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1   **Type of article: Major article**

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3   **Additional screening and treatment of malaria during pregnancy provides further protection**

4   **against malaria and non-malaria fevers during the first year of life**

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21   **Running title:** Decreased malaria risk in infants

22   **Brief summary of the article main's point**

23   This study examined the potential benefits of malaria in pregnancy (MiP) preventive strategies

24   in infants and showed, that enhanced screening and treatment, in addition to standard IPTp-SP,

25   may provide additional protection against malaria and non-malaria fevers in early infancy.

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28   797.

29   **Abstract**

30   **Background:** Although consensus on that malaria in pregnancy (MiP) increases the risk of malaria

31   in infancy, and eventually non-malaria fevers (NMFs), there is a lack of conclusive evidences of

32   benefits of MiP preventive strategies in infants.

33   **Methods:** In Burkina Faso, a birth cohort study was nested to a clinical trial assessing the

34   effectiveness of a community-based scheduled screening and treatment of malaria in combination

35   with intermittent preventive treatment with sulfadoxine-pyrimethamine (CSST/IPTp-SP) to

36   prevent placental malaria. Clinical episodes and asymptomatic infections were monitored over one-

37   year follow-up to compare the effect of CSST/IPTp-SP and standard IPTp-SP on malaria and

38   NMFs.

39   **Results:** Infants born during low-transmission season from mothers receiving CSST/IPTp-SP had

40   a 26% decreased risk of experiencing a first clinical episode (HR, 0.74 [95% CI, 0.55- 0.99]; $P =$

41   .047). CSST/IPTp-SP interacted with birth season and gravidity to reduce the incidence of NMFs.

42   No significant effects of CSST/IPTp-SP on the incidence of clinical episodes, parasite density and

43   *Plasmodium falciparum* infections were observed.

44   **Conclusions**: Our findings indicate that CSST/IPTp-SP strategy may provide additional protection

45   against both malaria and NMFs in infants during the first year of life, and suggest that malaria

46   control interventions during pregnancy could have long-term benefits in infants.

47

49

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].

56

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].

64

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

71 the impact of novel anti-malarial interventions during pregnancy, not only on pregnant women but

72 also on their infants, are urgently needed as information to date is scarce and inconclusive [12,13].

73

74 A multicentre cluster-randomized controlled trial (COSMIC) was recently conducted in three west-

75 African countries with high (Burkina Faso and Benin) and low (The Gambia) malaria transmission,

76 to assess the protective efficacy of adding community-scheduled screening and treatment of

77 malaria during pregnancy (CSST) to standard IPTp-SP (CSST/IPTp-SP). The CSST extension

78 strategy was implemented monthly by community health workers using rapid diagnostic test

79 (RDT). The aim of the combined CSST/IPTp-SP strategy was to provide additional opportunities

80 to detect and treat malaria infections during pregnancy and reduce the prevalence of PM [14].

81 In this study, we have followed infants born to mothers at the Burkina Faso site in the COSMIC

82 trial, with the aim to evaluate whether CSST/IPTp-SP compared to IPTp-SP alone was effective in

83 protecting infants from malaria and NMFs during the first year of life.

84

85 **Material and methods**

86 **Study site**

87 The study was conducted in Nanoro, a rural area with high malaria transmission located in the

88 central-west region of Burkina Faso. Malaria transmission occurs year-round and is highly

89 seasonal. Transmission peaks between July and December overlapping with the rainy season (July

90 to November). *P. falciparum* is the main species responsible for transmission, with a prevalence of

91 90%. The prevalence of peripheral and placental *P. falciparum* infections at delivery during the

92 high-transmission season is estimated at 32% and 34% respectively [15]. In 2015, approximately

93 7.4 million of clinical malaria cases and 32000 malaria related deaths were recorded in Burkina

94 Faso [16]. Out of 91154 clinical malaria episodes that occurred in Nanoro health district in 2016,

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

96

97 **Study design and participants**

98 This was an observational birth cohort study of infants born to women who participated in the

99 COSMIC trial (NCT01941264), described in detail elsewhere [14]. In COSMIC trial it was

100 assumed that CSST/IPTp-SP strategy would decrease PM by 30%. Following this assumption, and

101 considering the prevalence of malaria infection in a 12-month birth cohort study in Benin (35%)

102 [4], as at the time of recruitment there were no such data available in Burkina Faso, we assumed

103 that the impact of the intervention will decrease *P. falciparum* infection during the first year of life

104 by 30%, from 35% to 25%. With a 1:1 ratio to provide a power of at least 80% with 5% precision,

105 the required sample size was estimated to 350 newborns in each study arm. To account for loss to

106 follow-up and exclusion criteria at delivery, a total of 752 pregnant women from COSMIC trial

107 (376 in each arm) were enrolled between March 2014 and June 2015 onto the current study. Written

108 informed consent was obtained from all mothers and ethical approval was obtained from the

109 institutional ethics committees of Centre Muraz, Bobo Dioulasso, Burkina Faso (006-2014/CE-

110 CM), the Institute of Tropical Medicine, Antwerp, Belgium (953/14) and University Hospital in

111 Antwerp (UZA), Belgium (14/26/277).

112

113 **Recruitment and follow-up procedures**

114 Pregnant women participating in the COSMIC trial were invited to participate in the present study

115 at any antenatal care visits but the enrollment of mother-child pairs onto this study occurred at time

116 of delivery. Exclusion criteria at time of delivery were maternal death, stillbirth and presence of

117 major congenital malformation, chronic diseases or signs of cerebral asphyxia. Maternal peripheral

118 blood was collected by finger-prick to prepare blood smears and blood spots on filter paper for

119 subsequent malaria diagnosis. A tissue section was collected from the maternal side of the placenta

120 and preserved into 10% neutral buffered formalin at 4°C for histological examination within the

121 COSMIC trial. A drop of placental blood was also spotted on filter paper. Infants were followed

122 for 12 months and malaria infections were detected actively and passively. Active case detection

123 consisted of four cross-sectional surveys conducted at 3, 6, 9 and 12 months of age. For each

124 survey, blood films and blood spots on filter papers were collected for examination by light

125 microscopy (LM) and quantitative real-time polymerase chain reaction (qPCR), respectively. For

126 passive clinical case detection, mothers were encouraged to bring their offspring to the health

127 centre if they displayed any signs of illness. Infants presenting with fever (axillary temperature

128 ≥37.5°C) on examination or a history of fever in the previous 24 hours, were screened for malaria

129 infection using RDT (SD-Bioline Malaria Ag P.f®, Standard Diagnostic, Inc, Korea) and, if

130 positive, treated according to national guidelines. Malaria diagnosis was subsequently confirmed

131 by LM and qPCR.

132

133 **Laboratory methods**

134 LM readings were taken according to standard procedures by two independent experienced readers

135 [18]. A third reader was consulted in cases of discrepancies. Dried blood spots on filter papers were

136 used for DNA extraction (QIAamp 96 DNA blood kit, Qiagen, Germany) and, *P. falciparum*

137 detection and quantification by *Pf*-varATS qPCR using procedures described elsewhere [15]. The

138 limit of detection of the *Pf*-vasATS qPCR was estimated at 0.1 parasite/µL and qPCR was used as

139 reference malaria diagnostic test. Data on past history of malaria infections during pregnancy and

140 histological examinations of placental tissue data were obtained from the COSMIC trial [14].

141

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

166   analyses. A *P* value < .05 was considered to be statistically significant. The definitions of terms

167   and the different categories of prenatal malaria exposure used in the present study are shown in

168   Supplementary Table 1.

169

170   **Results**

171

172   **Study population**

173   The flow chart of the birth cohort study is shown in Figure 1. Out of the 761 infants (from 752

174   mothers) enrolled in the study, 669 (88%) completed the 12-months follow-up. Of the remaining

175   92 infants, 41 were lost to follow-up, 38 had consent withdrawn mainly because of migration from

176   the study area and 13 died (seven neonatal deaths and six within 1 to 12 months). In total, 734 of

177   the initial 761 infants were included in the survival analysis (367 in each COSMIC study arm). The

178   infants excluded from the analysis were the seven neonates who died before four weeks of age and

179   the 20 live twins although they completed follow-up. The cross-sectional surveys conducted at 3,

180   6, 9 and 12 months of age included 678 (92.4%), 631 (86%), 587 (80%) and 638 (86.9%) infants

181   respectively. Table 1 presents the characteristics of the mothers and infants as a whole and by MiP

182   intervention group. The baseline maternal and infant demographic and parasitological

183   characteristics were similar between the participants in the CSST/IPTp-SP and standard IPTp-SP

184   arms.

185

186   **Malaria infections**

187   Overall incidence of clinical malaria in the study population was 1.03 episodes per child-year (a

188   total of 717 cases over 8335.18 months at risk) and the median survival time from birth to the first

189   clinical malaria episode was 9.9 months. Nearly 59% (433/734) of the infants experienced at least

190   one clinical malaria episode during the first 12 months of life. The prevalence of asymptomatic

191   infections as detected by qPCR in the cross-sectional surveys was 17.70% (120/678) at 3 months,

192   20.13 (127/631) at 6 months, 18.40 (108/587) at 9 months and 30.41% (194/638) at 12 months.

193   Median qPCR-parasite density during clinical malaria episodes (10826.50 [IQR 876.50-38021.50]

194   parasites/µL) was significantly higher than that during asymptomatic infections (379 [IQR 1.94-

195   3932.5] parasites/µL, $P < .0001$).

196

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

198   Although time-to-first clinical malaria episode was not significantly different between the two

199   study arms in univariate and multivariate analyses (Table 2), birth season was found to interact and

200   significantly modify the effect of CSST/IPTp-SP on time-to-first clinical malaria episodes. We

201   observed that infants born to mothers in the CSST/IPTp-SP arm during malaria low-transmission

202   season had a 26% decreased risk of a first clinical malaria episode compared with those born to

203   mothers in the IPTp-SP arm (HR, 0.74 [95% CI, 0.55-0.99]; $P = .047$). By contrast, no significant

204   differences were observed between the two arms for infants born during malaria high-transmission

205   season (HR, 0.97 [95% CI, 0.76-1.25]; $P = .846$). That difference of the effect of CSST/IPTp-SP

206   on time-to-first clinical malaria episode according to birth season was further illustrated in Kaplan-

207   Meier survival curves (Figure 2).

208   We investigated whether the observed effect of CSST/IPTp-SP could be explained by differences

209   in prenatal malaria exposure. Table 3 shows that the increased risk of time-to-first clinical malaria

210   episode was associated with peripheral infection during pregnancy and past PM ($P = .009$ and P

211    $= .020$, respectively) but not with active PM ($P = .177$), after adjusting by gravidity, birth season

212   and LBW. Thus, the effect of CSST/IPTp-SP on time-to-first clinical malaria episode was more

9

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

214 PM.

215 The two COSMIC study arms had a similar effect on infant parasite density during the first clinical

216 malaria episode (6557.50 [IQR, 609.00-28484.50] parasites/µL in CSST/IPTp-SP vs. 4549.00

217 [IQR, 380.50-19627.75] parasites/µL in standard IPTp-SP). Similar lack of association was

218 observed on parasitaemia in all clinical episodes even after adjusting for gravidity and sex

219 (coefficient, 1.10 [95% CI, 0.82-1.47]; P = .526) (Supplementary Table 2).

220

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

222 Table 4 shows results of the analysis of the effect of the CSST/IPTp-SP strategy on the incidence

223 of clinical malaria episodes including repeated malaria attacks per infant during the first year of

224 life. We found no significant differences between CSST/IPTp-SP and IPTp-SP after adjusting for

225 birth season (adjusted IRR, 0.95 [95% CI, 0.82-1.10]; P = .459). Moreover, no significant

226 differences were observed in the stratified analysis by birth season (high-transmission season: IRR,

227 0.99 [95% CI, 0.82-1.20]; P = .941; low-transmission season IRR, 0.91 [95% CI, 0.72-1.14]; P =

228 .409).

229

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

231 In total, 553 of 690 infants (80.14%) experienced at least one symptomatic or asymptomatic

232 infection during the first year of life. The odds of experiencing a *P. falciparum* infection in infants

233 born to mothers in the CSST/IPTp-SP group was not significantly different from that of children

234 born to mothers in the IPTp-SP group, even after controlling for gravidity (adjusted OR, 1.20 [95%

235 CI, 0.82-1.74]; P = .348) (Supplementary Table 3). There were also no statistical differences

236   between the groups in terms of the proportions of asymptomatic infections in infants, regardless of

237   the time of screening (Supplementary Table 4).

238

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

240   A total of 805 NMFs episodes (incidence, 1.16 per child-year) were recorded in the 734 infants

241   analyzed during their first year of life. Table 5 presents the stratified analysis by birth season and

242   gravidity that were found to interact with CSST/IPTp-SP strategy to modify infant's susceptibility

243   to NMFs. Infants born to CSST/IPTp-SP mothers during malaria high-transmission season had a

244   significantly lower risk of developing NMFs than their counterpart born to mothers receiving the

245   standard IPTp-SP (adjusted IRR, .79 [95% CI, 0.64-0.98]; $P = .031$) (Table 5). When stratifying

246   the analysis by gravidity, only the offspring of multigravid women in the CSST/IPTp-SP group

247   were significantly protected against NMFs (adjusted IRR, .78 [95% CI, 0.63-0.97]; $P = .027$). We

248   did not observe any significant associations between prenatal malaria exposure and occurrence of

249   NMFs (Supplementary Table 5).

250

251   **Discussion**

252   In this prospective birth cohort study we found that, compared with standard IPTp-SP strategy,

253   CSST/IPTp-SP significantly increased time-to-first clinical malaria episode in infants born during

254   malaria low-transmission season, and protected against the occurrence of NMFs in those born

255   during malaria high-transmission season. Due to the transmission seasonality of malaria and NMFs

256   in Burkina Faso, CSST/IPTp-SP strategy appears to have different impact in women who had the

257   main course of their pregnancy during malaria high-transmission season (and most probably gave

258   birth during malaria low-transmission season) compared to those who were pregnant during

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

260   season).

261

262   Our findings showed, for the first time, that MiP preventive treatments may provide protection

263   against malaria in infants during the first year of life, in addition to the known protection against

264   adverse pregnancy outcomes. New MiP strategies should thus be evaluated not only for the

265   protective effect on pregnancy outcomes but also the potential additional long-term benefits in

266   infants. To our knowledge, only one study has compared the incidence of malaria in infants born

267   to mothers who received either IPTp-SP or screening and treatment with RDT and artemether-

268   lumefantrine (ISTp-AL) during antenatal care visits, without IPTp-SP [13]. The authors found no

269   differences in the risk of clinical malaria episodes during early life and observed that the two

270   interventions had similar performance in preventing PM [13]. In comparison, the strategy of

271   combining a CSST with the standard IPTp-SP increases the chances of clearing parasite infections

272   when the protective efficacy of IPTp-SP is waned, thus reducing the risk of long-term parasite

273   carriage during pregnancy. Indeed, thanks to the enhanced screening, the proportion of malaria

274   infections detected by RDT amongst women in the CSST/IPTp-SP arm was significantly higher

275   than that among women in the routine IPTp-SP arm (Table 1).

276

277   Remarkably, the impact of the CSST/IPTp-SP strategy on the risk of malaria in infancy was evident

278   in infants born to women who had the main course of their pregnancy during malaria high-

279   transmission season (and thus born during low-transmission season), while the two interventions

280   performed similarly when the main course of pregnancy was during the low-transmission season.

281   Therefore, we hypothesized that this effect of CSST/IPTp-SP is attributable to the additional

282   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

306   low-density infections [21]. We are tempted to speculate that the effect of the CSST/IPTp-SP

307   intervention could be increased with the use of high-sensitivity diagnostic tools capable of

308   detecting low-density infections. In this regard, high-sensitivity RDTs currently used in field

309   testing (PATH) [22] and molecular strategies such as LAMP [23], ~~which have a limit of detection~~

310   ~~of 10-20 parasites/µl and < 1 parasite/µL respectively,~~ are promising tools to improve screening

311   strategies.

312

313   It is noteworthy that the CSST/IPTp-SP intervention was also associated with a reduced incidence

314   of NMFs during the first year of life in infants born during malaria high-transmission season but

315   not in those born during malaria low-transmission season. Therefore, it is unlikely that the same

316   mechanism underlies the susceptibility to NMF since this protection, ~~which is in agreement with a~~

317   ~~previous study [24],~~ was independent of PM, even after exploring the association with different

318   categories of prenatal malaria exposure. Nevertheless, several studies have reported modulation of

319   the foetal immune system by malaria infections during pregnancy [19,25,26]. In addition to

320   malaria-specific immune responses, these modifications may also affect specific immunity to other

321   pathogens responsible of NMFs [27,28], such as those caused by blood stream infections,

322   gastrointestinal infections, acute respiratory infections, urinary tract infections and arbovirus

323   infections [29,30].

324   Moreover, the CSST/IPT-SP intervention was found to interact with gravidity to significantly

325   protect infants born to multigravid women but not these born to either secundigravid (non-

326   significant protection) or primigravid women (non-significant increased risk). These findings

327   suggest that, through the modification of prenatal malaria exposure (which may differ

328   depending on gravidity), the CSST/IPTp-SP intervention alters or prime the development of

329 fetal immune system affecting immunity to pathogens that are responsible of NMFs in early

330 infancy. To gain further an understanding of how MiP preventive interventions can modulate the

331 immune system to impact infant susceptibility to NMFs, new studies should include diagnosis of

332 NMFs.

333

334 **Conclusion**

335 In this study we have shown that, compared with standard IPTp-SP, CSST/IPTp-SP significantly

336 decreases the risk of experiencing a first clinical malaria episode and also interact with birth season

337 and gravidity to reduce the incidence of NMFs during the first year of life. Altogether, our findings

338 suggest that MiP preventive strategies that effectively reduce exposure to malaria parasites during

339 pregnancy could provide additional protection against both malaria and NMFs in early infancy and

340 therefore, have long-term benefits in infants.

341

342 **Notes:**

343

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

355

Presentation at meetings. Data of this study have been partly presented at the European Congress

of Tropical Medicine and International Health (ECTMIH) held from 16-20 October 2017 in

Antwerp, Belgium (Abstract number 8S1.1).

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458   **Tables**

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460   **Table 1: Baseline characteristics of study participants**

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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

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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.

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

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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

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506   **Figures**

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508   **Figure 1: Study flow diagram.** Infants born from pregnant women participating in a randomized-

509   cluster trial (CSST/IPTp-SP vs standard IPTp) [14] were recruited into the study at birth and

510   followed-up until 12 months of age. Lost to follow-up are infants who did not complete the 12-

511   months follow-up. They were considered as censored observations at the time of the last visit in

512   survival analysis. Neonatal deaths and twins were excluded from the analysis. *Not included in the

513   survival analysis. ** Included in the survival analysis.

514

515   **Figure 2: Kaplan-Meier survival curves for time-to-first clinical malaria episode during the**

516   **first year of life according to MiP prevention strategy by birth season.** Infants born during

517   malaria low transmission season (A) and born during malaria high transmission season (B) were

518   followed-up for 12 months.

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