANC contacts was defined as (1) not infected, (2) infected and RDT positive, or (3) infected and RDT negative. The association of infection status with maternal characteristics including gravidity, age, gestational age at enrollment, educational level, season of enrollment and socioeconomic status (calculated using principal components analysis of durable assets as described previously [17]) was then explored. Risk factors for P. falciparum infections not detected by an RDT were investigated using univariable and multivariable logistic regression comparing detected vs undetected infections. Finally, the association between malaria infections missed by RDT at routine ANC visits and the 3 primary study outcomes (low birth weight, Hb at the final ANC contact, and placental malaria infection) plus mean birth weight was explored using multivariable logistic regression (for binary outcomes) and linear regression (for continuous outcomes).

Ethics

The study and subsequent study amendments were approved by the London School of Hygiene and Tropical Medicine Ethics
Committee and by the ethics committees of all the partner African institutions which are listed in the Supplement. The trial was registered with ClinicalTrials.gov (NCT01084213) and the Pan African Clinical trials Registry (PACT20120200027122). Written, informed consent was obtained from all eligible women before enrollment.

RESULTS
The prevalence of malaria at enrollment, detected by microscopy or PCR, was highest in Burkina Faso (70.0%), followed by Ghana (58.6%), Mali (35.9%), and The Gambia (15.1%). Primigravidae were more likely than secundigravidae to be infected at first ANC visit at all centers.

RDT Positivity in Pregnant Women at Enrollment in Burkina Faso, Ghana, Mali, and The Gambia
At enrollment, the overall sensitivity of the RDT in detecting malaria infection was 87.4% (95% confidence interval [CI], 85.3%, 89.4%). Sensitivity was high in Burkina Faso (90.9% [95% CI, 87.5, 93.6]), Ghana (88.8% [95% CI, 85.0%, 91.9%]), and Mali (86.8% [95% CI, 81.2, 91.3]) but lower in The Gambia (59.3% [95% CI, 47.8, 70.1]) where geometric mean parasite density among blood slide positive women was lower than at the other centers (Table 1). Specificity was high at all centers (Table 1 and Supplementary Figure 1). More than half of all infections detected by an RDT at ANC contacts throughout pregnancy were detected at enrollment: 55.6% overall, and 54.1%, 53.6%, 61.5%, and 60.2% in Burkina Faso, Ghana, Mali, and The Gambia, respectively.

RDT Positivity During the Course of Pregnancy in Ghana
The prevalence of P. falciparum infection in Ghanaiian pregnant women was highest at enrollment and remained high throughout pregnancy (Table 2). Supplementary Figure 2 shows the overlap in the diagnosis of P. falciparum malaria by RDT, PCR, and blood smear microscopy at any routine ANC contact at which Ghanaian women were screened.

The sensitivity of the RDT against the combined measure of PCR and/or blood smear positivity at first ANC visit was 88.8% (95% CI, 85.0%, 91.9%), 83.7% (95% CI, 76.2%, 89.6%) at second ANC visit, 77.4% (95% CI, 67.0%, 85.8%) at third ANC visits, and 48.6% (95% CI, 31.4%, 66.0%) at delivery (Table 2 and Supplementary Figure 3). Specificity was generally high, >80% on all occasions in all gravidities but slightly higher in secundigravidae than in primigravidae (Table 2). During the course of pregnancy, 709 positive RDT tests were obtained, 380 of which (53.6%) were obtained at enrollment. Screening with an RDT at enrollment detected 339 of the 639 P. falciparum infections detected by microscopy or PCR during the course of pregnancy (53.1% [95% CI, 49.1%, 57.0%]). One or more infections were recorded subsequently in 172 of the 380 women (45.2%) who were positive at enrollment by RDT compared to 101 (36.2%) in the 272 women who had a negative RDT test at enrollment. There were 212 positive RDTs at subsequent ANC contacts among the 380 women who were RDT positive at the first ANC visit, compared to 117 positive RDTs among the 272 women RDT negative at baseline giving an incidence rate ratio of 1.30 (95% CI, 1.03, 1.63), P = .024.

The sensitivity of the RDT in detection of active malaria infection of the placenta was 73.1% (95% CI, 63.8%, 81.2%) overall and 82.1% (95% CI, 70.8%, 90.4%) in primigravidae (Table 3). The sensitivity of PCR in detection of placental malaria was slightly lower than that of the RDT: 67.0% (95% CI, 56.2%, 76.7%) overall and 74.1% (60.3%, 85.0%) in primigravidae. PCR combined with blood smear had a similar sensitivity to RDT: 73.8% (95% CI, 64.2%, 82.0%) overall and 80.0% (95% CI...
CI, 68.2%, 88.9%) in primigravidae. In sum, 15 and 14 of the 29 placental infections missed by RDT and by PCR, respectively, were recorded as acute infections (ie, parasite present with no malaria pigment, which may indicate a recent infection), suggesting that around half of these “missed” infections may have been acquired after the diagnostic test was done.

Risk Factors for RDT Positive and RDT Negative Malaria Infections

Infection at first ANC attendance was more likely among primigravidae, young women, mothers with a basic education, mothers enrolled in the late wet or early dry season, and mothers of low socioeconomic status (Supplementary Table 2). In crude analysis, gravidity, older age, and educational status were associated with

Table 2. Prevalence of Malaria Infection in Peripheral Blood at Different Time Points in Pregnant Ghanaian Women and Sensitivity and Specificity of an Rapid Diagnostic Test at These Time Points

| Gravidity       | Number Tested | Number Positive | % Positive by PCR or Blood Slide (95% CI) | PCR or Microscopy/RDT Result | RDT Sensitivity % | RDT Specificity % |
|-----------------|---------------|-----------------|------------------------------------------|-------------------------------|-------------------|-------------------|
| Peripheral blood|               |                 |                                          |                               |                   |                   |
| First ANC Visit | 592           | 347             | 58.6 (54.6, 62.5)                        | 30.6                          | 23                | 222               | 88.8 (85.0, 91.9) | 90.6 (86.2, 94.0) |
| Primigravidae   | 317           | 203             | 64.0 (58.6, 69.2)                        | 19.3                          | 15                | 99                | 95.1 (91.1, 97.6) | 86.8 (79.2, 92.4) |
| Secundigravidae | 272           | 142             | 52.2 (46.2, 58.1)                        | 113                           | 8                 | 122               | 79.6 (72.0, 85.9) | 93.8 (88.2, 97.3) |
| Second ANC Visit| 491           | 129             | 26.3 (22.6, 30.4)                        | 108                           | 39                | 53                | 83.7 (76.2, 90.6) | 89.2 (85.6, 92.2) |
| Primigravidae   | 265           | 74              | 27.9 (22.8, 33.7)                        | 66                            | 8                 | 38                | 89.2 (79.8, 95.2) | 85.3 (79.5, 90.0) |
| Secundigravidae | 224           | 55              | 24.6 (19.3, 30.7)                        | 42                            | 10                | 139               | 76.4 (63.0, 86.8) | 94.1 (89.4, 97.1) |
| Third ANC Visit | 384           | 84              | 21.9 (18.0, 26.3)                        | 65                            | 19                | 43                | 77.4 (67.0, 85.8) | 85.7 (81.2, 89.4) |
| Primigravidae   | 201           | 51              | 25.4 (19.8, 31.9)                        | 40                            | 11                | 27                | 78.4 (64.7, 88.7) | 82.0 (74.9, 87.8) |
| Secundigravidae | 182           | 32              | 17.6 (12.7, 23.9)                        | 24                            | 8                 | 16                | 75.0 (56.6, 88.5) | 89.3 (83.3, 93.8) |
| Delivery        | 158           | 35              | 22.2 (16.3, 29.4)                        | 17                            | 18                | 106               | 48.6 (31.4, 66.0) | 86.2 (78.8, 91.7) |
| Primigravidae   | 80            | 20              | 25.0 (16.6, 35.9)                        | 11                            | 9                 | 11                | 55.0 (31.5, 76.9) | 81.7 (69.6, 90.5) |
| Secundigravidae | 78            | 15              | 19.2 (11.8, 27.9)                        | 6                             | 9                 | 6                 | 40.0 (16.3, 67.7) | 90.5 (80.4, 96.4) |

The number of women with missing gravidity status was first visit, 3; second visit, 2; third visit, 1; delivery, 0.

Abbreviations: ANC, antenatal clinic; CI, confidence interval; PCR, polymerase chain reaction; RDT, rapid diagnostic test.

a Positivity in peripheral blood is defined as either a positive PCR result or a positive blood slide.

Table 3. The Prevalence of Placental Malaria, and the Sensitivity and Specificity of an Rapid Diagnostic Test, Polymerase Chain Reaction (PCR) or PCR/Microscopy Undertaken at any Antenatal Contact in Predicting Active Malaria Infection of the Placenta Detected by Histology in Ghanaian Women

| Gravidity       | Number Tested | Number Positive | Percent Positive by Histology | Histology/Diagnostic |
|-----------------|---------------|-----------------|-------------------------------|----------------------|
|                 |               |                 | % Positive by Histology (95% CI) | +/−                  |
| All Women       | 425           | 122             | 73.1 (63.8, 81.2)             | 28.7 (24.6, 33.2)    |
| Primigravidae   | 243           | 76              | 82.1 (70.8, 90.4)             | 31.3 (25.7, 37.4)    |
| Secundigravidae | 181           | 46              | 58.5 (42.1, 73.7)             | 25.4 (19.6, 32.3)    |
| Placental Malaria |             |                 | Sensitivity % (95% CI)        | Specificity % (95% CI) |
| Diagnostic      |               |                 |                               |                       |
| RDT positive at any ANC contact | | | 25.5 (20.3, 31.2) | 25.5 (20.3, 31.2) |
| Primigravidae   | 55            | 12              | 82.1 (70.8, 90.4)             | 19.3 (13.2, 26.7)    |
| Secundigravidae | 24            | 17              | 58.5 (42.1, 73.7)             | 33.3 (24.9, 42.6)    |
| Microscopy positive at any ANC contact | | | 42.9 (36.8, 49.2) | 42.9 (36.8, 49.2) |
| Primigravidae   | 46            | 21              | 88.7 (56.2, 79.4)             | 37.8 (29.8, 46.2)    |
| Secundigravidae | 19            | 22              | 46.3 (30.7, 62.6)             | 48.7 (39.4, 58.1)    |
| PCR positive at any ANC contact | | | 34.2 (28.1, 40.7) | 34.2 (28.1, 40.7) |
| Primigravidae   | 40            | 14              | 71.8 (50.3, 82.8)             | 28.2 (21.3, 35.3)    |
| Secundigravidae | 19            | 15              | 55.9 (37.9, 72.8)             | 38.0 (28.8, 47.8)    |
| PCR or blood slide positive at any ANC Contact | | | 25.2 (20.0, 30.1) | 25.2 (20.0, 30.1) |
| Primigravidae   | 52            | 13              | 80.0 (68.2, 88.9)             | 21.6 (15.1, 29.4)    |
| Secundigravidae | 24            | 14              | 63.2 (46.0, 78.2)             | 28.9 (20.8, 38.2)    |

1 woman with a placental malaria result had missing data on gravidity.

Abbreviations: ANC, antenatal clinic; CI, confidence interval; PCR, polymerase chain reaction; RDT, rapid diagnostic test.

a Positivity for placental malaria is defined as the detection of malaria parasites in the placenta on histological examination.
infection missed by RDT (Table 4), but after adjusting for co-


covariates, only gravidity remained weakly associated, with missed infections being more frequent in secundigravidae than primigravidae OR 1.85 (1.00, 3.43) (P = .050).

**DISCUSSION**

Intermittent screening and treatment provides a possible approach to the management of malaria in pregnancy in certain epidemiological situations. There are, however, concerns that infections present at too low a density to be detected with an RDT could have an adverse effect on the outcome of pregnancy.

In Ghana, the sensitivity of a PLDH/HRP2 combination RDT in detection of malaria infection of peripheral blood was high throughout pregnancy until delivery when there was a substantial drop. The reason for the lower sensitivity of the RDT at delivery is likely to be the fact that the proportion of low-density infections was higher at this time than earlier in pregnancy because of treatment of RDT positive infections and possibly some acquisition of immunity as the pregnancy progressed. Mean

**Table 4. Risk Factors for an Rapid Diagnostic Test (RDT) Negative Malaria Infection, as Defined by a Positive Blood Film or RDT Test, in Pregnant Ghanaian Women at Any Stage During Pregnancy**

|                             | Infected*, RDT+ve | Infected*, RDT- | Crude OR | P Value | Adjusted OR | P Value |
|-----------------------------|-------------------|----------------|----------|---------|-------------|---------|
|                             | No.    | %       | No.    | %       |             |         |           |         |           |             |         |           |
| Gravidity                   |        |         |        |         |             |         |           |         |           |             |         |           |
| 1                           | 239    | 88.8    | 30     | 11.2    | [reference] |         | . .       |         | [reference] | . .       |         | . .       |
| 2                           | 148    | 76.7    | 45     | 23.3    | 2.42 (1.46, 4.02) | .001   | 1.85 (1.00, 3.43) | .050 |
| Gestational age at enrollment |        |         |        |         |             |         |           |         |           |             |         |           |
| <20 wks                     | 176    | 86.7    | 27     | 13.3    | [reference] |         | . .       |         | [reference] | . .       |         | . .       |
| 20–24 wks                   | 176    | 81.5    | 40     | 18.5    | 1.48 (0.87, 2.52) | .147   | 1.62 (0.92, 2.85) | .094 |
| 25–30 wks                   | 37     | 82.2    | 8      | 17.8    | 1.41 (0.59, 3.35) | .437   | 1.62 (0.65, 4.04) | .300 |
| Age group                   |        |         |        |         |             |         |           |         |           |             |         |           |
| under 18                    | 35     | 89.7    | 4      | 10.3    | [reference] |         | . .       |         | [reference] | . .       |         | . .       |
| 18–20                       | 180    | 89.1    | 22     | 10.9    | 1.07 (0.35, 3.30) | .907   | 0.95 (0.30, 3.02) | .934 |
| 21–24                       | 108    | 76.6    | 33     | 23.4    | 2.67 (0.89, 0.80) | .081   | 1.80 (0.54, 6.01) | .339 |
| 25 y +                      | 66     | 80.5    | 16     | 19.5    | 2.12 (0.66, 6.83) | .208   | 1.37 (0.37, 5.05) | .639 |
| Education                   |        |         |        |         |             |         |           |         |           |             |         |           |
| 1-None                      | 76     | 75.2    | 25     | 24.8    | [reference] |         | . .       |         | [reference] | . .       |         | . .       |
| 2-Basic                     | 264    | 87.1    | 39     | 12.9    | 0.45 (0.26, 0.79) | .005   | 0.63 (0.34, 1.17) | .139 |
| 3-Secondary                 | 40     | 80      | 10     | 20      | 0.76 (0.33, 1.74) | .516   | 0.89 (0.36, 2.25) | .812 |
| 4-Tertiary                  | 9      | 90      | 1      | 10      | 0.34 (0.04, 2.80) | .314   | 0.42 (0.05, 3.89) | .443 |
| Season enrolled             |        |         |        |         |             |         |           |         |           |             |         |           |
| early wet                   | 139    | 85.3    | 24     | 14.7    | [reference] |         | . .       |         | [reference] | . .       |         | . .       |
| late wet                    | 95     | 81.9    | 21     | 18.1    | 1.28 (0.67, 2.43) | .450   | 1.18 (0.60, 2.33) | .630 |
| early dry                   | 68     | 82.9    | 14     | 17.1    | 1.19 (0.58, 2.45) | .632   | 1.12 (0.53, 2.39) | .769 |
| late dry                    | 87     | 84.5    | 16     | 15.5    | 1.07 (0.54, 2.12) | .857   | 1.07 (0.52, 2.23) | .851 |
| SES                         |        |         |        |         |             |         |           |         |           |             |         |           |
| 1-wealthiest                | 67     | 83.8    | 13     | 16.3    | [reference] |         | . .       |         | [reference] | . .       |         | . .       |
| 2-wealthy                   | 71     | 88.6    | 11     | 13.4    | 0.80 (0.34, 1.91) | .612   | 0.86 (0.35, 2.11) | .738 |
| 3-medium                    | 88     | 88.9    | 11     | 11.1    | 0.64 (0.27, 1.53) | .318   | 0.76 (0.31, 1.90) | .558 |
| 4-poor                      | 74     | 77.1    | 22     | 22.9    | 1.53 (0.72, 2.88) | .272   | 1.88 (0.53, 6.28) | .132 |
| 5-poorest                   | 88     | 83      | 17     | 13      | 1.05 (0.48, 2.30) | .895   | 1.08 (0.46, 2.52) | .868 |

*p-values from Wald test.

Abbreviations: OR, odds ratio; PCR, polymerase chain reaction; RDT, rapid diagnostic test; SES, socio-economic status.

*a Infected = a positive PCR test or blood film.
Table 5. Associations Between Rapid Diagnostic Test (RDT) +ve and RDT –ve Malaria Infections, as Defined by Positive Microscopy or Polymerase Chain Reaction, and Various Outcome of Pregnancy

|                    | Uninfecteda | Infected, RDT+veb | Infected, RDT–vec |
|--------------------|-------------|-------------------|------------------|
| No.                | %           | No.               | %                |
| No active placental malaria | 64          | 70.3              | 154              | 71               |
| Active placental malaria | 27          | 29.7              | 63               | 29               |
| Adjusted OR (95% CI), P-value | [reference] | 0.97 (1.57, 1.66) | 0.911            |
| Adjusted OR (95% CI), P-value | [reference] | 0.93 (1.51, 1.68) | 0.807            |
| Normal birth weight | 97          | 78.2              | 257              | 81.8             |
| Low birth weight   | 27          | 21.8              | 57               | 18.2             |
| Adjusted OR (95% CI), P-value | [reference] | 0.80 (1.48, 1.33) | 0.386            |
| Adjusted OR (95% CI), P-value | [reference] | 0.84 (1.47, 1.50) | 0.552            |
| Hb at final antenatal clinic visit | 10.90 | 1.27              | 10.70            | 1.20             |
| Mean SD            | 2.73        | 0.50              | 2.73             | 0.49             |
| Mean SD            | 0.38        | 0.38              |                  |                  |
| Mean SD            | 0.38        | 0.38              |                  |                  |

Abbreviations: ANC, antenatal clinic; CI, confidence interval; Hb, hemoglobin; OR, odds ratio; PCR, polymerase chain reaction; RDT, rapid diagnostic test; SD, standard deviation.

a Uninfected. This group of women were negative for malaria infection by PCR, RDT, and microscopy when ever tested at a routine ANC visit.

b Infected RDT +ve. This group of women had a malaria infection detected by PCR or by microscopy, which was also detected by RDT.

c Infected RDT –ve. This group of women had a malaria infection detected by PCR or by microscopy, which was not detected by RDT.

d Active malaria infection. This is defined as the detection of malaria parasites in the placenta on histological examination with or without the presence of pigment and/or inflammatory cells.

e Adjusted OR/difference: multivariable model adjusting for gravidity, gestational age at enrollment, age, education level, socio-economic status, and season of delivery.

parasite densities were measured at different time points during pregnancy in women who had a positive blood film and did not show a significant decrease at delivery (data not shown), but this finding does not reflect the fact that at the time of delivery, a high proportion of infections may have been present at a density that was too low to be detected by microscopy but which were detected by PCR. The sensitivity of the RDT used in our study was high in 3 countries, but our study was restricted to analysis of P. falciparum infections in primigravidae and secundigravidae. Different results might have been obtained in multigravidae, in whom parasite densities are generally lower than in primigravidae, and in areas where P. vivax is prevalent, as RDTs are generally less sensitive in detecting P. vivax than P. falciparum infections.

The sensitivity of the RDT in detection of active malaria infection of the placenta in our study was 73.1% overall and 82.1% in primigravidae (Table 3). Previous studies of the sensitivity of RDTs in detecting "pregnancy malaria," reviewed by Fried et al [18], have shown variable results, in part because different end-points have been used including peripheral blood smear, placental blood smear, placental histology or a combination of these end-points. As expected, HRP2 tests have generally shown higher sensitivity than pLDH assays because of persistence of the HRP 2 antigen and because this antigen can access the circulation from sequestered parasites [19]. In 2 previous studies in which an HRP2 test on peripheral blood was used to detect placental malaria as defined by histology, the RDT had a sensitivity of 78% and 81% [20, 21], percentages similar to the 73% found in our study. PCR assays have, in general, shown a higher sensitivity in detection of placental malaria than an RDT [18], but in our study the sensitivity of PCR on peripheral blood was slightly lower than that of the RDT, despite the ability of the PCR assay used in our study to detect very low density infections (approximately 10 parasites per µL). The reason for the generally good performance of the RDT used in this study in comparison with PCR was not that the RDT had a low sensitivity.

We did not find that RDT negative/PCR positive infections were associated with any adverse effect on the outcome of pregnancy, perhaps contributing to the reason why ISTp-AL was found to be noninferior to IPTp-SP in our trial. However, only a small number of Ghanaian women (75) had a RDT negative/PCR positive infection, so the study was not powered to detect a small effect. Many previous studies have shown an association between a positive blood film, RDT or PCR assay and 1 or more adverse outcomes of pregnancy. Whether infections that are PCR positive but RDT or microscopy negative are associated with a poor outcome of pregnancy is less certain and is an important issue if ISTp is to be considered for implementation in some epidemiological situations. Some studies have shown an association between submicroscopic infections and maternal anemia [22–24] or low birth weight [25, 26], but other studies have not shown any association between PCR positive but RDT or microscopy negative infections and an adverse
outcome of pregnancy [27, 28] as in our study. The reasons for this discrepancy is uncertain but it may reflect differences in the characteristics of the study population, the number of subjects studied, and the sensitivity of the RDT and PCR assays used.

It has been suggested that ISTp could be adopted in areas where IPTp-SP is currently deployed but where the level of transmission has fallen to such a level that few pregnant women are at risk. However, in such situations, the cost per case of malaria prevented would be high. A possible, more cost effective, approach could be to restrict screening to first ANC visits as, in this study, this approach detected over 50% of all infections diagnosed in the pregnancy. Women who tested positive at enrollment were at increased risk of a further infection, and so these women could be followed with further screening. However, the lowest sensitivity of the RDT seen in this study was at the site with the lowest transmission; it is not clear if this is a causal association due to lower average parasite densities, but this is a possible explanation for this observation. It is also unclear if sensitivity would decline further over the course of pregnancy in lower transmission settings, and this needs to be investigated. As the incidence of malaria falls in many previously highly endemic areas, new approaches to the control of malaria in pregnancy need to be developed and evaluated.

Supplementary Data
Supplementary materials are available at http://cid.oxfordjournals.org.

Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes
Acknowledgments. We thank the women who participated in the trial, the many field, laboratory, and data management staff at each of the trial sites who contributed to its successful outcome and the Ministries of Health which supported this study. We thank Manuela Clatte for her administrative support for the trial. We also thank members of the Data Safety and Monitoring Board (Prof GAT Targett [chair], Dr GA Quansah-Asare, Prof F Little, Prof O Nyan, Dr C Ouedraogo and Prof S Sow). Raouf Osseni and Adama Gansane were the trial's clinical monitors. High-quality placental histology slides were prepared by the staff of the Department of Pathology, Korle Bu Hospital, Accra, Ghana and Dr Jaume Ordi assisted with standardisation of placental histology. We thank Prof Steve Taylor for help in quality control of the polymerase chain reaction (PCR) assay.

Authors role. J. E. W., S. L. Q., T. A., A. O., H. T., and K. B. supervised the field studies. F. N. did the PCR assays, and A. W. was responsible for data management. M. C., P. Milligan, and J. E. W. undertook the statistical analyses. P. Magnussen, F. O. t. K., D. C., and B. G. contributed to the study design and overall conduct of the trial. M. C., B. G., and H. T. drafted the manuscript. All authors participated in the review of study results and approved the final manuscript.

Financial support. The study was funded by the European Developing Country Clinical Trials Programme (grant number IP.2007.31080.003) and by the Malaria in Pregnancy Consortium which is funded through a grant from the Bill & Melinda Gates Foundation to the Liverpool School of Tropical Medicine. M. C. is supported by a fellowship jointly funded the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References
1. Eisele TP, Larsen DA, Anglewicz PA, et al. Malaria prevention in pregnancy, birth-weight, and neonatal mortality: a meta-analysis of 32 national cross-sectional data-sets in Africa. Lancet Infect Dis 2012; 12:942–9.
2. McClure EM, Goldenberg RL, Dent AE, Meshnick SR. A systematic review of the impact of malaria prevention in pregnancy on low birth weight and maternal anaemia. Int J Gynaecol Obstet 2013; 121:103–9.
3. World Health Organisation. Updated WHO policy recommendation: intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP). Geneva: World Health Organisation, 2012.
4. Gutman J, Mwandama D, Wiegand RE, Ali D, Mathanga DP, Skarbinski J. Effectiveness of intermittent preventive treatment with sulfadoxine-pyrimethamine during pregnancy on maternal and birth outcomes in Machinga district, Malawi. J Infect Dis 2013; 208:907–16.
5. Sridaran S, McClintock SK, Syphard LM, Herman KM, Barnwell JW, Udhyakumar V. Anti-folate drug resistance in Africa: meta-analysis of reported dihydrofolate reductase (dfrh) and dihydropterote synthase (dhp) mutant genotype frequencies in African Plasmodium falciparum parasite populations. Malar J 2010; 9:247.
6. Arnauttive A, Ades V, Watukara A, et al. Intermittent preventive therapy with sulfadoxine-pyrimethamine for malaria in pregnancy: a cross-sectional study from Tororo, Uganda. PLoS One 2013; 8:e23073.
7. Likwela JL, D’Alessandro U, Lokwa BL, Meuris S, Dromaix MW. Sulfadoxine-pyrimethamine resistance and intermittent preventive treatment during pregnancy: a retrospective analysis of birth weight data in the Democratic Republic of Congo (DRC). Trop Med Int Health 2012; 17:322–9.
8. Minja DT, Schmiegelow C, Mmbando B, et al. (2013) Plasmodium falciparum mutant haplotype infection during pregnancy associated with reduced birthweight, Tanzania. Emerf Infect Dis 2013; 9:1446–54.
9. Harrington WE, Morrison R, Fried M, Duffy PE. Intermittent preventive treatment in pregnant women is associated with increased risk of severe malaria in their offspring. PLoS One 2013; 8:e56183.
10. Harrington WE, Mutabingwa TK, Muehlenbachs A, et al. Competitive facilitation of drug-resistant Plasmodium falciparum malaria parasites in pregnant women who receive preventive treatment. Proc Natl Acad Sci U S A 2009; 106:9027–32.
11. Clinicaltrials.gov. NCT01103063 - Evaluate Azithromycin Plus Chloroquine And Sulphadoxine Plus Pyrimethamine Combinations For Intermittent Preventive Treatment Of Falciparum Malaria Infection In Pregnant Women In Africa. 2014. Available at: http://www.clinicaltrials.gov/ct2/show/NCT01103063?term=NCT01103063&rank=1. Accessed 1 April 2015.
12. González R, Mombo-Ngoma G, Ouidoalgo S, et al. Intermittent preventive treatment of malaria in pregnancy with mefloquine in HIV-negative women: a multicentre randomised controlled trial. PLoS Med 2014; 11:e1001733.
13. Desai M, Gutman J, Llanza M, Mutabingwa T. Intermittent screening and treatment (IST) or intermittent preventive treatment (IPT) with dihydroartemisinin-piperaquine versus IPT with sulphadoxine-pyrimethamine for the control of malaria in pregnancy in western Kenya: a randomized controlled superiority trial. Lancet 2015; 385:220–4.
14. Eisele TP, Larsen DA, Anglewicz PA, et al. Malaria prevention in pregnancy, birth-weight, and neonatal mortality: a meta-analysis of 32 national cross-sectional data-sets in Africa. Lancet Infect Dis 2012; 12:942–9.
15. Tagbor H, Bruce J, Agbo M, Greenwood B, Chandramohan D. Intermittent screening and treatment versus intermittent preventive treatment of malaria in pregnancy: a randomised controlled non-inferiority trial. PLoS One 2010; 5:e14425.
16. Tagbor H, Cairns M, Bojang K, et al. A non-inferiority, individually randomized trial of intermittent screening and treatment versus intermittent preventive treatment in the control of malaria in pregnancy. PLoS One 2015; 10:1371.
17. Sweyen C, Yekemans J, Bruls M, et al. Development of standardized laboratory methods and quality processes for a phase III study of the RTS,S/AS01 candidate malaria vaccine. Malar J 2011; 10:e223.
18. Fried M, Muehlenbachs A, Duffy PE. Diagnosing malaria in pregnancy: an update. Expert Rev Anti Infect Ther 2012; 10:1777–87.
19. Katzenberg JH, Tahita CM, Versteegh IAJ, et al. Antigen persistence of rapid diagnostic tests in pregnant women in Nanoro, Burkina Faso, and the implications for the diagnosis of malaria in pregnancy. Trop Med Int Health 2012; 17:550–7.
20. Mayor A, Moro L, Angeleri R, et al. How hidden can malaria be in pregnancy? Diagnosis by microscopy, placental histology, polymerase chain reaction and detection of histidine-rich protein 2 in plasma. Clin Infect Dis 2012; 54:1561–8.
21. Kyabayinze DJ, Tibenderana JK, Nassali M, et al. Placental Plasmodium falciparum malaria infection: operational accuracy of HRP2 rapid diagnostic tests in a malaria endemic setting. Malar J 2012; 10:e306.

22. Mockenhaupt FP, Rong B, Till H, et al. Submicroscopic Plasmodium falciparum infections in pregnancy in Ghana. Trop Med Int Health 2000; 5:167–73.

23. Cottrell G, Moussilou A, Luty AJF, et al. Submicroscopic Plasmodium falciparum infections are associated with maternal anemia, premature births and low birth weight. Clin Infect Dis 2015; 60:1481–8.

24. Adegnika AA, Verweij JJ, Agranijii ST, et al. Microscopic and sub-microscopic Plasmodium falciparum infection, but not inflammation caused by infection, is associated with low birth weight. Am J Trop Med Hyg 2006; 75:798–803.

25. Mohammed AH, Salih MM, Elhassan EM, et al. Submicroscopic Plasmodium falciparum malaria and low birth weight in an area of unstable malaria transmission in Central Sudan. Malar J 2013; 12:172.

26. Mankhambo I, Kanjala M, Rudman A, Lema VM, Rogerson SJ. Evaluation of the OptiMAL rapid antigen test and species-specific PCR to detect placental Plasmodium falciparum infection at delivery. J Clin Microbiol 2002; 40:155–8.

27. Cohee LM, Kalilani-Phiri I, Boudova S, et al. Submicroscopic malaria infection during pregnancy and the impact of intermittent preventive treatment. Malar J 2014; 13:274.

28. Singh N, Bharti PK, Singh MP, et al. What is the burden of sub-microscopic malaria in pregnancy in central India. Pathog Glob Health 2015; 109:30–8.