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Intermittent screening using ultra-sensitive malaria rapid diagnostic test and treatment with pyronaridine-artesunate compared to standard preventive treatment with sulfadoxine-pyrimethamine for malaria prevention in pregnant women in Kinshasa, DRC

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Abstract

Background The declining effectiveness of Intermittent Preventive Treatment in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) due to the emergence of *Plasmodium falciparum* resistance highlights the need for alternative malaria prevention strategies in pregnant women. A novel approach was proposed: screening with an ultra-sensitive rapid diagnostic test and treating positive with pyronaridine-artesunate (ISTp-uRDT-PA). This trial compared the impact of both strategies on maternal malaria and anaemia, abortion, intrauterine death, birth weight, preterm delivery.

Methods This non-inferiority trial, conducted in Kinshasa, enrolled pregnant women in their second and third trimesters. Participants in the IPTp-SP arm (n = 124) received SP at monthly antenatal visit as per guidelines, while those in the ISTp-uRDT-PA arm (n = 126) were screened monthly with an uRDT and treated with PA if positive. Primary outcomes included asymptomatic parasitaemia (uRDT positive without fever) or symptomatic parasitaemia (uRDT positive with fever or history of fever, and parasite density by microscopy during pregnancy.

Results Asymptomatic parasitaemia by uRDT during pregnancy was similar in both arms (20.8% in IPTp-SP vs 21.0% in ISTp-uRDT-PA). At delivery, asymptomatic parasitaemia was 51% higher in ISTp-uRDT-PA arm compared to IPTp-SP (cRR = 1.51 [95% CI 0.76–3.00], p = 0.24). Symptomatic parasitaemia by uRDT at delivery showed no significant difference. Malaria by microscopy at enrolment was detected in 34.4% of women. Malaria by microscopy during pregnancy was 9.6% in IPTp-SP and 10.1%. ISTp-uRDT-PA (p = 0.19), decreasing to 3.2% and 0.9%, respectively, at delivery

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(p = 0.24). Mean haemoglobin concentration at enrolment was 10.1 g/dl in the IPTp-SP and 9.8 g/dl in the ISTp-uRDT-PA with no significant difference in maternal anaemia at delivery (7%; cRR = 1.07 [95% CI 0.87–1.31], p = 0.52). No significant differences were found for spontaneous abortions and in utero death in both arms. The risk of a premature newborn declined by 14% in ISTp-uRDT-PA compared to the IPTp-SP arm (cRR = 0.86 [95% CI 0.29–2.85], p = 0.79) while low-birth-weight was not significantly higher (cRR = 1.74 [95% CI 0.86–3.53], p = 0.12).

Conclusion ISTp-uRDT-PA was non inferior to IPTp-SP and can be considered as a future alternative for IPTp-SP in case this intervention can no longer be used due to high SP resistance.

Clinical trials registration: NCT04783051.

Keywords Sulfadoxine-Pyrimethamine, Pyronaridine-artesunate, Ultra-sensitive rapid diagnostic test, Malaria in pregnancy, Democratic Republic of the Congo

Background

In sub-Saharan Africa, 12.7 million pregnancies were at risk of malaria infection in 2022 [1]. Malaria infections during pregnancy significantly contribute to adverse outcomes both during pregnancy and at birth [2, 3]. To prevent this, the World Health Organization (WHO) recommends administering three or more doses of intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) one month apart during the second and third trimester of pregnancy [4]. IPTp-SP addresses the potential misdiagnosis of malaria in pregnant women by treating them, regardless of their malaria infection status, with SP during antenatal care (ANC) visits. This strategy aims to reduce malaria-related morbidities by targeting maternal *Plasmodium* infections [5]. However, there is increasing concern that the spread of SP-resistant *P. falciparum* strains may pose a significant threat to the efficacy of this strategy in the (near) future [6–8]. SP resistance occurs through the combined effect of point-mutations in the dihydrofolate reductase (*dhfr*) and the dihydropteroate synthase (*dhps*) gene and together these decrease the susceptibility *of P. falciparum* to pyrimethamine and sulfadoxine, respectively. Infections having the triple mutant *dhfr*, i.e., a combination of N51I, C59R and S108N mutations, are common throughout Africa. When this triple mutation in *dhfr* is combined with double-mutant *dhps* (A437G and K540E), the risk of SP treatment failure is very high [9, 10]. Additionally, mutations in the Pf*dhps* gene, including *dhps*K540E and *dhps*A581G, contribute to haplotypes that confer resistance of *P. falciparum* to SP [11, 12]. In the Democratic Republic of Congo (DRC), the resistance profile to SP remains moderate, with the prevalence of the Pf*dhps* K540E mutation estimated at 32.6% [13].

Intermittent Screening and Treatment during pregnancy (ISTp) involves regular malaria testing with rapid diagnostic tests (RDTs) during ANC visits and treating positive cases with artemisinin-based combination therapy (ACT). This approach has been proposed due to high levels of SP resistance [14, 15]. ISTp aims to minimize the overuse of antimalarials, thereby reducing drug pressure on malaria parasites [16]. Nonetheless, the efficacy of ISTp largely depends on the diagnostic accuracy of the method used. A meta-analysis that included research conducted in West Africa, a region where the malaria parasites are still sufficiently sensitive to SP, as well as in Malawi and Kenya where SP resistance is high, suggested that conventional RDTs (co-RDTs) to detect a *Plasmodium* antigens lack the required sensitivity for considering ISTp as a suitable alternative intervention, even in regions with high SP resistance [17].

To enhance the ISTp strategy, the present study used ultrasensitive RDTs (uRDTs), which are believed to have a better sensitivity, compared to standard co-RDTs, for the detection of low *P. falciparum* parasitaemia [18–20]. The uRDTs have a detection limit of approximately 80 pg/mL, which is ten times lower than of co-RDTs and prolonged incubation time of 20 min [19, 21]. Upon detection of *Plasmodium* parasites with uRDT, treatment with Pyronaridine-Artesunate (PA) was initiated. PA is a new antimalarial, that has been approved by the European Medicines Agency for the treatment of uncomplicated acute malaria and is also registered in several African countries [22, 23]. It is also among the treatments recommended by the National Malaria Control Program (NMCP) of the Democratic Republic of the Congo (DRC) for the treatment of uncomplicated malaria [24]. PA is seen as an alternative treatment to other artemisinin-based combinations, whose efficacy is threatened by emerging resistance [25]. The combination of ISTp with uRDT and treatment with PA (ISTp-uRDT-PA) is thus anticipated to significantly improve the effectiveness of ISTp. Therefore, the study aimed to compare the conventional IPTp-SP strategy, as routinely implemented by the NMCP of the DRC, with ISTp-uRDT-PA, for the prevention of maternal malaria and anaemia, abortion, intrauterine deaths, low birth weight (LBW), and preterm birth in a malaria-endemic area in Kinshasa (DRC).

Methods

Study design

The study was implemented at "Maternité Esengo" located in Kisenso, one the 24 municipalities of Kinshasa, where malaria transmission is intense and perennial. A cross-sectional study involving pregnant women in this region showed a maternal malaria prevalence of around 30% [26].

The study was conducted from May, 2021 to June, 2022. The trial protocol can be accessed online [27]. This clinical trial has been registered: clinicaltrials.gov/study/NCT04783051.

This was a 2-arm, 1:1 ratio, randomized, non-inferiority trial including pregnant women in their 2nd semester, specifically at week 16 and above of pregnancy. Participants were assigned to either the ISTp-uRDT-PA arm or the IPTp-SP arm based on a pre-generated randomization list associated with identification code. The schedule of study visits was designed to coincide with standard ANC visits. Participants in the ISTp-uRDT-PA arm were screened using an uRDT and treated with PA upon a positive test result. Those assigned to the IPTp-SP arm received the standard regimen recommended by the NMCP of DRC at week 16, 28, 32, and 36 of their pregnancy [24].

Ethical consideration, data protection and confidentiality

The study strictly adhered to fundamental ethical principles and complied with applicable national and international regulations. Before the start of the study, the protocol was reviewed and approved by the National Ethics Committee of the DRC (approval reference: 169/CNES/BN/PMMF/2019 of March 13, 2020) and the Congolese Pharmaceutical Regulatory Authority (ACOREP) (approval reference: MS1253/P/DKK/01096/2020, 9 October 2020). The collection of personal information was strictly limited to what was needed for achieving the study's objectives. To protect participant's confidentiality, each participant was assigned a unique study identification code, ensuring that no names or personal identifiers were recorded in the database or disclosed in any subsequent publications.

Participants were enrolled on a voluntary basis after the research team has provided a comprehensive explanation of the study either in French or the local language, Lingala, and written informed consent was obtained in either of these two languages too. Each participant signed or placed a fingerprint to indicate her approval. The close follow-up of recruited pregnant women in this trial ensured that participants received a high standard of care during their pregnancy and delivery.

Study participants

The trial specifically targeted pregnant women attending ANC at the study site. Eligibility criteria for participants included a gestational age of 16 weeks or more, age of 18 years or older, residency within the study's catchment area, willingness to adhere to the study's principles and, to give birth at the maternity ward "Maternité Esengo" (Kisenso municipality). Exclusion criteria encompassed not meeting the above-mentioned inclusion criteria, a history of allergies to SP or ACT, ongoing prophylaxis with cotrimoxazole, or medical conditions necessitating hospital admission (such as severe malaria or high-risk pregnancies).

Sample size

This trial was designed as a non-inferiority trial in order to be able to determine whether ISTp-uRDT-PA would be an equal effective strategy in case ITPp-SP could no longer be used due to future emerging resistance against SP. The sample size required to detect a 10% difference in outcomes with a significance level of 5% and a power of 80% for assessing the non-inferiority of IST to IPTp-SP was determined to be 220 participants in total (for details see: [27]). To establish the non-inferiority of ISTp-uRDT-PA versus IPTp-SP, with a margin not exceeding a 10% difference in the percentages of women experiencing anaemia, malaria, and LBW, and accounting for an anticipated 10% loss to follow-up and/or non-compliance, the final sample size was adjusted at 250 participants (125 per arm).

Study treatments

Pyronaridine—Artesunate (PA; Shin Poong Pharmaceutical Company, South Korea) comes as a film-coated tablet containing 180 mg of pyronaridine tetraphosphate and 60 mg of artesunate. The recommended oral dosage is once daily for three days, tailored to body weight: 24– < 45 kg requires 2 tablets per day; 45– < 65 kg 3 tablets per day; ≥ 65 kg, 4 tablets per day [28].

Sulfadoxine-pyrimethamine (SP: Roche Laboratories, Switzerland) comes as a tablet containing 500 mg of sulfadoxine and 25 mg of pyrimethamine. In context of IPTp, the recommended dose for individuals weighing 31 to 45 kg is 2 tables, and for women weighing > 45 kg, it is three tablets [29].

Study procedures

Enrolment

Details on the study visits are presented in detail in the protocol paper [27]. At enrolment, demographic data, medical history, and obstetric history were recorded. The gestational age was determined on the basis of

the date of the last menstrual period for all women. A peripheral blood sample was collected for uRDT, thick and thin blood smear for malaria microscopy, and haemoglobin (Hb) concentration measurement using Hemocue© (HemoCue AB, Ängelholm, Sweden). Next, participants were randomized to either the IPTp-SP arm or the ISTp-uRDT-PA arm.

IPTp-SP arm

Participants in the IPTp-SP arm received a recommended single dose of SP, administered by a study nurse at every ANC visit. Follow-up visits for participants in the IPTp-SP arm were scheduled monthly. In principle, SP was administered at weeks 16, 28, 32, and 36 of pregnancy as recommended by the NMCP. Participants were encouraged to attend unscheduled visits if they experienced any health issues. Blood samples and tests (microscopy, uRDT, Hb) conducted at enrolment were also repeated during the follow-up. Concomitant treatment was administered according to the symptoms of each woman during the visits. Adverse events (AEs), and serious adverse events (SAEs) to evaluate the safety and tolerability of the treatments were reported. All pregnant women in the IPTp-SP arm presenting symptoms of uncomplicated malaria, were also additionally tested with uRDT, and if found positive, received treatment with artesunate lumefantrine (AL) according to NMCP guidelines. In case of severe malaria, injectable artesunate was used [24].

ISTp-uRDT-PA arm

Follow-up visits for the pregnant women in the ISTp-uRDT-PA arm were also scheduled monthly and the study participants were also encouraged to attend unscheduled visits if they experienced any health issues. Blood sampling and testing were done at enrolment and at subsequent visits. Concomitant treatment was provided according to woman 's symptoms observed during the visits. Additionally, AEs and SAEs were also reported. Participants in the ISTp-uRDT-PA arm were tested for malaria by uRDT at every ANC visit and if positive by uRDT, the participants in the ISTp-uRDT-PA arm received a three-days course of PA tablets. The first dose of PA was administered by study nurse and the subsequent doses were given to women to take them at home. Instructions were provided for self-medication, and in case of vomiting within 30–60 min post intake, the full dose of PA was repeated. In case of the development of severe malaria, women in the ISTp-uRDT-PA arm were also treated with injectable artesunate.

Procedures for pregnancy outcomes

For both study arms, pregnancy outcomes were recorded as soon as possible after delivery. At delivery, a clinical examination of the newborns was performed including assessment for visible congenital anomalies, and measurement of birth weight. Maternal and fetal peripheral blood was collected for further testing (uRDT, microscopy and Hb). For women who gave birth outside the health centre, active follow-up was conducted to collect certain pregnancy-related outcomes.

Laboratory procedures

Malaria microscopy

Thick and thin blood smears were stained using 10% Giemsa solution and examined by experienced microscopists. The microscopy findings did not influence treatment decisions. Each slide was independently read in duplicate, and the microscopists were blinded to the uRDT results and study arms assignments. Any discrepancies, whether in terms of positivity *versus* negativity or species differentiation, were judged by a third, blinded reader. Quantification of *Plasmodium* infection and parasitaemia in thick smears was based on the examination of 200 leukocytes, with results expressed per 8000 parasites per microlitre. A slide was deemed negative if no asexual stage of *Plasmodium* were identified after reviewing 100 microscopic fields.

uRDT

For the detection of malaria parasites in peripheral blood samples uRDTs (Alere Malaria Ag *P. falciparum* ultra-sensitive; batches 05LDF006B, 05LDG001B, 05BDDG043, 05LDG001A, Alere/Abbott, Republic of Korea, now called NxTek™ Eliminate Malaria *P. falciparum* RDT) were used as per manufacturer's instructions [21]. The test results were interpreted by two independent readers after 20 min according to the manufacturer's instructions. In case of discrepancies, a third reader interpreted the test and his decision was decisive.

Haemoglobin

Hb concentrations were determined using the Hemocue Hb 301 digital analyzer (HemoCue AB, Ängelholm, Sweden). Maternal peripheral blood samples were collected for Hb measurement at enrolment and during each subsequent ANC visit, whether scheduled or unscheduled. At the time of delivery, Hb concentrations were measured in both maternal and fetal peripheral blood samples. Maternal anaemia was categorized on the basis of Hb concentrations: severe anaemia for Hb < 8.0 g/dL; and moderate anaemia for Hb between

8.0 and 10.9 g/dL. Neonatal anaemia is defined as Hb concentration < 13.0 g/dL.

Data management and storage

All collected data were initially documented on the paper case report form (CRF) before being double entered into the Research Electronic Data Capture system. Data collection and management were executed using REDCap electronic data capture tools, hosted by the University of Antwerp (Belgium). Study monitors conducted periodic site visits to confirm the reliability of data entered in the electronic CRFs against source documents. The final database was compiled after addressing and resolving any discrepancies or queries.

Outcomes

Primary outcomes

The primary outcomes were: (1) the detection of asymptomatic parasitaemia (*P. falciparum* presence in peripheral blood detected by uRDT with a temperature < 37.5 °C) or symptomatic parasitaemia (*P. falciparum* presence in peripheral blood detected by uRDT with either a temperature ≥ 37.5 °C or a history of fever in the last 24 h) during pregnancy and at delivery, and () *P. falciparum* parasite density during pregnancy and at delivery as assessed by microscopy.

Secondary outcomes

During pregnancy, anaemia and the incidence of spontaneous abortion (natural termination of pregnancy before 20 weeks gestation), and intrauterine death (loss of pregnancy after 20 weeks gestation) were assessed. At delivery neonatal mortality or morbidity (preterm birth: birth before 37 weeks gestation; LBW: birth weight < 2500 g) were assessed.

AE reported during the entire study period were recorded to assess safety. AEs were considered serious if they met any of the following criteria: the event resulted in death, required hospitalization, constituted a congenital anomaly, was life-threatening, or caused disability.

Statistics

Main outcomes analysis was performed as per-protocol population (PP) and modified intention-to-treat population (mITT) as well. The PP analysis included data only from women who strictly adhered to their assigned randomization arm and of whom the main study outcomes were recorded. Specifically, women had attended a minimum of one visit after enrolment, during which they received at least one course of SP (IPTp-SP arm) or were screened at least once using an uRDT at scheduled visits (ISTp-uRDT-PA arm) after enrolment. Additionally, they have been assessed for maternal malaria and maternal anaemia at 36–40 weeks gestation.

In the modified intent-to-treat analysis (mITT), data from all eligible women who were randomized, received at least one study intervention, and contributed to the outcome were included. This meant that women had to have received at least one course of SP (for IPTp-SP arm) or been screened at least once using an uRDT at scheduled visits (for ISTp-uRDT-PA arm) and tested for maternal malaria and maternal anaemia at 36–40 weeks gestation, at time of delivery.

Using the generalized linear model, study arms were first compared for binary responses with unadjusted log-binomial models in PP and mITT populations experiencing each outcome for the trial arms, and the associated 2-sided 95% CI for the risk ratio. Next, outcomes were adjusted for women age, insecticide treated bed net (ITN) use and gravidity. Univariate and multivariate analyses were run for actors associated with anaemia at delivery and low weight. Continued variables were reported as mean, standard deviation or median, and Interquartile range. Descriptive statistics were used to summarize the data for safety analysis. The safety populations included all enrolled women who received at least one dose of study medication and all-live-born babies.

All analysis were done using Stata version 17 (StataCorp, College Station, Texas).

Results

Demographic and clinical characteristics at enrolment

A total of 285 pregnant women were screened, and 250 were randomly assigned to either the IPTp-SP arm (n = 124) or the ISTp-uRDT-PA arm (n = 126) (Fig. 1). Among the 35 pregnant women excluded during the screening, 22 did not meet the inclusion criteria, 10 refused to be enrolled in the trial and 3 were excluded for other reasons (refusal from the husband, family, and/or in-laws).

During follow-up, 72 pregnant women in the IPTp-SP arm received 3 doses of SP, 26 received 2 doses, and 26 received a single dose. Seventy-nine (79) pregnant women in the ISTp-uRDT-PA arm received PA treatment because they were found malaria positive: 42 received it once, 22 twice and 15 more than twice. Additionally, 47 pregnant women did not receive PA (uRDT negative) during the whole study period.

Pregnant women in both study arms had similar characteristics at enrolment (Table 1). Almost half the women in both arms were under 25 years of age, with an overall mean age of 26.5 ± 6.3 years. Thirty-seven point six percent (37.6%) of pregnant women were primigravida, 36.4% secundigravida and 26.0% were multigravida.

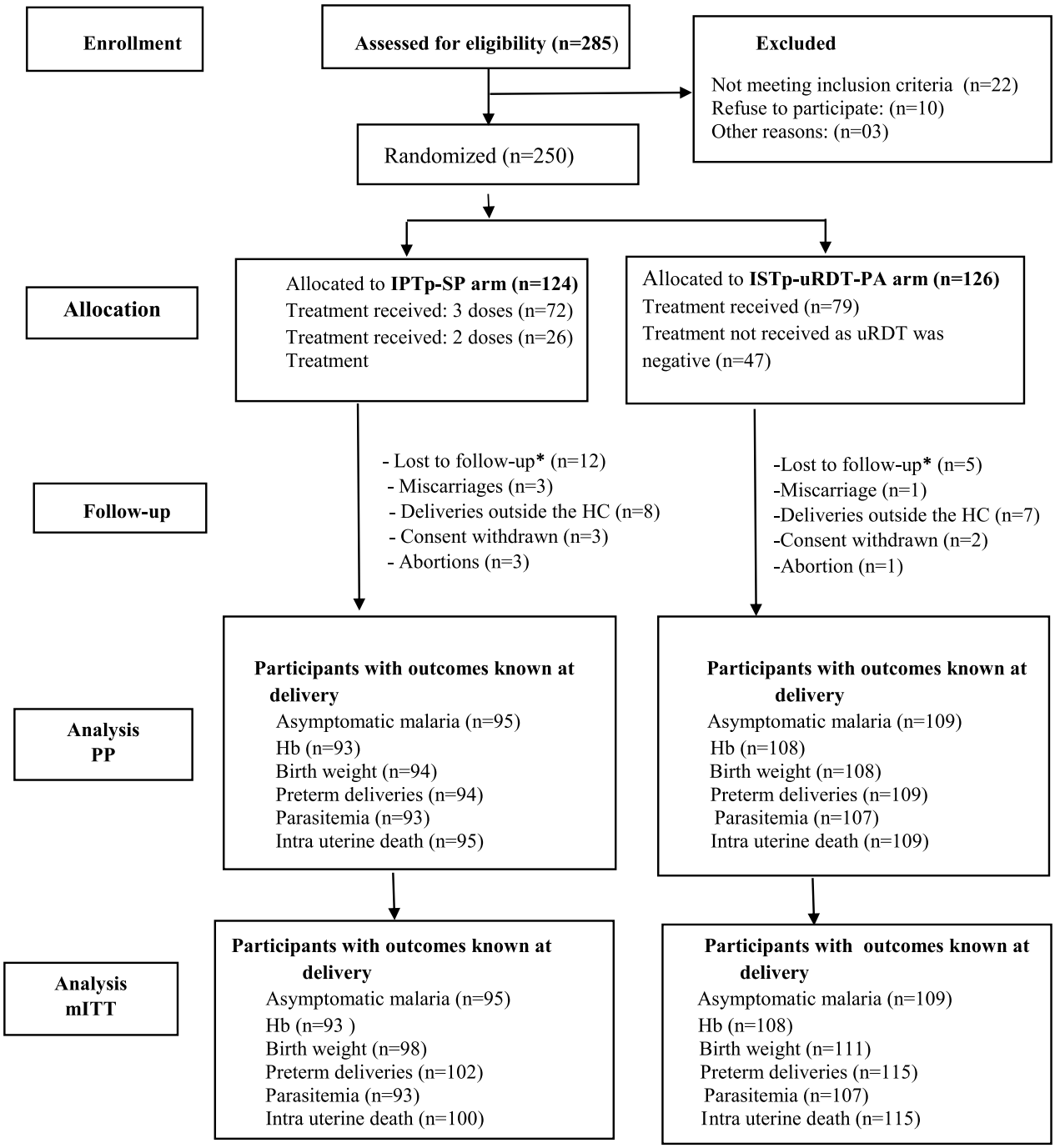

Fig. 1 Flow chart showing participants at enrolment and during the follow-up. Abbreviations: n: number; Hb: haemoglobin; PP: per protocol analysis; mITT: modified intent-to-treat analysis

Forty-two point two percent (42.2%) were nulliparous, and 12.8% were multiparous. Twenty-eight percent (28.0%) of women slept under an insecticide treated bed net the night before enrolment. The overall mean Hb concentration in the IPTp-SP arm was 10.1 g/dl [95% CI (8.9–11.8)] compared to 9.8 g/dl [95% CI (8.2–11.3)] in the ISTp-uRDT-PA arm. However, this difference was not statistically significant ($p=0.15$). Fifteen women (12.1%) in the ISTp-uRDT-PA arm and thirteen (11.0%) in the IPTp-SP arm had a Hb concentration < 8.0 g/dl. The prevalence of malaria, as determined by microscopy at enrolment, was 34.4% overall, with 36.5% in the

Table 1 Baseline characteristics of the pregnant women assigned to the different study arms

	Study arm		P-value	Total
	ISTp-uRDT-PA n (%)	IPTp-SP n (%)		
Maternal age (in years)	(n = 126)	(n = 124)		(n = 250)
≤ 25	65 (52.0)	59 (47.6)	0.53	124 (50.0)
26–30	29 (23.0)	27 (21.8)	0.81	56 (22.4)
≥ 31	31 (25.0)	38 (30.6)	0.28	69 (27.6)
Mean (SD)	25.9 (6.2)	27.0(6.3)	0.16	26.5 (6.3)
Used an insecticide treated bed net previous night	34 (27.0)	36 (29.0)	0.72	70 (28.0)
Pregnancy number (gravidity)				
Primigravidae	49 (38.9)	45 (36.3)	0.67	94 (37.6)
Secundigravidae	51 (40.5)	40 (32.3)	0.18	91 (36.4)
Multigravidae	26 (20.6)	39 (31.4)	0.51	65 (26.0)
Parity				
Nulliparous	57 (45.2)	49 (39.5)	0.36	106 (42.2)
Primiparous	25 (19.8)	24 (19.35)	0.92	49 (19.6)
Secundiparous	32 (25.4)	31 (25)	0.94	63 (25.2)
Multiparous	12 (9.5)	20 (16.1)	0.12	32 (12.8)
Pregnancy age (Weeks)				
16–20	97 (77.0)	92 (74.2)	0.61	189 (75.6)
21–28	29 (23.0)	32 (25.8)	0.59	61 (24.4)
Laboratory findings				
Thin smear positive	46 (36.5)	40 (32.2)	0.48	86 (34.4)
Parasitaemia (p/µL) Median (Min–Max)	3,558 (23–18,125)	3,920 (152–25,580)		3,722 (23–25,580)
Asymptomatic parasitaemia	60 (47.6)	57 (46.0)	0.84	117 (46.8)
Symptomatic malaria	31 (24.6)	37 (29.8)	0.35	68 (27.2)
Heamoglobin (g/ dL)	(n = 124)	(n = 118)		(n = 242)
≥ 11.0	25 (20.2)	36 (30.5)	0.06	61 (25.2)
8.0–10.9	84 (67.7)	69 (58.4)	0.13	153 (63.2)
< 8.0	15 (12.1)	13 (11.0)	0.79	28 (11.6)
Mean	9.8 (1.5)	10.1 (1.1)	0.07	9.9 (1.6)

N: Number; SD: standard deviation; Min: minimal; Max: maximum; IPTp-SP: intermittent preventive treatment with Sulfadoxine-Pyrimethamine; ISTp-uRDT-PA: intermittent screening with ultra-sensitive RDT and treatment with Pyronaridine-artesunate

ISTp-uRDT-PA arm and 32.2% in the IPTp-SP arm. Furthermore, the prevalence of malaria infection among women in both the ISTp-uRDT-PA and IPTp-SP arms, as detected by uRDT, was similar at 49.2%.

Study outcomes according to per protocol (PP) analysis

At the end of the follow-up period, the following evaluable records were available for the various outcomes from the study cohort of 250 women: delivery Hb concentration for 201 women (80.4%), birth weight for 202 newborns (80.8%), asymptomatic parasitaemia for 204 participants (81.6%), parasite density at the delivery for 200 women (80.0%), a live birth for 227 cases (90.8%), intrauterine death for 215 participants (86.0%), and prematurity for 202 study cases (80.8%). The number of pregnant women included in the PP population for birth weight estimation were slightly lower than in the mITT analysis (80.8% versus 83.6%). The study also observed withdrawal of 22 participants, with 15 from the IPTp-SP arm and 7 from the ISTp-uRDT-PA arm.

Primary outcomes

Detection of asymptomatic or symptomatic parasitaemia

At enrolment, the prevalence of asymptomatic parasitaemia was 47.6% (60/126) in the ISTp-uRDT-PA arm and 45.9% (57/124) in the IPTp-SP arm with no statistically significant difference between the two arms (p = 0.84) (Table 1). During different follow-up visits, the overall prevalence of asymptomatic parasitaemia was comparable between the two arms, at 20.8% for the IPTp-SP arm and 21.0% for the ISTp-uRDT-PA arm (Table 2). At delivery, the risk of asymptomatic parasitaemia was 51% higher in ISTp-uRDT-PA (17.4%) compared to IPTp-SP arm (11.6%), however this increased risk was not

Table 2 Comparison of asymptomatic parasitaemia detected by uRDT in enrolled women during pregnancy and at delivery

	Total		PP analysis IPTp-SP		ISTp-uRDT-PA		p-value
	n	Positive (%)	n	Positive (%)	n	Positive (%)	
At enrolment	250	123 (49.2)	124	61 (49.2)	126	62 (49.2)	0.99
Visit 1	223	25 (11.2)	110	14 (12.7)	113	11 (9.7)	0.47
Visit 2	220	36 (16.4)	106	16 (15.1)	114	20 (17.5)	0.62
Visit 3	193	22 (11.4)	92	12 (13.0)	101	10 (9.9)	0.49
Visit 4	145	16 (12.4)	69	6 (8.7)	76	10 (13.2)	0.39
Visit 5	75	4 (5.3)	39	2 (5.1)	36	2 (5.6)	0.93
Visit 6	21	2 (9.5)	12	2 (16.7)	9	0 (0)	0.19
Visit 7	5	1 (20.0)	4	1 (25.0)	1	0 (0)	0.57
Unscheduled visit	83	25 (30.1)	40	10 (25.0)	43.0	15 (34.9)	0.32
During pregnancy	1215	254 (20.9)	596	124 (20.8)	619	130 (21.0)	0.99
Delivery	204	30 (14.7)	95	11 (11.6)	109	19 (17.4)	0.24

IPTp-SP: intermittent preventive treatment with Sulfadoxine-Pyrimethamine; IST-Us-PA: intermittent screening and treatment with Pyronaridine-artesunate
PP:per protocol analysis

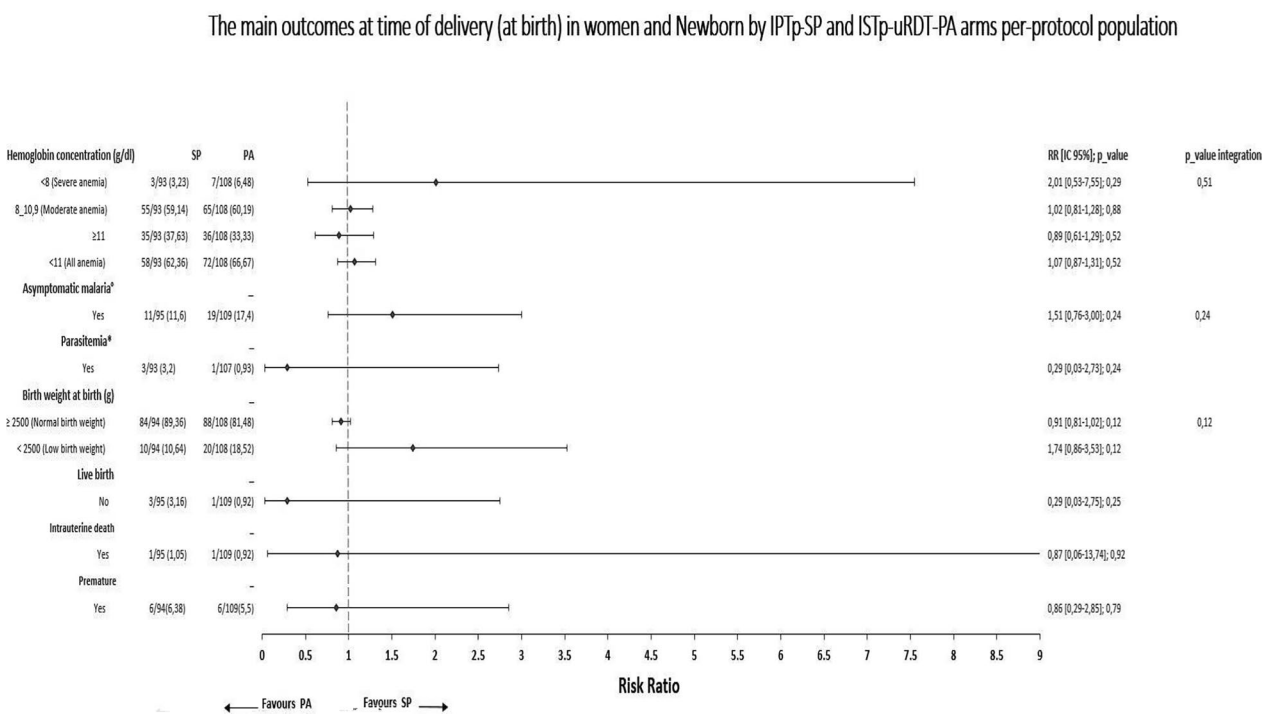

Fig. 2 Maternal outcomes: anaemia, malaria, asymptomatic parasitaemia, parasitaemia, birthweight at birth, live at birth, intrauterine death, premature. *p*-value represents difference between Hb, asymptomatic and parasitaemia strata; ***** Maternal plasmodium infection detected by microscopy **°** Maternal plasmodium infection detected by uRDT without fever (temperature < 37.5 °C) or history of fever in the last 24 h. SP: sulfadoxine-pyrimethamine; PA: Pyronaridine artesunate; IPTp-SP, intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine; ISTp-uRDT-PA, intermittent screening and treatment in pregnancy with Pyronaridine-artesunate; RR: risk ratio; Hb: haemoglobin; g: grams; g/dL: gram per decilitre

statistically significant (crude Risk Ratio, cRR = 1.51 [95% CI 0.76–3.00], p = 0,24) (Fig. 2). After adjusting for covariates (maternal age, gravidity, ITN use), the increased risk of asymptomatic parasitaemia remained statistically non-significant (adjusted risk ratio, aRR = 1.60 [95% CI 0.80–3.18], p = 0,18).

At enrolment, the prevalence of symptomatic parasitaemia was 29.8% (37/124) in the IPTp-SP arm and 24.6% (31/126) in the ISTp-uRDT-PA arm, and the difference was not statistically significant (p = 0.35). At delivery, symptomatic malaria detected by uRDT was observed in just one woman in the IPTp-SP arm, while none of the women in the ISTp-uRDT-PA arm was symptomatic.

Plasmodium falciparum parasite density

At enrolment, the prevalence of malaria detected by microscopy was 32.2%, with a geometric mean parasite density (GMPD) of 7.02 parasite/µL (95%CI 6.38–7.65) in the IPTp-SP arm and 36.5% with a GMPD of 7.55 parasite/µL (95%CI 7.17–7.93) in the ISTp-uRDT-PA arm. The parasite densities at enrolment were not significantly different between the two study arms (p = 0.15). Throughout pregnancy, the overall prevalence of malaria detected by microscopy was not statistically significant different (p = 0.8) between the IPTp-SP (9.9%) and ISTp-uRDT-PA (10.5%), respectively (Table 3). The GMPD during the pregnancy was 7.4 parasite/µL in the ISTp-SP arm and 7.73 in ISTp-uRDT-PA arm (p = 0.29). Parasite positivity rates detected by microscopy during the pregnancy were equivalent across both arms (Table 3). At delivery, malaria prevalence detected by microscopy reduced to 3.2% in the IPTp-SP arm, with only 3 women testing positive by microscopy, with a (GMPD) of 5.13 (95%CI 4.89–5.51) whereas one positive case (GMPD of 5.09) was detected in the ISTp-uRDT-PA (cRR = 0.29 [95%CI 0.03–2.73], p = 0.24) (Fig. 2). Adjusted results were consistent, with no statistically difference observed (aRR = 0.26 [95% CI 0.03–2.46], p = 0.24).

Secondary outcomes

Maternal haemoglobin concentration

There were no significant differences in Hb concentrations between the two study arms. During pregnancy, the overall cumulative prevalence of severe anaemia (Hb < 8.0 g/dl) was 6.1% in ISTp-uRDT-PA and 2.1% in IPTp-SP (p = 0.23). Moderate anaemia (8–10.9 g/dl) was observed at a similar rate, with 45.2% in IPTp-SP and 45.9% in ISTp-uRDT-PA (p = 0.44). The cumulative mean Hb concentration during pregnancy (mean of all monthly visits during pregnancy) was 10.3 g/dl in ISTp-uRDT-PA and 10.6 g/dl IPTp-SP, (p = 0.33).

After running univariate and multivariate logistic regression, neither allocation to a study arms, maternal age, gestation, gravidity nor baseline parasitaemia were associated with anaemia at delivery. Although the risk of anaemia in ISTp-uRDT-PA arm was 7.0% higher compared to women in IPTp-SP arm, but this was not statistically different (cRR = 1.07 [95%CI 0.87–1.31], p = 0.53 (Additional Table 1). After adjusting for maternal age, gravidity and ITN use, the risk remained non-significant (aRR = 1.06 [95%CI 0.87–1.31], p = 0.52. The risk of moderate anaemia was slightly higher (2.0%) in the ISTp-uRDT-PA arm compared to the IPTp-SP arm (cRR = 1.02 [95%CI 0.81–1.28], p = 0.88). Adjusted analysis confirmed the non-significance (aRR = 1.03 [95%CI: 0.82–1.30], p = 0.27 Similarly, the risk of having anaemia at delivery was slightly higher in ISTp-uRDT-PA (66.67%) arm compared to IPTp-SP arm (62.36%). However, this increased risk of having anaemia at delivery was not statistically significant (Crr = 1.07 [95%CI 0.87–1.31], p = 0.52) (Fig. 2). Adjusted results remained consistent with no-significant

Table 3 Parasite density expressed as parasites per µl blood and determined by microscopy in women during different study visits

Visit	IPTp-SP n	PP analysis			Geometric Mean	SD	p-value
		Geometric Mean	SD	ISTp-uRDT-PA n			
Enrolment	40	7.02	1.98	46	7.55	1.28	0.15
Visit 1	4	7.39	3.14	2	6.68	1.06	0.68
Visit 2	5	8.63	1.95	4	7.49	1.54	0.33
Visit 3	1	8.69	0	0	0		
Visit 4	4	8.09	1.23	1	11.63	0	
Visit 5	0	0		1	7.69	0	
Unscheduled Visit	5	8.39	2.44	11	8.42	1.72	0.98
During pregnancy	59	7.4	2.06	65	7.73	1.36	0.29
Delivery	3	5.13	0.33	1	0.35	5.09	0.92
Newborn visit	0	0		1	5.99	0	
Cumulative number	62	7.29	2.07	67	7.69	1.46	0.20

Only data from women who were found positive by microscopy were used to determine the geometric means of parasite density

SD: standard deviation; n = number; SD: standard deviation; n = number; IPTp-SP: intermittent preventive treatment with Sulfadoxine-Pyrimethamine; IST-Us-PA: intermittent screening and treatment with Pyronaridine-artesunate; PP: per-protocol analysis

difference observed (aRR = 1.07 [95%CI 0.87–1.31], p = 0.51. The risk of pregnant women having severe anaemia at delivery was 2.0 times higher in ISTp-uRDT-PA arm (6.5%) compared to women in IPTp-SP arm (3.2%) (cRR = 2.1 [95%CI 0.53–7.55], p = 0.29) (Fig. 2). However, the increased risk remained non-significant after adjustment (aRR = 1.91 [95%CI 0.51–7.15], p = 0.34.

Fetal loss

Spontaneous abortions (spontaneous loss of pregnancy before 20 weeks of gestation) were observed in 1/109 (0.9%) women in the ISTp-uRDT-PA arm and 3/95 (3.2%) women in the IPTp-SP arm (difference was not statistically significant: p = 0.25) (Table 4). In utero deaths (loss of pregnancy after 20 weeks of gestation) were observed in 1/95 (1.1%) and 1/109 (0.9%) in the ISTp-uRDT-PA and IPTp-SP arms, respectively, and this difference was not statistically significant (cRR = 0.87 [95%CI 0.06–13.74], p = 0.92)(Fig. 2).

Prematurity

Prematurity was observed in 6.4% in the IPTp-SP arm and 5.1% in the ISTp-uRDT-PA arm (Table 4). The risk of pregnant women allocated to the ISTp-uRDT-PA arm of giving to premature newborn birth was 14.0% higher compared to IPTp-SP arm, but this difference was not statistically significant (cRR = 0.86 [95%CI 0.29–2.85], p = 0.79) (Fig. 2). Adjusted results remained consistent showing no-significant difference (aRR = 0.82 [95%CI 0.27–2.46], p = 0.72).

Birth weight

The overall prevalence of LBW was 14.9% among all study participants. Ten newborns (10/94; 10.6%) in the IPTp-SP arm had LBW compared to 18.5% (20/108) in the ISTp-uRDT-PA arm. The mean birth weight was statistically not different (p = 0.128) between the IPTp-SP arm (3031.9 g ± 506.6 g) and the ISTp-uRDT-PA arm (2941 g ± 433.3 g) (Table 2).

Table 4 Key outcome characteristics of the pregnant women enrolled at delivery stratified per Study arm

Outcomes	PP analysis Treatment	Total
	ISTp-uRDT-PA n (%)	IPTp-SP n (%)
Birth issues		
Weigth		
Low birth weight	20/108 (18.5)	10/94 (10.6) 30 (14.8)
Normal birth weight	88/108 (81.5)	84/94 (89.4) 172 (85.1)
Mean (SD)	2,941.1 (443.3)	3,031.9 (506.6) 2,983.4 (474.8)
Preterm Birth < 37 week	6/109 (5.5)	6/94 (6.4) 12 (5.9)
In utero death[a]	1/109 (0.9)	1/95(1.0) 2/206 (1.0)
Abortion[b]	1/109 (0.9)	3 /95(3.1) 4/204(1.9)
Maternal Laboratory		
Microscopy	1/107 (0.9)	3/93 (3.2) 4/200 (2.0)
Asymptomatic parasitaemia	19/107 (17.8)	11/93 (11.8) 30/200 (15)
Heamoglobin (g/ dL)		
≥ 11.0	36/108 (33.3)	35/93 (37.6) 71/201 (35.3)
8.0–10.9	65/108 (60.2)	55/93 (59.1) 120/201 (59.7)
< 8.0	7/108 (6.5)	3/93 (3.2) 10/201 (5.0)
Mean (SD)	10.3(1.5)	10.4 (1.5) 10.3 (1.5)
Laboratory findings on newborn		
Microscopy	1/107 (0.9)	1/96 (1.0) 2/203 (1.0)
Heamoglobin (g/ dL)		
Hb ≥ 13.0	103/105 (98.1)	88/91 (61.2) 191/196 (97.4)
Hb < 13.0	2/105 (1.9)	3/91 (3.3) 5/196 (2.6)
Mean (SD)	17.4 (3.3)	17.9 (2.6) 17.7 (2.6)

%: percent; SD: standard deviation; < :inferior; ≥ **:** greater than or equal to; SD: standard deviation; n = number; IPTp-SP: intermittent preventive treatment with Sulfadoxine-Pyrimethamine; IST-uRDT-PA: intermittent screening and treatment with Pyronaridine-artesunate; g/dL: gram per decilitre; PP: PP: per-protocol analysis

[a] Natural termination of pregnancy before 20 weeks gestation, n: number

[b] loss of pregnancy after 20 weeks gestation

Baseline parasitaemia, age, gravidity and gestation of pregnant women were not significantly associated with LBW of their newborn (Additional Table 2). The risk of having a low-birth-weight newborn was 74% higher in the ISTp-uRDT-PA arm than in the IPTp-SP arm, but the association between the study arm and LBW was not statistically significant (cRR = 1.74 [95%CI 0.86–3.53], p = 0.12) (Fig. 2). Conversely, after adjusting for maternal age, gravidity, and ITN use, newborns in ISTp-uRDT-PA arm had 40% lower risk of LBW compared to those in IPTp-SP arm although this difference also remained non-significant (aRR = 0.62 [95%CI 0.30–1.25],p = 0.18.

Fetal viability and neonatal anaemia

The incidence of non-viable newborn births in the IPTp-SP arm was 3.16%, compared to 0.92% in the ISTp-uRDT-PA arm. However, the difference in the risk of delivering a non-viable newborn between the two study arms was not statistically significant different (RR = 0.29 [95%CI 0.03–2.75], p = 0.25) (Fig. 2). The prevalence of neonatal anaemia (Hb < 13 g/dl) was higher in IPTp-SP arm (3.3%) *versus* 1.9% in the ISTp-uRDT-PA arm.

Other safety outcomes

In the IPTp-SP arm, 30 participants experienced serious adverse events, including one case of skin rash that led to the discontinuation of SP treatment and one case of congenital malformation (cleft lip and palate) (Table 5) (Additional Table 3). Eleven serious adverse events were reported by participants in the ISTp-uRDT-PA arm. Comparative analysis did not reveal a statistically significant differences between the two study arms concerning the incidence of premature deliveries, abortions, or intrauterine deaths. No maternal deaths were reported.. The majority of minor adverse events observed from the last visit before delivery included headaches, physical weakness, abdominal pain, and anorexia. These adverse events were not significantly different between both study arms, with exception of reproductive and breast disorders which showed a statistically significant difference (p = 0.02). Additionally, a higher, but not statically significant (p = 0.36), incidence of fever was noted among the IPTp-SP arm (16 cases) compared to the ISTp-uRDT-PA arm (11 cases) (Table 6). A full presentation of all SAEs and AEs related to the use of PA in pregnant women will be presented in the paper that describe the outcomes of the PYRAPREG project [22].

Modified intention to treat analysis (mITT)

The mITT analysis showed similar results to those of the PP analysis for both primary and secondary outcomes. No significant differences were found for the mains outcomes at delivery compared to the PP analysis as well.

Discussion

This trial is the first to compare the standard of care (IPTp-SP) with intermittent screening and treatment (IST) for malaria control in pregnancy using uRDTs and PA as the treatment drug for test-positive women (IST-uRDT-PA). All previous trials comparing ISTp used co-RDTs and other ACTs, including dihydroartemisinin-piperaquine (ISTp-DP) as the treatment drug. This study adds new information to the limited landscape of evidence on the role of test-and-screen strategies in sub-Saharan Africa.

In general, the pregnant women enrolled in the current study adhered well to both strategies as there were relatively few lost to follow-up (< 10%) during the whole study. There was motivation among women to participate in regular malaria screening, likely due to the perceived

Table 5 Overview of adverse events in enrolled women

Adverse event, n (%)	PP analysis	
	IPTp-SP n = 124	ISTp-uRDT-PA n = 126
Number of AEs	97	98
Study participants with AEs	21 (16.9)	19 (15.1)
Study participants with SAEs	19 (15.3)	11 (8.7)
Study participants with treatment-related AES	0	0
Number of deaths	0	0
Study participants who discontinued treatment due to AES	1 (0.8)	0
Study participants who temporarily discontinued treatment due to AES	0	1 (0.8)

SD: standard deviation; n = number; IPTp-SP: intermittent preventive treatment with sulfadoxine-Pyrimethamine; IST-uRDT-PA: intermittent screening and treatment with Pyronaridine-artesunate; SAE: serious adverse event; AEs: adverse events

PP: per-protocol analysis

Table 6 Comparison of adverse events among enrolled women

Preferred term[a]	IPTp-SP		PP analysis				
			ISTp-uRDT-PA		Total		p-value
	(n = 96)		(n = 93)		(n = 189)		
	n	%	n	%	n	%	
Nervous system disorders	16	16.7	17	18.3	33	17.5	0.77
Headache	14	14.6	12	12.9	26	13.8	0.74
Tinnitus	0	0.0	1	1.1	1	0.5	0.31
Dizziness	1	1.0	4	4.3	5	2.7	0.16
Behavioural change	1	1.0	0	0.0	1	0.5	0.32
Gastrointestinal disorders	25	26.0	23	24.7	48	25.4	0.84
Anorexia	7	7.3	8	8.6	15	7.9	0.74
Abdominal pain	7	7.29	10	10.75	17	9.0	0.4
Nausea	4	4.2	2	2.2	6	3.2	0.43
Diarrhoea	2	2.1	0	0.0	2	1.1	0.16
Vomiting	5	5.2	3	3.2	8	4.2	0.49
General disorders	25	26.0	15	16.1	40	21.2	0.09
Asthenia	9	9.4	4	4.3	13	6.9	0.17
Fever	16	16.7	11	11.8	27	14.3	0.34
Muscle, skeletal and connective tissue disorders	11	11.5	14	15.1	25	13.2	0.47
Hypogastralgia	0	0.0	2	2.2	2	1.1	0.15
Arthralgia	3	3.1	4	4.3	7	3.7	0.67
Rib pain	0	0.0	1	1.1	1	0.5	0.3
Pelvic pain	3	3.1	1	1.1	4	2.1	0.33
Low back pain	1	1.0	3	3.2	4	2.1	0.3
Lumbohypogastralgia	4	4.2	3	3.2	7	3.7	0.73
Respiratory disorders	11	11.5	11	11.8	22	11.6	0.94
Cough	4	4.2	5	5.4	9	4.8	0.69
Cold	3	3.1	2	2.2	5	2.7	0.68
Flu	4	4.2	4	4.3	8	4.2	0.96
Reproductive and breast disorders	3	3.1	11	11.8	14	7.4	0.02
Left breast abscess	0	0.0	1	1.9	1	0.5	0.31
Vaginal discharge	3	3.1	7	7.5	10	5.3	0.18
Leukorrhoea	0	0.0	3	3.2	3	1.6	0.08
Pregnancy conditions	3	3.1	0	0.0	3	1.6	0.08
Abortion threat	3	3.1	0	0.0	3	1.6	0.08
Skin and subcutaneous tissue disorders	3	3.1	7	7.5	10	5.3	0.18
Pruritus	2	2.1	5	5.4	7	3.7	0.23
Skin rash	1	1.0	2	2.2	3	1.6	0.54

n = number of the events; IPTp-SP: intermittent preventive treatment with Sulfadoxine-Pyrimethamine; IST-uRDT-PA: intermittent screening and treatment with Pyronaridine-artesunate

[a] MedDRA preferred term; PP: Per protocol analysis

risks that malaria could pose to their infants, as conveyed by the study personnel. This inclination towards regular screening has also been documented in other screening and treatment studies [30].

For the primary study outcomes it was noted that symptomatic and asymptomatic parasitaemia detected by uRDT as well as parasite density detected by microscopy in pregnant women did not significantly differ during the study in both study arms. However, it was observed that parasite density detected by microscopy at delivery was slightly more prevalent, but not statistically significant higher, in the IPTp-SP arm compared to the ISTp-uRDT-PA arm. This observation is consistent with the study of Kayiba et *al.*, which demonstrated that IPTp-SP

did not reduce maternal malaria at delivery [13]. This reduced efficacy of IPTp-SP may be attributed to a possible diminished prophylactic effectiveness of the drug possibly due to increasing resistance to SP among the parasite population in the study. The presence and magnitude of SP resistance in the study area have not been formally investigated in the context of this study, but are considered to be within acceptable limits, with P*fdhps* K540E mutation at 32.6%, falling within the 30% to < 90% for SP efficacy [13, 31]. In contrast, results from Malawi showed a significantly higher prevalence of malaria at delivery in the ISTp-DP arm compared to IPTp-SP [32]. This discrepancy between the current study and the one from Malawi could be attributed, next to the use of different artemisinin-based combinations to treat positive cases, to the deployment of uRDT in this study, offering higher sensitivity compared to co-RDTs used in the Malawi study [21], thereby enabling enhanced detection and more systematic treatment of malaria in this study. Furthermore, the use of microscopy for diagnosis at delivery, known for its limited sensitivity in diagnosing malaria in pregnant women, may also be another explanation [21]. Importantly, it was found that in both arms the study participants had little malaria detected by microscopy at delivery (3.2% in IPTp-SP arm and 0.9% in the ISTp-uRDT-PA arm), which is remarkably low compared to the overall prevalence of 34.4% at enrolment. This suggests that both interventions can control malaria infections pretty well.

The incidence of asymptomatic parasitaemia at delivery was higher, but not significantly, in the ISTp-uRDT-PA arm (17.4%) compared to the IPTp-SP arm (11.6%). This finding follows the trend from a study in Malawi with high SP resistance, which also reported a higher risk of malaria in the ISTp-DP arm (9.6% by RDT) compared to the IPTp-SP arm (7.4%) [32]. The same result was also observed in Kenya, where the prevalence of malaria at delivery (detected by RDT) was higher in the ISTp-DP arm than in the IPTp-SP arm [33]. PA is used to interrupt the progression of existing infections and prevent new infections. However, the administration of PA was contingent upon a positive uRDT result, which was performed monthly. This screening frequency might allow the emergence and persistence of another malaria infection between consecutive visits, especially in area with high transmission rates, such as DRC. This could explain the higher, yet not significant, asymptomatic parasitaemia rates at delivery in the ISTp-uRDT-PA arm *versus* the IPTp-SP arm. Additionally, parasitaemia detected by uRDT showed no statistically significant difference between the two study arms during pregnancy. Both strategies performed similarly in clearing parasitaemia among pregnant women in the study area. This observation contrasts with the findings of a study conducted in Kenya where ISTp-DP demonstrated a higher incidence of malaria infections compared to IPTp-SP [33].

It was observed that the prevalence and risk of maternal anaemia was the same in both arms (ISTp-uRTD-PA and IPTp-SP) during the whole study period. A similar study conducted in West Africa, comparing ISTp-AL to IPTp-SP, reported consistent findings [34]. However, it was noted that mean Hb concentration observed in the study participants was significantly lower than that reported for women in a study that assessed ISTp-AL *versus* IPTp-SP for malaria in pregnancy [16]. Possible explanations for this discrepancy include: (i) the malaria infections encountered were mild, often asymptomatic, and did not require hospitalization, yet they contributed to a reduction in Hb concentration; (ii) the provision of insecticide-treated nets during prenatal visits might have contributed to this outcome; (iii) the regular monthly appointments potentially improved awareness and compliance with iron supplementation, as advised by healthcare providers.

Research conducted in four West African countries by Tagbor et *al*. identified a consistent prevalence of LBW across both study arms, with 15.1% in the IPTp-SP arm and 15.6% in the ISTp-AL arm [34]. These results align with the overall prevalence of LBW found in both arms reported in the current study. Infants born from women in the IPTp-SP arm seemed to have a higher average birth weight (18.5%) compared to those in the ISTp-uRDT-PA arm (10.6%), despite a higher incidence of peripheral parasitaemia at birth in the IPTp-SP arm. This suggests that, against expectations, the prevalence of parasitaemia in the IPTp-SP arm did not lead to a higher rate of LBW newborns. This result is conform clinical trials comparing ISTp-DP and IPTp-SP [33, 35]. The SP treatment may exert a beneficial impact on birth weight through mechanisms beyond its antimalarial action. Specifically, the broad antimicrobial properties of SP, effective against sexually transmitted infections (STI) and potentially beneficial to the intestinal and vaginal microbiome, could offer additional protection against bacterial infections harmful to newborns [36]. Moreover, this protection confirmed by SP against STIs and reproductive tract infection could explain the low rate of adverse events related to the reproductive system observed in this study, and is in-line as reported by Chico et al. [37].

It is important to note that unlike IPTp-SP, ISTp in principle reduces the overuse of antimalarials and thereby decreases drug pressure on malaria parasites, which is considered very beneficial in this era of increasing malaria drug resistance [16]. This benefit can be further potentiated by using an ACT that has not yet

been or should be used by the NMCP for other strata of the population other than pregnant women [27]. As a newly approved antimalarial, PA could be an ideal candidate for this purpose in DRC. Since its approval for use in malaria-endemic countries in 2015, PA has been deployed in the field to treat malaria in children and adults, but not in pregnant women [38].

A limitation of the present study lies in the fact that placental malaria, diagnosed through histopathological examination of placental biopsies, was not performed. Although initially planned, this procedure was suspended during screening due to the risk of causing unjustified concerns about the collection of placental biopsies in the community. These kind of concerns have also been observed in other studies [39]. In order facilitate the enrolment in the present study, the research team stopped these collections. Additionally, relying on the date of the last menstrual period and uterine height instead of ultrasound to estimate the gestational age could have led to inaccurate classification of premature birth cases in some infants, although this is not expected to introduce major bias between arms.

Furthermore, not all women in the IPTp-SP arm received the recommended number of SP doses. This is a reflection of the real-world setting in which the study was conducted, and where delayed ANC is common [40]. However, the current study, through its structured follow-ups, facilitated the SP administration that did occur, and without this intervention the uptake might have been lower.

Lastly, during the current study it became evident that the diagnostic performance of the uRDT is slightly higher sensitivity compared to the co-RDT, but that this difference is not significant [21]. Alternative diagnostic tests, such as PCR, which was not used in this study, could perhaps increase the efficacy of the proposed ISTp-uRDT-PA strategy, as PCR is considered the gold standard for detecting low parasitaemia and occults infections [20, 41]. However, molecular diagnostics might be difficult to implement in routine practice in resource limited settings, but simplified formats are being developed [42]

To summarize, the present study showed that ISTp-uRDT-PA is as effective as the currently implemented IPTp-SP strategy in preventing the negative effects of a malaria infection during pregnancy on both mother and child. There was no significant difference in terms of symptomatic and asymptomatic parasitaemia as well as parasite density detected in pregnant women by microscopy in both the ISTp-uRDT-PA arm as well as the IPTp-SP arm. Furthermore, the prevalence and risk of maternal anaemia was the same in both arm during the whole study. Other secondary outcomes, such as the incidence of spontaneous abortion and intrauterine death, were not significantly different in both study arms. Also the impact on newborns, e.g. birth weight and neonatal anaemia, was not different in both arms. Importantly, although (some severe) adverse events were reported for the study participants, no statistically significant differences between the two study arms concerning the incidence of these were found. PA was generally well-tolerated in the present study, aligning with the safety profiles of PA in children under five years of age and the general population [43, 44].

Finally, with respect to future implementation of the ISTp strategy it should be noted that this preventive approach, whether with PA or another ACT, is likely to be more expensive than IPTp-SP. This constraint needs to be balanced against the non-financial benefit of avoiding the administration of a drug (i.e.SP) to many pregnant women in settings where SP resistance increases and its prophylactic effect will be diminished, and as a consequence the IPTp-SP strategy may have reduced efficacy in preventing the negative outcomes of malaria in pregnancy. Thus, studies on cost-effectiveness, as well as on the acceptability of ISTp-uRDT-PA by women and healthcare providers are needed.

Conclusions

In this region of Kinshasa, ISTp-uRDT-PA was found to be non-inferior to the current IPTp-SP regimen, demonstrating a comparable effect on malaria, maternal anaemia, and adverse birth outcomes relative to IPTp-SP. ISTp-uRDT-PA can be an alternative in settings where SP resistance is high and IPTp-SP may lose its effectiveness.

Abbreviations

ACT	Artemisinin-based combination therapy
AE	Adverse events
AL	Artesunate lumefantrine
ANC	Antenatal care
CI	Confidence interval
co-RDT	Conventional RDTs
CRF	Case report form
dhfr	Dihydrofolate reductase
dhps	Dihydropteroate synthase
DRC	Democratic Republic of the Congo
Hb	Haemoglobin
g/dL	Gram per decilitre
GMPD	Geometric mean parasite density
ITN	Insecticide-treated bed net
IPTp	Intermittent Preventive Treatment in pregnancy
ISTp	Intermittent screening treatment in pregnant women
IPTp-SP	Intermittent Preventive Treatment in pregnancy with Sulfadoxine-Pyrimethamine
ISTp-uRDT-PA	Intermittent screening and treatment with pyronaridine-artesunate
LBW	Low birth weight
mITT	Modified intention-to-treat population
NMCP	National Malaria Control Programme
PA	Pyronaridine-artesunate
pg/mL	Picogram per millilitre
PP	Per-protocol analysis
uRDT	Ultra-senstive rapid diagnostic test

SP	Sulfadoxine-Pyrimethamine
SAEs	Serious adverse events
RR	Risk ratio
STI	Sexually transmitted infections
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12936-025-05260-6.

Additional file 1: Table 1. Factors associated with anaemia during delivery visits.

Additional file 2: Table 2. Factors associated with low birth weight in newborns delivered by enrolled women.

Additional file 3: Table 3. Serious adverse events by study arms.

Additional file 4: Table 4. Comparison of uRDT and microscopy results for the detection of a malaria infection in study participants during the different visits (Data not shown).

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Author contributions
VM and HT conceived and designed the experiments. MK, FL, PL, HM, VM and JK performed the experiments. TK, JK, VM, HM, HS analyzed the data. FL, MK, JK, VM, PL and HM contributed reagents/materials/analysis tools. JK wrote the first draft of the manuscript. JK, FL, MK, HM, LZ, TK, HS, KK, PM, PL, HT and VM contributed to the writing of the manuscript. MK, FL, VM, and JK enrolled patients. All authors agreed with the manuscript's results and conclusions.

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Availability of data and materials
Data sets produced during this trial can be obtained from the corresponding author upon the upon the reasonable request. No datasets were generated or analysed during the current study.

Declarations

Ethics and consent to participate
The study was reviewed and approved by the National Ethics Committee of the DRC (approval reference: 169/CNES/BN/PMMF/2019 of March 13, 2020) and the Congolese Pharmaceutical Regulatory Authority (ACOREP) (approval reference: MS1253/P/DKK/01096/2020 of October 9, 2020).

Consent for publications
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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