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Additional screening and treatment of malaria during pregnancy provides further protection against malaria and non-malaria fevers during the first year of life

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Running title: Decreased malaria risk in infants

Brief summary of the article main's point

This study examined the potential benefits of malaria in pregnancy (MiP) preventive strategies in infants and showed, that enhanced screening and treatment, in addition to standard IPTp-SP, may provide additional protection against malaria and non-malaria fevers in early infancy.

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Abstract

Background: Although consensus on that malaria in pregnancy (MiP) increases the risk of malaria in infancy, and eventually non-malaria fevers (NMFs), there is a lack of conclusive evidences of benefits of MiP preventive strategies in infants.

Methods: In Burkina Faso, a birth cohort study was nested to a clinical trial assessing the effectiveness of a community-based scheduled screening and treatment of malaria in combination with intermittent preventive treatment with sulfadoxine-pyrimethamine (CSST/IPTp-SP) to prevent placental malaria. Clinical episodes and asymptomatic infections were monitored over one-year follow-up to compare the effect of CSST/IPTp-SP and standard IPTp-SP on malaria and NMFs.

Results: Infants born during low-transmission season from mothers receiving CSST/IPTp-SP had a 26% decreased risk of experiencing a first clinical episode (HR, 0.74 [95% CI, 0.55- 0.99]; P = .047). CSST/IPTp-SP interacted with birth season and gravidity to reduce the incidence of NMFs. No significant effects of CSST/IPTp-SP on the incidence of clinical episodes, parasite density and *Plasmodium falciparum* infections were observed.

Conclusions: Our findings indicate that CSST/IPTp-SP strategy may provide additional protection against both malaria and NMFs in infants during the first year of life, and suggest that malaria control interventions during pregnancy could have long-term benefits in infants.

Key words: Malaria, *Plasmodium falciparum*, pregnancy, prevention, infancy, non-malaria fevers

Introduction

Malaria in pregnancy (MiP) causes significant adverse health effects in both mothers and their newborn children and, is estimated to account for between 75000 and 200000 infant deaths a year[1]. While maternal mortality is often associated with severe maternal anaemia, infant deaths are due to complications during pregnancy, such as foetal growth restriction, foetal anaemia, pre-term deliveries and congenital malaria [1].

The hallmark of MiP due to *Plasmodium falciparum* is placental malaria (PM), characterized by sequestration of infected erythrocytes in intervillous spaces of the placenta through binding to chondroitin sulfate A on syncytiotrophoblast [2]. Increasing evidence from birth cohort studies have shown that PM is associated with increased susceptibility to malaria during infancy [3–8], probably due to the acquisition of immune tolerance to parasite antigens during in utero exposure. Likewise, the development of a tolerogenic environment as a result of PM might also be the cause for increased infant-susceptibility to non-malaria fevers (NMFs) [9].

Intermittent preventive treatment with at least three full sulfadoxine-pyrimethamine (IPTp-SP) doses (administered at least one month apart), combined with the use of insecticide treated bed-nets (ITNs), is currently the recommended strategy for preventing MiP in most malaria endemic countries, as it reduces the risk of PM at delivery, neonatal mortality and infants' low birth weight (LBW) [10]. However, the effectiveness of this IPTp-SP based intervention is threatened by the growing spread of parasites with increasing resistance to SP [11]. In this context, studies assessing

the impact of novel anti-malarial interventions during pregnancy, not only on pregnant women but also on their infants, are urgently needed as information to date is scarce and inconclusive [12,13].

A multicentre cluster-randomized controlled trial (COSMIC) was recently conducted in three west-African countries with high (Burkina Faso and Benin) and low (The Gambia) malaria transmission, to assess the protective efficacy of adding community-scheduled screening and treatment of malaria during pregnancy (CSST) to standard IPTp-SP (CSST/IPTp-SP). The CSST extension strategy was implemented monthly by community health workers using rapid diagnostic test (RDT). The aim of the combined CSST/IPTp-SP strategy was to provide additional opportunities to detect and treat malaria infections during pregnancy and reduce the prevalence of PM [14].
In this study, we have followed infants born to mothers at the Burkina Faso site in the COSMIC trial, with the aim to evaluate whether CSST/IPTp-SP compared to IPTp-SP alone was effective in protecting infants from malaria and NMFs during the first year of life.

Material and methods

Study site

The study was conducted in Nanoro, a rural area with high malaria transmission located in the central-west region of Burkina Faso. Malaria transmission occurs year-round and is highly seasonal. Transmission peaks between July and December overlapping with the rainy season (July to November). *P. falciparum* is the main species responsible for transmission, with a prevalence of 90%. The prevalence of peripheral and placental *P. falciparum* infections at delivery during the high-transmission season is estimated at 32% and 34% respectively [15]. In 2015, approximately 7.4 million of clinical malaria cases and 32000 malaria related deaths were recorded in Burkina Faso [16]. Out of 91154 clinical malaria episodes that occurred in Nanoro health district in 2016,

approximately 55% were detected among infants below five years of age [17].

Study design and participants

This was an observational birth cohort study of infants born to women who participated in the COSMIC trial (NCT01941264), described in detail elsewhere [14]. In COSMIC trial it was assumed that CSST/IPTp-SP strategy would decrease PM by 30%. Following this assumption, and considering the prevalence of malaria infection in a 12-month birth cohort study in Benin (35%) [4], as at the time of recruitment there were no such data available in Burkina Faso, we assumed that the impact of the intervention will decrease *P. falciparum* infection during the first year of life by 30%, from 35% to 25%. With a 1:1 ratio to provide a power of at least 80% with 5% precision, the required sample size was estimated to 350 newborns in each study arm. To account for loss to follow-up and exclusion criteria at delivery, a total of 752 pregnant women from COSMIC trial (376 in each arm) were enrolled between March 2014 and June 2015 onto the current study. Written informed consent was obtained from all mothers and ethical approval was obtained from the institutional ethics committees of Centre Muraz, Bobo Dioulasso, Burkina Faso (006-2014/CE-CM), the Institute of Tropical Medicine, Antwerp, Belgium (953/14) and University Hospital in Antwerp (UZA), Belgium (14/26/277).

Recruitment and follow-up procedures

Pregnant women participating in the COSMIC trial were invited to participate in the present study at any antenatal care visits but the enrollment of mother-child pairs onto this study occurred at time of delivery. Exclusion criteria at time of delivery were maternal death, stillbirth and presence of major congenital malformation, chronic diseases or signs of cerebral asphyxia. Maternal peripheral blood was collected by finger-prick to prepare blood smears and blood spots on filter paper for

subsequent malaria diagnosis. A tissue section was collected from the maternal side of the placenta and preserved into 10% neutral buffered formalin at 4°C for histological examination within the COSMIC trial. A drop of placental blood was also spotted on filter paper. Infants were followed for 12 months and malaria infections were detected actively and passively. Active case detection consisted of four cross-sectional surveys conducted at 3, 6, 9 and 12 months of age. For each survey, blood films and blood spots on filter papers were collected for examination by light microscopy (LM) and quantitative real-time polymerase chain reaction (qPCR), respectively. For passive clinical case detection, mothers were encouraged to bring their offspring to the health centre if they displayed any signs of illness. Infants presenting with fever (axillary temperature ≥37.5ºC) on examination or a history of fever in the previous 24 hours, were screened for malaria infection using RDT (SD-Bioline Malaria Ag P.f®, Standard Diagnostic, Inc, Korea) and, if positive, treated according to national guidelines. Malaria diagnosis was subsequently confirmed by LM and qPCR.

Laboratory methods

LM readings were taken according to standard procedures by two independent experienced readers [18]. A third reader was consulted in cases of discrepancies. Dried blood spots on filter papers were used for DNA extraction (QIAamp 96 DNA blood kit, Qiagen, Germany) and, *P. falciparum* detection and quantification by *Pf*-varATS qPCR using procedures described elsewhere [15]. The limit of detection of the *Pf*-vasATS qPCR was estimated at 0.1 parasite/µL and qPCR was used as reference malaria diagnostic test. Data on past history of malaria infections during pregnancy and histological examinations of placental tissue data were obtained from the COSMIC trial [14].

Data analysis and definition of terms

Data were double entered into the study databases (OpenClinica, community version or Excel, Microsoft, USA) and analyzed with STATA 12.0 (StataCorp, USA). The baseline characteristics of mother-child pairs were compared between the study arms. Continuous data normally distributed and categorical variables were analyzed using the t-test and the χ^2 test, respectively. Non-normally distributed data were described using median and interquartile ranges (IQR).

The risk of malaria and NMFs during the first year of life was analyzed using the following outcomes: time from birth to the first clinical malaria episode, incidence of clinical malaria episodes, parasite density, prevalence of *P. falciparum* infection and incidence of NMFs. The main exposure was the MiP intervention (CSST/IPTp-SP or IPTp-SP). The effect of prenatal malaria exposure -defined as maternal peripheral and placental infections- on outcome was investigated in a separate analysis. Maternal age, gravidity, ITN usage by mothers, birth season and newborn sex were considered as potential confounders. Twin infants were excluded from the analysis.

The effect of the interventions on time-to-first clinical malaria episode was analyzed using Cox proportional univariate and multivariate analyses, while Kaplan-Meier analysis was used to calculate the survival curves. Because MiP preventive strategies may have a causal effect on pregnancy outcomes and subsequently an effect on the risk of malaria in infancy, neither low birth weight (LBW) nor maternal peripheral and PM infections at delivery were used as covariates in the multivariate Cox proportional models. The effect of the interventions on the incidence of clinical malaria episodes and NMFs was investigated using Poisson regression or negative binomial models depending on the goodness-of-fit tests with results expressed as incidence rate ratios (IRRs with 95% confidence interval (CI)). Linear regression analysis was used for log-transformed parasite densities and logistic regression models for *P. falciparum* infection excluding censored infants. Variables with a *P* value < .1 in univariate analyses were included in multivariable

analyses. A *P* value < .05 was considered to be statistically significant. The definitions of terms and the different categories of prenatal malaria exposure used in the present study are shown in Supplementary Table 1.

Results

Study population

The flow chart of the birth cohort study is shown in Figure 1. Out of the 761 infants (from 752 mothers) enrolled in the study, 669 (88%) completed the 12-months follow-up. Of the remaining 92 infants, 41 were lost to follow-up, 38 had consent withdrawn mainly because of migration from the study area and 13 died (seven neonatal deaths and six within 1 to 12 months). In total, 734 of the initial 761 infants were included in the survival analysis (367 in each COSMIC study arm). The infants excluded from the analysis were the seven neonates who died before four weeks of age and the 20 live twins although they completed follow-up. The cross-sectional surveys conducted at 3, 6, 9 and 12 months of age included 678 (92.4%), 631 (86%), 587 (80%) and 638 (86.9%) infants respectively. Table 1 presents the characteristics of the mothers and infants as a whole and by MiP intervention group. The baseline maternal and infant demographic and parasitological characteristics were similar between the participants in the CSST/IPTp-SP and standard IPTp-SP arms.

Malaria infections

Overall incidence of clinical malaria in the study population was 1.03 episodes per child-year (a total of 717 cases over 8335.18 months at risk) and the median survival time from birth to the first clinical malaria episode was 9.9 months. Nearly 59% (433/734) of the infants experienced at least

one clinical malaria episode during the first 12 months of life. The prevalence of asymptomatic infections as detected by qPCR in the cross-sectional surveys was 17.70% (120/678) at 3 months, 20.13 (127/631) at 6 months, 18.40 (108/587) at 9 months and 30.41% (194/638) at 12 months. Median qPCR-parasite density during clinical malaria episodes (10826.50 [IQR 876.50-38021.50] parasites/μL) was significantly higher than that during asymptomatic infections (379 [IQR 1.94-3932.5] parasites/μL, $P < .0001$).

Effect of CSST/IPTp-SP strategy on time-to-first clinical malaria episode

Although time-to-first clinical malaria episode was not significantly different between the two study arms in univariate and multivariate analyses (Table 2), birth season was found to interact and significantly modify the effect of CSST/IPTp-SP on time-to-first clinical malaria episodes. We observed that infants born to mothers in the CSST/IPTp-SP arm during malaria low-transmission season had a 26% decreased risk of a first clinical malaria episode compared with those born to mothers in the IPTp-SP arm (HR, 0.74 [95% CI, 0.55-0.99]; $P = .047$). By contrast, no significant differences were observed between the two arms for infants born during malaria high-transmission season (HR, 0.97 [95% CI, 0.76-1.25]; $P = .846$). That difference of the effect of CSST/IPTp-SP on time-to-first clinical malaria episode according to birth season was further illustrated in Kaplan-Meier survival curves (Figure 2).

We investigated whether the observed effect of CSST/IPTp-SP could be explained by differences in prenatal malaria exposure. Table 3 shows that the increased risk of time-to-first clinical malaria episode was associated with peripheral infection during pregnancy and past PM ($P = .009$ and $P = .020$, respectively) but not with active PM ($P = .177$), after adjusting by gravidity, birth season and LBW. Thus, the effect of CSST/IPTp-SP on time-to-first clinical malaria episode was more

likely to be attributable to the impact on malaria infection during pregnancy rather than to active PM.

The two COSMIC study arms had a similar effect on infant parasite density during the first clinical malaria episode (6557.50 [IQR, 609.00-28484.50] parasites/μL in CSST/IPTp-SP vs. 4549.00 [IQR, 380.50-19627.75] parasites/μL in standard IPTp-SP). Similar lack of association was observed on parasitaemia in all clinical episodes even after adjusting for gravidity and sex (coefficient, 1.10 [95% CI, 0.82-1.47]; *P* = .526) (Supplementary Table 2).

Effect of CSST/IPTp-SP strategy on the incidence of clinical malaria episodes

Table 4 shows results of the analysis of the effect of the CSST/IPTp-SP strategy on the incidence of clinical malaria episodes including repeated malaria attacks per infant during the first year of life. We found no significant differences between CSST/IPTp-SP and IPTp-SP after adjusting for birth season (adjusted IRR, 0.95 [95% CI, 0.82-1.10]; *P* = .459). Moreover, no significant differences were observed in the stratified analysis by birth season (high-transmission season: IRR, 0.99 [95% CI, 0.82-1.20]; *P* = .941; low-transmission season IRR, 0.91 [95% CI, 0.72-1.14]; *P* = .409).

Effect of CSST/IPTp-SP strategy on *P. falciparum* infections

In total, 553 of 690 infants (80.14%) experienced at least one symptomatic or asymptomatic infection during the first year of life. The odds of experiencing a *P. falciparum* infection in infants born to mothers in the CSST/IPTp-SP group was not significantly different from that of children born to mothers in the IPTp-SP group, even after controlling for gravidity (adjusted OR, 1.20 [95% CI, 0.82-1.74]; *P* = .348) (Supplementary Table 3). There were also no statistical differences

between the groups in terms of the proportions of asymptomatic infections in infants, regardless of the time of screening (Supplementary Table 4).

Effect of CSST/IPTp-SP strategy on the incidence of NMFs

A total of 805 NMFs episodes (incidence, 1.16 per child-year) were recorded in the 734 infants analyzed during their first year of life. Table 5 presents the stratified analysis by birth season and gravidity that were found to interact with CSST/IPTp-SP strategy to modify infant's susceptibility to NMFs. Infants born to CSST/IPTp-SP mothers during malaria high-transmission season had a significantly lower risk of developing NMFs than their counterpart born to mothers receiving the standard IPTp-SP (adjusted IRR, .79 [95% CI, 0.64-0.98]; P = .031) (Table 5). When stratifying the analysis by gravidity, only the offspring of multigravid women in the CSST/IPTp-SP group were significantly protected against NMFs (adjusted IRR, .78 [95% CI, 0.63-0.97]; P = .027). We did not observe any significant associations between prenatal malaria exposure and occurrence of NMFs (Supplementary Table 5).

Discussion

In this prospective birth cohort study we found that, compared with standard IPTp-SP strategy, CSST/IPTp-SP significantly increased time-to-first clinical malaria episode in infants born during malaria low-transmission season, and protected against the occurrence of NMFs in those born during malaria high-transmission season. Due to the transmission seasonality of malaria and NMFs in Burkina Faso, CSST/IPTp-SP strategy appears to have different impact in women who had the main course of their pregnancy during malaria high-transmission season (and most probably gave birth during malaria low-transmission season) compared to those who were pregnant during

malaria low-transmission season (and most probably gave birth during malaria high-transmission season).

Our findings showed, for the first time, that MiP preventive treatments may provide protection against malaria in infants during the first year of life, in addition to the known protection against adverse pregnancy outcomes. New MiP strategies should thus be evaluated not only for the protective effect on pregnancy outcomes but also the potential additional long-term benefits in infants. To our knowledge, only one study has compared the incidence of malaria in infants born to mothers who received either IPTp-SP or screening and treatment with RDT and artemether-lumefantrine (ISTp-AL) during antenatal care visits, without IPTp-SP [13]. The authors found no differences in the risk of clinical malaria episodes during early life and observed that the two interventions had similar performance in preventing PM [13]. In comparison, the strategy of combining a CSST with the standard IPTp-SP increases the chances of clearing parasite infections when the protective efficacy of IPTp-SP is waned, thus reducing the risk of long-term parasite carriage during pregnancy. Indeed, thanks to the enhanced screening, the proportion of malaria infections detected by RDT amongst women in the CSST/IPTp-SP arm was significantly higher than that among women in the routine IPTp-SP arm (Table 1).

Remarkably, the impact of the CSST/IPTp-SP strategy on the risk of malaria in infancy was evident in infants born to women who had the main course of their pregnancy during malaria high-transmission season (and thus born during low-transmission season), while the two interventions performed similarly when the main course of pregnancy was during the low-transmission season. Therefore, we hypothesized that this effect of CSST/IPTp-SP is attributable to the additional reduction in parasite exposure that was not prevented by the standard IPTp-SP owing to the fact

283 that maternal peripheral infections were associated with an increased risk of malaria in infants
284 (Table 3). The observation that past PM and peripheral infections during pregnancy, but not active
285 PM, were associated with a risk of clinical malaria suggests that preventing infections at early stage
286 of pregnancy may have a critical effect on future infant susceptibility. Most studies to date have
287 focused in looking at the association between PM and malaria risk, probably thus missing the
288 overall effect of preventing all infections [3–8]. Our findings are supported by a recent study that
289 showed that the timing of prenatal malaria exposure had a pivotal role on the polarization of the
290 foetal immune responses [19]. In this context, the study that we are currently conducting on the
291 relationships between types of prenatal malaria exposure, innate immune responses at birth and the
292 subsequent risk of malaria in early infancy will provide further understanding on how maternal
293 infections modulate newborn's immune responses and affect risk of malaria during the first year
294 of life.
295 CSST/IPTp-SP strategy significantly increased time from birth to the first clinical malaria episode
296 compared with the standard IPTp-SP in infants born during the low transmission season,
297 however this effect did not translate into a significant reduction of malria incidence in the
298 same group. Although the difference was non-significant, the overall clinical malaria incidence
299 was also reduced in these infants suggesting that a MiP intervention with a stronger effect size
300 on time to first infection would further reduce malaria incidence in infancy. In this study, the
301 observed effect of the CSST/IPTp-SP intervention may have been limited by the sensitivity of the
302 RDT used during screenings (limit of detection of approximately 200 parasites/μL) [20]. It is thus
303 reasonable to assume that the lack of effect observed in infants born to mothers who were mostly
304 pregnant during the low-transmission season might be due to the reduction of transmission intensity
305 during this time and to the possibility that RDTs might have missed a considerable proportion of

low-density infections [21]. We are tempted to speculate that the effect of the CSST/IPTp-SP intervention could be increased with the use of high-sensitivity diagnostic tools capable of detecting low-density infections. In this regard, high-sensitivity RDTs currently used in field testing (PATH) [22] and molecular strategies such as LAMP [23], ~~which have a limit of detection of 10-20 parasites/µl and < 1 parasite/µL respectively,~~ are promising tools to improve screening strategies.

It is noteworthy that the CSST/IPTp-SP intervention was also associated with a reduced incidence of NMFs during the first year of life in infants born during malaria high-transmission season but not in those born during malaria low-transmission season. Therefore, it is unlikely that the same mechanism underlies the susceptibility to NMF since this protection, ~~which is in agreement with a previous study [24]~~, was independent of PM, even after exploring the association with different categories of prenatal malaria exposure. Nevertheless, several studies have reported modulation of the foetal immune system by malaria infections during pregnancy [19,25,26]. In addition to malaria-specific immune responses, these modifications may also affect specific immunity to other pathogens responsible of NMFs [27,28], such as those caused by blood stream infections, gastrointestinal infections, acute respiratory infections, urinary tract infections and arbovirus infections [29,30].

Moreover, the CSST/IPT-SP intervention was found to interact with gravidity to significantly protect infants born to multigravid women but not these born to either secundigravid (non-significant protection) or primigravid women (non-significant increased risk). These findings suggest that, through the modification of prenatal malaria exposure (which may differ depending on gravidity), the CSST/IPTp-SP intervention alters or prime the development of

fetal immune system affecting immunity to pathogens that are responsible of NMFs in early infancy. To gain further an understanding of how MiP preventive interventions can modulate the immune system to impact infant susceptibility to NMFs, new studies should include diagnosis of NMFs.

Conclusion

In this study we have shown that, compared with standard IPTp-SP, CSST/IPTp-SP significantly decreases the risk of experiencing a first clinical malaria episode and also interact with birth season and gravidity to reduce the incidence of NMFs during the first year of life. Altogether, our findings suggest that MiP preventive strategies that effectively reduce exposure to malaria parasites during pregnancy could provide additional protection against both malaria and NMFs in early infancy and therefore, have long-term benefits in infants.

Notes:

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Potential conflicts of interest. The authors declare no competing interests.

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infections among children admitted to a rural hospital in Burkina Faso. PLoS One **2014**; 9: e89103.

Tables

Table 1: Baseline characteristics of study participants

Category, characteristics	Total study participants (N=734)	Standard IPTp-SP (N=367)	CSST/IPTp-SP (N=367)	*P* value
Maternal characteristics				
Age—years, Mean±SD	26.4±6.2	26.0±6.2	26.7±6.2	.139
Gravidity—no. (%)				.674
Primigravid	132 (18.0)	68 (18.5)	64 (17.4)	
Secundigravid	117 (15.9)	62 (16.9)	55 (15.0)	
Multigravid	485 (66.1)	237 (64.6)	248 (67.6)	
SP doses uptake, Mean±SD	2.68±0.97	2.64 ±0.98	2.72±0.97	.236
Total maternal peripheral infections detected by RDT*—no.(%)	159 (21.7)	50 (13.6)	109 (29.7)	< .001
Maternal peripheral infection at deliver by qPCR (MPI)**—no. (%)	152 (21.3)	84 (23.7)	68 (18.9)	.119
Placental malaria (PM)**—no.(%)				

By qPCR	160 (22.8)	86 (24.4)	74 (21.3)	.328
By histology (acute, chronic, past infections)	447 (67.3)	215 (67.7)	232 (67.1)	.878
Gestational age at delivery—no. (%)				.199
Pre-term delivery (28-36 weeks)	31 (4.2)	12 (3.3)	19 (5.2)	
Term delivery (>36 weeks)	703 (95.8)	355 (96.7)	348 (94.8)	
Insecticide treated net (ITN) use—no. (%)	574 (78.2)	278 (75.7)	296 (80.7)	.108
Infant characteristics				
Sex—no. (%)				.301
Male	356 (48.5)	171 (46.6)	185 (50.4)	
Female	378 (51.5)	196 (53.4)	182 (49.6)	
Birth season—no. (%)				.254
High-transmission (July-December)	455 (62.0)	235 (64.0)	220 (59.9)	
Low-transmission (January-June)	279 (38.0)	132 (36.0)	147(40.1)	
Birth weight—g, Mean±SD	3012.7±422.2	2984.8±411.1	3040.6±431.7	.073
Low birth weight (<2500) —no. (%)	60 (8.2)	35 (9.5)	25 (6.8)	.178

NOTE. IPTp-SP, Intermittent Preventive Treatment during pregnancy with Sulfadoxine-Pyrimethamine; CSST/IPTp-SP, community-based scheduled screening and treatment of malaria during pregnancy with artemether-lumefantrine plus standard IPTp-SP for malaria in pregnancy prevention and control; SD, Standard Deviation; g, gram. *Positive RDT cases diagnosed (at home visits (CSST/IPTp-SP arm)) and unscheduled visits (both IPTp-SP and CSST/IPTp-SP arms)) and confirmed by light microscopy. ** Missing: MPI detected by qPCR (N=713): Standard IPTp-SP =353, CSST/IPTp-SP=359; PM detected by qPCR (N=701): Standard IPTp-SP =353, CSST/IPTp-SP= 348; PM detected by histology (N=664): Standard IPTp-SP=318, CSST/IPTp-SP= 346; CM (N=703): Standard IPTp-SP=352, CSST/IPTp-SP=351.

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Table 2: Effect of CSST/IPTp-SP on time-to-first clinical malaria episode by Cox proportional hazards analysis

Characteristic	Risk factor	Unadjusted		Adjusted*	
		HR (95% CI)	***P* value**	**HR (95% CI)**	***P* value**
Overall cohort analysis					
MiP Intervention strategy	Standard IPTp-SP	1		1	
	CSST/IPTp-SP	0.90 (0.75-1.09)	.293	0.88 (0.73-1.07)	.205
Gravidity	Multigravid	1		1	
	Secundigravid	1.25 (0.97-1.61)	.081	1.22 (0.94-1.56)	.129
	Primigravid	0.98 (0.75-1.28)	.863	0.97 (0.75-1.27)	.851
Mother's age	>30	1			
	21-30	1.07 (0.85-1.35)	.572		
	≤20	1.03 (0.77-1.36)	.856		
ITN use	No	1			
	Yes	1.10 (0.85-1.44)	.462		
Birth season	Low-transmission	1		1	
	High-transmission	0.69 (0.57-0.84)	.001	0.69 (0.56-0.83)	.001
Newborn sex	Male	1			
	Female	0.90 (0.75-1.09)	.284		

Stratified analysis (CSST/IPTp-SP vs standard IPTp-SP (ref))			
Birth season	Low-transmission	0.74 (0.55-0.99)	.047
	High-transmission	0.97 (0.76-1.25)	.846

NOTE. MiP, malaria in pregnancy; ITN, insecticide treated net; HR, hazard ratio; CI, confidence interval

*Adjusted by variables that showed a *P* value < .1 in univariate analysis

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477 **Table 3. Cox proportional analysis assessing the effect of prenatal malaria exposure on time**

478 **to first clinical malaria episode**

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Characteristic	Risk factor	Unadjusted		Adjusted*	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
Prenatal malaria exposure	Non-infected mothers	1		1	
	Infected mothers	1.56 (1.09-2.23)	.016	1.62 (1.12-2.32)	.009
	Past PM mothers	1.39 (1.04-1.86)	.026	1.42 (1.06-1.91)	.020
	Active PM mothers	1.12 (0.78-1.61)	.550	1.30 (0.89-1.91)	.177
LBW	No	1		1	
	Yes	1.55 (1.13-2.13)	.007	1.55 (1.10-2.18)	.011
Gravidity	Multigravid	1		1	
	Secundigravid	1.25 (0.97-1.61)	.081	1.13 (0.86-1.47)	.384
	Primigravid	0.98 (0.75-1.27)	.863	0.97 (0.73-1.30)	.865
Mother's age	>30	1			
	21-30	1.07 (0.85-1.35)	.572		
	≤20	1.03 (0.77-1.36)	.856		
Birth season	Low-transmission	1			
	High-transmission	0.69 (0.57-0.84)	.001	0.69 (0.56-0.86)	.001
Newborn sex	Male	1			

	Female	0.90 (0.75-1.09)	.284
ITN use	No	1	
	Yes	1.10 (0.85-1.44)	.462

NOTE. PM, placental malaria; ITN, insecticide treated net; LBW, low birth weight; CI, confidence interval; HR, hazard ratio. *Adjusted by variables that showed a *P* value < .1 in univariate analyses.

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483 **Table 4: Effect of CSST/IPTp-SP on clinical malaria incidence during the first 12 months**

484 **of life by Poisson regression analysis**

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Characteristic	**Risk factor**	**Univariate**		**Multivariate***	
		IRR (95% CI)	***P* value**	**IRR (95% CI)**	***P* value**
MiP preventive strategy	Standard IPTp-SP	1		1	
	CSST/IPTp-SP	0.94 (0.82-1.10)	.479	0.95 (0.82-1.10)	.459
Gravidity	Multigravid	1			
	Secundigravid	1.11 (0.91-1.35)	.291		
	Primigravid	0.97 (0.78-1.19)	.756		
Mother's age	>30	1			
	21-30	1.01 (0.84-1.20)	.932		
	≤20	0.97 (0.78-1.20)	.763		
ITN use	No	1			
	Yes	0.99 (0.83-1.18)	.892		
Birth season	Low-transmission	1		1	
	High-transmission	0.88 (0.75-1.02)	.091	0.91 (0.79-1.06)	.229
Newborn sex	Male	1			
	Female	0.98 (0.85- 1.14)	.811		

NOTE. MiP, malaria in pregnancy; ITN, insecticide treated net; IRR, incidence rate ratio; CI, confidence interval

* Adjusted by variables that showed a *P* value < .1 in univariate analysis.

Table 5: Stratified analyses of the effect of CSST/IPTp-SP on the incidence of NMFs

CSST/IPTp-SP vs IPTp-SP (ref)		Unadjusted stratum-specific IRR (95% CI)	*P* value	Adjusted* stratum-specific IRR (95% CI)	*P* value
By birth season	Low-transmission	1.00 (0.76-1.32)	.977	1.02 (0.77-1.34)	.887
	High-transmission	0.80 (0.64-0.99)	.039	0.79 (0.64-0.98)	.031
By gravidity	Multigravid	0.81 (0.65-0.99)	.042	0.78 (0.63-0.97)	.027
	Secundigravid	0.82 (0.55-1.20)	.281	0.83 (0.56-1.23)	.357
	Primigravid	1.35 (0.92-1.99)	.130	1.35 (0.92-1.99)	.119

NOTE. IRR, incidence rate ratio; ref, reference

*Adjusted by gravidity or by birth season

Figures

Figure 1: Study flow diagram. Infants born from pregnant women participating in a randomized-cluster trial (CSST/IPTp-SP vs standard IPTp) [14] were recruited into the study at birth and followed-up until 12 months of age. Lost to follow-up are infants who did not complete the 12-months follow-up. They were considered as censored observations at the time of the last visit in survival analysis. Neonatal deaths and twins were excluded from the analysis. *Not included in the survival analysis. ** Included in the survival analysis.

Figure 2: Kaplan-Meier survival curves for time-to-first clinical malaria episode during the first year of life according to MiP prevention strategy by birth season. Infants born during malaria low transmission season (A) and born during malaria high transmission season (B) were followed-up for 12 months.