



HAL
open science

Sustained clinical benefit of malaria chemoprevention with sulfadoxine-pyrimethamine (SP) in pregnant women in a region with high SP resistance markers

Glória Matambisso, Nanna Brokhattingen, Sónia Maculuve, Pau Císterro, Henriques Mbeve, Anna Escoda, Gizela Bambo, Boaventura Cuna, Cardoso Melembe, Nelo Ndimande, et al.

► To cite this version:

Glória Matambisso, Nanna Brokhattingen, Sónia Maculuve, Pau Císterro, Henriques Mbeve, et al.. Sustained clinical benefit of malaria chemoprevention with sulfadoxine-pyrimethamine (SP) in pregnant women in a region with high SP resistance markers. *Journal of Infection*, 2024, 88 (5), pp.106144. 10.1016/j.jinf.2024.106144 . hal-04749079

HAL Id: hal-04749079

<https://hal.science/hal-04749079v1>

Submitted on 22 Oct 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



Sustained clinical benefit of malaria chemoprevention with sulfadoxine-pyrimethamine (SP) in pregnant women in a region with high SP resistance markers

Glória Matambisso^a, Nanna Brokhattingen^b, Sónia Maculuve^a, Pau Císteros^b, Henriques Mbeve^a, Anna Escoda^b, Gizela Bambo^a, Boaventura Cuna^a, Cardoso Melembe^a, Nelo Ndimande^a, Kevin K.A. Tetteh^c, Chris Drakeley^c, Benoit Gamain^d, Chetan Chitnis^e, Virander Chauhan^f, Llorenç Quintó^{a,b}, Eusébio Macete^{a,g,1}, Alfredo Mayor^{a,b,h,i,*}

^a Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Moçambique

^b ISGlobal, Barcelona Institute for Global Health, Hospital Clínic-Universitat de Barcelona, Barcelona, Spain

^c Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK

^d Sorbonne Université, INSERM, CNRS, Centre d'Immunologie et des Maladies Infectieuses, CIMI-Paris, Paris, France

^e Malaria Parasite Biology and Vaccines, Department of Parasites & Insect Vectors, Institut Pasteur, Paris, France

^f Malaria Group, International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India

^g National Directorate of Health, Ministry of Health, Maputo, Mozambique

^h Spanish Consortium for Research in Epidemiology and Public Health (CIBERESP), Madrid, Spain

ⁱ Department of Physiologic Sciences, Faculty of Medicine, Universidade Eduardo Mondlane, Maputo, Mozambique

ARTICLE INFO

Article history:

Accepted 21 March 2024

Available online 2 April 2024

Keywords:

Malaria

Pregnancy

Chemoprevention

Intermittent preventive treatment

Sulfadoxine-pyrimethamine

Antimalarial resistance

SUMMARY

Objective: The effectiveness of intermittent preventive treatment of malaria in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) is threatened by increasing SP-resistance in Africa. We assessed the level of SP-resistance markers, and the clinical and parasitological effectiveness of IPTp-SP in southern Mozambique. **Methods:** *P. falciparum* infection, antimalarial antibodies and *dhfr/dhps* SP-resistance mutants were detected by quantitative polymerase chain reaction (qPCR), suspension array technology and targeted deep sequencing, respectively, among 4016 HIV-negative women in Maputo province (2016–2019). Univariate and multivariate regression models were used to assess the association between taking the recommended three or more IPTp-SP doses (IPTp3+) and parasitological and clinical outcomes.

Results: 84.3% (3385/4016) women received three or more IPTp-SP doses. The prevalence of quintuple mutants at first antenatal care (ANC) visit was 94.2%. IPTp3+ was associated with a higher clearance rate of qPCR-detected infections from first ANC visit to delivery (adjusted odds ratio [aOR]=5.9, 95% CI: 1.5–33.3; $p = 0.012$), lower seroprevalence at delivery of antibodies against the pregnancy-specific antigen VAR2CSA_{DBL3.4} (aOR=0.72, 95% CI: 0.54–0.95; $p = 0.022$), and lower prevalence of low birth weight deliveries (aOR: 0.61, 95% CI: 0.41–0.90; $p = 0.013$).

Conclusion: A sustained parasitological effect of IPTp-SP contributes to the clinical effectiveness of IPTp3+ in areas with high prevalence of SP-resistance markers.

© 2024 The Author(s). Published by Elsevier Ltd on behalf of The British Infection Association. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Introduction

Medicines that prevent *Plasmodium* infection and its consequences can contribute to reduce the malaria burden.¹ Preventive chemotherapy requires giving a full treatment course of an antimalarial medicine to vulnerable populations (generally pregnant women, infants and children under 5 years of age) at designated time points during the period of greatest malarial risk, regardless of whether the person is infected with malaria. Despite the encouraging results

* Correspondence to: Barcelona Institute of Global Health (ISGlobal), Hospital Clínic, Universitat de Barcelona, Rosselló 149, CEK building, 1st floor, 08036 Barcelona, Spain.

E-mail address: alfredo.mayor@isglobal.org (A. Mayor).

¹ Present address: Mozambique's Pharmaxis Farmac SA, Maputo, Mozambique.

obtained in several clinical trials, concerns over generation of drug resistance, impairment of immunity, safety and cost led to a slowdown in the adoption of chemopreventive approaches.^{1–3}

Malaria chemoprevention among pregnant women in malaria endemic countries aims to reduce the severe consequences of malaria infections in mothers and unborn children,⁴ which are associated with the ability of *Plasmodium falciparum* to colonize the placenta.⁴ VAR2CSA expressed by the parasite binds to chondroitin sulfate A in the placenta, and immunity acquired during successive pregnancies against this antigen has been suggested to explain the gravidity-dependent reduction in susceptibility to the infection.⁴ Since the initial use of chloroquine for chemoprophylaxis in the 1970s and 1980s, several clinical trials testing the efficacy of different treatment doses of sulfadoxine-pyrimethamine (SP) to reduce low birth weight (LBW) lead WHO to recommend in 2012 the use of monthly SP doses, at least a month apart, after the first trimester.⁵

The efficacy of SP for treatment is affected by the frequency of *dihydrofolate reductase* (*pfdhfr*; N51I, C59R, and S108N), and *dihydropteroate synthetase* (*pfdhps*; A437G and K540E) mutations in the parasite.⁶ The effectiveness of IPTp has been also suggested to decline with an increasing population prevalence of these mutations,⁷ and a loss of IPTp-SP efficacy has been associated in some studies with the emergence of sextuple mutant parasites harboring the additional *pfdhps* A581G mutation.^{8–10} The sustained benefit of IPTp with SP in areas with high levels of the quintuple *pfdhfr/dhps* mutants^{11–13} has been attributed to the antibiotic properties of sulfadoxine.^{14–16} Larger non-malarial effects in multigravidae¹⁷ have been suggested to reflect a greater attributable fraction of non-malarial causes of low birthweight compared with primigravidae.¹⁴ Overall, the insufficient evidence to withhold chemoprevention with SP in areas where the prevalence of quintuple mutants is high led WHO to keep the recommendation of using SP chemoprevention, independently of the prevalence of *pfdhps* and *pfdhfr* mutants, while research in this field keeps ongoing.⁵

The non-malaria effect theory to explain the sustained impact of IPTp with SP on birth outcomes in areas of high prevalence of *pfdhfr/dhps* quintuple mutants^{11–13} suggests an additional possible protective effect of SP in these resistance contexts. However, there is little evidence that the frequency of molecular markers predicts the effect of IPTp with SP on the clearance and prevention of *P. falciparum* infections, as most studies have focused on clinical outcomes.³ Here, we aimed to assess the parasitological and clinical impact of the recommended dosage of IPTp-SP during a three-year prospective observational study conducted in southern Mozambique where *pfdhfr/dhps* quintuple mutants have almost reached fixation.¹⁸ Parasitological outcomes included *P. falciparum* infections at delivery, clearance of parasite infections from first antenatal care (ANC) visit to delivery, and antibodies against VAR2CSA, as a serological surrogate of parasite exposure during pregnancy intended to amplify signals of parasite infection in low transmission areas.^{18,19} The study relied on the reported use of IPTp, given the absence of placebo-controlled trial data following the introduction of IPTp-SP as policy across Africa. To minimize biases common in observational studies,²⁰ we adjusted for a range of possible confounders including timely adherence to first ANC consultation, which has been associated with high IPTp uptake,²¹ and considered differences in malaria exposure that could contribute to the clinical outcomes under study. Finally, we used interaction analysis to assess gravidity-dependent differences in the impact of IPTp uptake.

Methods

Study area and population

The analysis is based on a three-year prospective observational study, conducted between November 2016 and November 2019 at

ANC and delivery clinics in Manhiça Sede District Hospital, Ilha Josina Health Center (Manhiça district), and Magude Health Center (Magude district), in Maputo province, southern Mozambique. The climate in the region is subtropical with a hot and rainy season from November to April and a cool and dry season during the rest of the year. Malaria transmission is moderate-to-low in Magude and Manhiça District, with high transmission pockets such as Ilha Josina.^{22,23} IPTp with SP was first implemented in Mozambique in 2006, delivered free of charge to all pregnant women.²⁴ In 2014, the national guidelines were updated and implemented countrywide according to the IPTp3 + WHO recommendation.⁵

Recruitment and data collection

Pregnant women at their first routine ANC visit and at delivery were invited to participate in the study if resident in the study area. If consenting, women at their first ANC visit and at delivery were requested to donate finger prick blood onto filter paper (dried blood spots [DBS]) together with routine tests. A brief form, including visit date, age of mother, gravidity, gestational age based on the fundal height measurement and date of last menstruation, use of insecticide treated nets (ITN) and indoor residual spraying (IRS), area of residence and recent or past movements were filled in. HIV status was recorded from the maternal health card and, if not available, a HIV serological rapid test was done following standard procedures. At delivery, the schedule of IPTp-SP intake was reconstructed from the information in the booklet of routine ANC of each pregnant woman. The new-borns were physically examined and weighed using digital scales calibrated weekly. Maternal hemoglobin levels were determined from whole blood collected by finger prick using HemoCue® (Hb 201 + HemoCue AB, Angelholm, Sweden).

Ethical considerations

Written informed consent was obtained from all participating women aged 18 or over. For pregnant women under 18 years of age (12–17), it was necessary for them to be accompanied by their parents, relatives or guardians for deciding about the participation of the minor in the study. The written informed assent was requested to the minor and the written informed consent to the accompanying adult. To be included in the study, both had to give consent. All study protocols were approved by Manhiça Health Research Center's institutional ethics committee, Hospital Clínic of Barcelona's Ethics Committee and the Mozambican National Committee for Bioethics in Health.

Parasitological determinations

DNA extracted from the DBS following the Chelex method was used for detection and quantification of *P. falciparum* by real-time quantitative PCR (qPCR) targeting *P. falciparum* 18S rRNA.¹⁸ qPCR-positive samples were amplicon-sequenced at the Wellcome Sanger Institute (Hinxton, UK) as previously described²⁵ (Supplementary methods). Genotypes were called for *pfdhfr* and *pfdhps* variants using *bcftools* and custom scripts in R.²⁵ For each codon, samples were classified as wild-type, mutant, mixed if both wild-type and mutant alleles were detected, or missing if samples failed to produce a valid genotype. Samples with no mixed genotypes were retained for the *pfdhfr/dhps* haplotype reconstruction and downstream statistical analysis.

Immunoglobulins G determinations

Immunoglobulin G (IgG) eluted from available DBS were quantified using the xMAP technology and the Luminex 100/200 System as previously described.^{18,19} The multiplex suspension array panel

included pregnancy-specific (VAR2CSA) antigens (Duffy binding-like recombinant domains DBL3–4 as well as peptides targeting the NTS region [P1] and ID1 [P8 and PD]) and non-pregnancy specific antigens (full-length *P. falciparum* reticulocyte-binding homolog protein 2 and 5 [PFRH2 and PFRH5], 19-kDa fragment of the merozoite surface protein-1 [MSP1], region II/F2 of erythrocyte-binding antigen-175 [EBA175], gametocyte exported protein 18 [GEXP18], acyl CoA synthetase 5 [ACS5], early transcribed membrane protein 5 [ETRAMP5], heat shock protein 40 [HSP40] and thrombospondin-related apical merozoite protein [PfTRAMP]; [Supplementary Methods](#)). The seropositivity thresholds were defined as the geometric mean plus 2 standard deviations of the first component from two-component normal mixture distributions of mean fluorescent intensity values (R package *mixtools*).

Data analysis

As IPTp-SP should not be given to pregnant women receiving cotrimoxazole,⁵ we restricted the analysis to HIV-uninfected women with information about IPTp use and data on first ANC visit and delivery. Women were classified as primigravidae (first pregnancy) or multigravidae (at least one previous pregnancy). Age was categorized as either younger than 18 years or 18 years and older, and season as rainy (November to April) or dry (May to October). Hemoglobin levels below 11 g/dL and new-born weight below 2500 grams were classified as anemia and LBW, respectively.

Categorical and continuous data were compared between IPTp dosing groups (less than three [IPTp3-] and three or more [IPTp3+] SP doses) using Chi-squared test or Fisher's exact test, and Student *t*-test, respectively. Univariate regression analyses were conducted to assess the association of IPTp groups with *P. falciparum* positivity rate by qPCR (PfPR_{qPCR}) at delivery and first ANC visit, clearance of *P. falciparum* infections from first ANC visit to delivery, maternal hemoglobin levels and anemia, new-born weight and LBW, and seroprevalence against *P. falciparum* antigens. Subsequently, a multivariate analysis including clinic, study period, season, gravidity, gestational stage at ANC and at delivery visit, reported use of IRS and ITNs, was performed. The hypothesis of a gravidity-dependent impact of IPTp was assessed including an interaction term between gravidity and IPTp groups in the models. All statistical analyses were performed with R studio software (version 1.0.143).

Results

Characteristics of study participants

Among the 8558 delivery visits recorded from November 2016 to October 2019, 309 were excluded from the study, mainly due to changes of residence area ($n = 199$). 66% (5465/8249) of the delivery visits were by HIV-negative women with information about the IPTp-SP intake ([Supplementary Fig. 1](#)). Of these, 4016 (73.5%) also had a first ANC study record (1423 [35.4%] from Magude, 2227 [55.5%] from Manhiça and 366 [9.1%] from Ilha Josina), and were included in the analysis ([Supplementary Fig. 1](#)). No major differences were observed between the 5465 HIV-uninfected women recruited in the study and the 4016 included in the analysis, nor between the women from the three study centers ([Supplementary Table 1](#)).

Mean gestational age at first ANC visit was 20.9 weeks (SD: 5.3), with 150 (3.7%), 3305 (82.3%) and 561 (14%) women having their visit during the first, second and third trimester, respectively ([Supplementary Table 1](#)). Among the 4016 women, 1284 (32%) were primigravidae, 428 (10.7%) less than 18 years of age, and 4005 (99.7%) delivered in the third trimester (mean gestational age: 38.8 weeks, SD: 2.2). ITN use and IRS was reported among 3581 (97.1%) and 2800 (75.9%) women out of the 3688 from whom data was available. The median number of IPTp-SP doses was four, ranging

Table 1
Number of IPTp-SP doses received during pregnancy at each health center.

Doses	All	Magude	Manhiça	Ilha Josina
	N = 4016	N = 1423	N = 2227	N = 366
	n (%)	n (%)	n (%)	n (%)
0	96 (2.4)	23 (1.6)	46 (2.1)	27 (7.4)
1	160 (4.0)	48 (3.4)	100 (4.5)	12 (3.3)
2	375 (9.3)	129 (9.1)	228 (10.2)	18 (4.9)
3	900 (22.4)	339 (23.8)	512 (23.0)	49 (13.4)
4	1090 (27.1)	416 (29.2)	602 (27.0)	72 (19.7)
5	954 (23.8)	328 (23.0)	512 (23.0)	114 (31.1)
6	388 (9.7)	128 (9.0)	192 (8.6)	68 (18.6)
7	51 (1.3)	11 (0.8)	35 (0.6)	5 (1.4)
8	2 (0.0)	1 (0.1)	0 (0.0)	1 (0.3)

from zero to eight ([Table 1](#)), with 84.3% (3385/4016) women having received three or more IPTp-SP doses. Primigravity, being less than 18 years and early attendance for the first ANC visit were more frequent among women who received 3 or more doses compared to those with fewer doses ([Table 2](#)).

Impact of IPTp3+ on parasitological outcomes

A total of 2999 and 2810 blood samples collected from pregnant women at first ANC visit and delivery, respectively, were analysed by qPCR. The prevalence of *P. falciparum* infection decreased from 7.7% (232/2999) at first ANC visit to 1.9% (55/2810) at delivery ($p < 0.001$). No statistically significant difference was observed in PfPR_{qPCR} at first ANC visit between subsequent IPTp-SP uptake groups ($p = 0.859$; [Supplementary Table 2](#)), although a trend was observed towards higher prevalence of *P. falciparum* infection at delivery in the IPTp3+ group (2.7% [12/440]) compared with the IPTp3- group (1.8% [42/2370], $p = 0.180$; [Table 3](#)). The analysis stratified by gravidity showed a statistically significant lower prevalence

Table 2
Baseline characteristics of the HIV-uninfected women included in the study, by IPTp-SP uptake group. Women were included if information about IPTp-SP intake, first antenatal visit and delivery was available. The p-values were obtained through the Chi-squared test and Fisher's exact test for categorical variables, and unpaired *t*-test for continuous variables.

	< 3 doses N = 631	≥3 doses N = 3385	p
Clinic, n (%)			
Magude	200 (31.7)	1223 (36.1)	0.085
Manhiça	374 (59.3)	1853 (54.7)	
Ilha Josina	57 (9.0)	309 (9.1)	
Year, n (%)			
Nov. 2016–Oct. 2017	169 (26.8)	762 (22.5)	0.037
Nov. 2017–Oct. 2018	252 (39.9)	1358 (40.1)	
Nov. 2018–Oct. 2019	210 (33.3)	1265 (37.4)	
Village, n (%)			
Yes	597 (94.6)	3195 (94.4)	0.821
No	34 (5.4)	190 (5.6)	
Season, n (%)			
Rainy	276 (43.7)	1415 (41.8)	0.365
Dry	355 (56.3)	1970 (58.2)	
Age, n (%)			
< 18 years	46 (7.3)	382 (11.3)	0.003
≥18 years	585 (92.7)	3003 (88.7)	
Gravidity, n (%)			
Primigravidae	129 (20.4)	1155 (34.1)	<0.001
Multigravidae	502 (79.6)	2230 (65.9)	
RPR, n (%)			
Positive	8 (1.3)	35 (1.0)	0.533
Negative	623 (98.7)	3350 (99.0)	
Gestational weeks at first ANC visit, mean (SD)	25.03 (5.68)	20.08 (4.80)	<0.001
Gestational weeks at delivery, mean (SD)	38.06 (3.32)	38.92 (1.95)	<0.001

RPR, rapid plasma reagin; SD, standard deviation.

Table 3

Parasitological and clinical outcomes at delivery by IPTp-SP uptake groups. Multivariate models adjusted by clinic, study period, season, gravidity, gestational stage at first ANC visit, use of ITNs and IRS reported at delivery and gestational age at delivery. p-values for comparing IPT-SP uptake groups obtained from Chi-squared test and Fisher's exact test for categorical variables, and unpaired t-test for continuous variables. Odds ratios and p-values were obtained from univariate and multivariate models adjusted by clinic, study period, season, gravidity, gestational stage at first ANC visit and delivery, and ITN and IRS reported use at delivery.

	N	<3 doses	≥3 doses	p ^a	Crude effect ^b (95% CI)	p ^c	Adjusted effect ^b (95% CI)	p ^d
<i>Pf</i> infection by qPCR, n (%)	2810	12/440 (2.7)	42/2370 (1.8)	0.180	0.64 (0.34; 1.28)	0.195	0.80 (0.39; 1.73)	0.559
IgG seropositivity, n (%)								
DBL34	2560	110/391 (28.1)	419/2169 (19.3)	<0.001	0.61 (0.48; 0.78)	<0.001	0.72 (0.54; 0.95)	0.022
P1	2648	26/407 (6.4)	73/2241 (3.3)	0.002	0.49 (0.31; 0.79)	0.004	0.54 (0.32; 0.91)	0.019
P8	2633	24/402 (6.0)	83/2231 (3.7)	0.035	0.61 (0.38; 0.99)	0.045	0.61 (0.36; 1.05)	0.065
PD	2633	26/404 (6.4)	125/2229 (5.6)	0.510	0.86 (0.56; 1.36)	0.505	0.84 (0.53; 1.38)	0.479
P39	2618	31/402 (7.7)	113/2216 (5.1)	0.035	0.64 (0.43; 0.98)	0.042	0.66 (0.42; 1.06)	0.078
MSP1	2640	177/405 (43.7)	974/2235 (43.6)	0.963	0.99 (0.80; 1.23)	0.962	1.06 (0.84; 1.35)	0.614
EBA175	2631	147/404 (36.4)	724/2227 (32.5)	0.128	0.84 (0.67; 1.05)	0.130	0.94 (0.73; 1.20)	0.601
PfPRH2	2614	57/395 (14.4)	198/2219 (8.9)	<0.001	0.58 (0.42; 0.80)	0.001	0.64 (0.45; 0.92)	0.013
PfPRH5	2585	59/395 (14.9)	264/2190 (12.1)	0.111	0.58 (0.42; 0.80)	0.001	0.84 (0.60; 1.18)	0.308
ACS5	2546	114/384 (29.7)	550/2162 (25.4)	0.081	0.81 (0.64; 1.03)	0.083	0.80 (0.61; 1.05)	0.104
GEXP18	2629	16/405 (4.0)	66/2224 (3.0)	0.295	0.74 (0.433; 1.33)	0.301	0.71 (0.40; 1.36)	0.284
Etramp	2641	55/406 (13.5)	264/2235 (11.8)	0.324	0.71 (0.53; 0.96)	0.026	0.86 (0.61; 1.22)	0.379
PfTramp	2639	70/405 (17.3)	353/2234 (15.8)	0.454	0.90 (0.68; 1.20)	0.452	0.84 (0.62; 1.15)	0.273
HSP40	2596	65/395 (16.5)	305/2201 (13.9)	0.174	0.81 (0.61; 1.10)	0.178	0.83 (0.60; 1.16)	0.275
Newborn weight (g), mean (SD)	3894	3083.2 (487.8)	3136.0 (444.9)	0.014	52.82 (13.56; 92.08)	0.008	58.08 (18.33; 97.83)	0.004
Low birth weight, n (%)	3894	55/602 (9.1)	169/3292 (5.1)	<0.001	0.54 (0.39; 0.74)	<0.001	0.61 (0.41; 0.90)	0.013
Maternal hemoglobin (g/dL), mean (SD)	3999	11.03 (1.46)	11.18 (1.50)	0.018	0.15 (0.02; 0.28)	0.020	0.04 (-0.09; 0.17)	0.533
Maternal anemia, n (%)	3999	285/625 (45.6)	1346/3374 (39.9)	0.008	0.79 (0.67; 0.94)	0.008	0.88 (0.72; 1.07)	0.204

SD, standard deviation.

^a, Chi-squared test for categorical variables and t-test for continuous variables.

^b, Odds Ratio for categorical variables and difference in the average values for continuous variables.

^c, Univariate regression models.

^d, Adjusted multivariate regression models.

Table 4

P. falciparum infection detected by qPCR at first antenatal care visit and at delivery, by IPTp-SP uptake group and gravidity. The p-values were obtained by two-proportion Z-test and multivariate models adjusted by clinic, study period, season, gravidity, gestational stage at first ANC visit and delivery, and reported use of ITN and IRS.

	All		Delivery				Multigravidae								
	N	qPCR+	qPCR-	p ^a	p ^b	N	qPCR+	qPCR-	p ^a	p ^b	N	qPCR+	qPCR-	p ^a	p ^b
		n (%)	n (%)				n (%)	n (%)				n (%)	n (%)		
qPCR+ANC															
<3 doses	29	8 (27.6)	21 (72.4)	<0.001	0.012	7	1 (14.3)	6 (85.7)	1.000	0.723	22	7 (31.8)	15 (68.2)	<0.001	0.009
≥3 doses	164	9 (5.5)	155 (94.5)			71	7 (9.9)	64 (90.1)			93	2 (2.2)	91 (97.8)		
qPCR-ANC															
<3 doses	323	4 (1.2)	319 (98.8)	0.619	0.499	60	0 (0.0)	60 (100)	0.598	0.994	263	4 (1.5)	259 (98.5)	0.804	0.920
≥3 doses	1743	30 (1.7)	1713 (98.3)			550	9 (1.6)	541 (98.4)			1193	21 (1.8)	1172 (98.2)		

^a, Two-proportion Z-test.

^b, Multivariate logistic regression model.

of *P. falciparum* infection at delivery among IPTp3+ multigravidae (1.5% [24/1576]) compared to IPTp3- multigravidae (3.1% [11/351]; $p = 0.041$), although the interaction was not statistically significant ($p = 0.165$; [Supplementary Table 3](#)). The proportion of qPCR-positive women at first ANC visit who were uninfected at delivery was higher among the IPTp3+ group (94.5% [155/164]) than among the IPTp3- group (72.4% [21/29]; aOR=5.9, 95% CI: 1.5–33.3; $p = 0.012$; [Table 4](#)). When stratifying by gravidity, this difference was only evident among multigravidae (aOR=1.8, 95% CI: 0.0–50.0, $p = 0.723$ for primigravidae and 20.0, 95% CI: 2.7–333.3, $p = 0.009$ for multigravidae; p for the interaction=0.088). The proportion of qPCR-negative women at ANC who were positive at delivery was similar in both IPTp-SP uptake groups ($p = 0.619$; [Table 4](#)).

Impact of IPTp3+ on serological outcomes

IgGs were measured in 2644–2745 (depending on the antigen) first ANC samples, and in 2546–2648 delivery samples. VAR2CSA_{DBL34} seroprevalence at delivery was lower in the IPTp3+ group (aOR=0.72; 95% CI: 0.54–0.95; $p = 0.022$; [Table 3](#)), but no statistically significant differences were observed at first ANC visit ($p = 0.133$; [Supplementary Table 2](#)). Similar results were observed for

VAR2CSA_{P1} and PfPRH2 ([Table 3](#)). The analysis stratified by gravidity showed a reduction in VAR2CSA_{DBL34}, VAR2CSA_{P8}, PfPRH2 and GEXP18 seroprevalences associated with the uptake of three or more doses among multigravidae women, but not among primigravidae ([Supplementary Table 3](#)).

Impact of IPTp3+ on new-born birthweight

Birth weight was higher in the IPTp3+ group (mean=3136 g, SD=444.89) compared to the IPTp3- group (mean=3083 g, SD=488; $p = 0.014$; [Table 3](#)), with a mean increase of 58 g (95%CI: 18–98; $p = 0.004$) observed in the multivariate model ([Table 3](#)). Similarly, IPTp3+ was associated with a 39% reduction in risk of LBW deliveries (aOR=0.61; 95% CI: 0.41–0.90; $p = 0.013$; [Table 3](#)). Same increase of birthweight and reductions in LBW were observed regardless of gravidity ([Supplementary Table 3](#)).

Impact of IPTp3+ on maternal anemia

Lower mean maternal hemoglobin and anemia was observed in women who received less than three IPTp-SP doses (hemoglobin: 11.03 ± 1.46 g/dL; anemia: 45.6% [285/625]) than in the IPTp3+ group

(11.18 ± 1.50 g/L, $p = 0.018$; 39.9% [1346/3374], $p = 0.008$; Table 3). However, the difference was not statistically significant in the multivariate model (Table 3).

Molecular markers of SP-resistance

pfdhfr and *pfdhps* sequences were obtained from 346 *P. falciparum* samples (249 from first ANC visit and 97 from delivery). Prevalence of polymorphisms in *pfdhfr* and *pfdhps* was above 95%. After excluding samples with missing or mixed genotypes, 94.2% (130/138) first ANC samples presented a quintuple mutant haplotype (Supplementary Table 4). However, *pfdhps* A581G mutation was not detected in any of the samples analyzed. No statistically significant difference was observed in the prevalence of *pfdhfr/dhps* mutation at the first ANC visit and delivery (Supplementary Table 4).

Discussion

This three-year prospective observational study shows that the use of three or more doses of IPTp-SP was associated with increased clearance of *P. falciparum* infections detected at first ANC visit, a lower seropositivity against VAR2CSA, and increased new-born weight, in spite of the very high prevalence of *pfdhfr/dhps* quintuple mutants associated with SP-resistance. A sustained parasitological effect of IPTp-SP to clear *P. falciparum* infections, combined with the antibiotic properties of sulfadoxine,^{14–16} may contribute to the beneficial effects of the intervention even in settings where the quintuple mutants have reached saturation. However, late attendance to first ANC consultations (21 weeks of gestation, far from WHO recommendations of starting ANC consultations during the first trimester of pregnancy²⁶) and failure to receive three or more IPTp-SP doses (16% of the women participating in the study)²⁷ are still major barriers for the successful implementation of IPTp-SP.

The study area in southern Mozambique is characterized by a low prevalence of qPCR-detected *P. falciparum* infections, both at first ANC visit (7.7%) and at delivery (1.9%), and by a high prevalence (94%) of parasites carrying *pfdhfr/dhps* quintuple mutants. However, we found no evidence of the occurrence *pfdhps* A581G mutation which, in addition to quintuple mutations, has been associated with the loss of IPTp-SP efficacy in some studies.^{8–10} In this epidemiological context, IPTp3+ is associated with a 39% reduction in the risk of LBW, as has been reported in medium-to-high SP-resistance areas.^{11,12,15,28} The antibiotic properties of sulfadoxine may contribute to sustain this beneficial impact in areas with high levels of SP-resistance mutations.^{14–16} However, we did not observe the difference by gravidity in the beneficial effect of IPTp3+ on new-born birth-weight detected in a previous study,¹⁴ which was explained by a greater attributable fraction of non-malarial causes of low birth-weight compared with primigravidae. Moreover, our study shows a higher clearance rate of *P. falciparum* infections detected at first ANC visit and lower seroprevalence of pregnancy-specific antibodies in the IPTp3+ group, indicating that SP retains sufficient effectiveness to reduce exposure to *P. falciparum* during pregnancy.¹² This parasitological effect of IPTp3+ was more evident among multigravid women, which may be explained by the contribution of immunity acquired against placental-type parasites during previous pregnancies²⁹ or to a higher number of multigravid than primigravid women in the study. Further studies are needed to assess the role of anti-malarial immunity on the preventive effect of IPTp, as well as the impact that reductions in antibody-mediated immunity may have on the risk of *P. falciparum* infections during subsequent pregnancies.

Overall, the inferences on causality can only be tentative in the present study, mainly due to two limitations. First, we cannot discard “adherer effects” due to broader health awareness or improved health status among those who use health services.²⁰ Second, it is

not possible to disentangle the contribution of earlier ANC care attendance on the beneficial effect of IPTp3+.¹⁵ Although we rigorously adjusted for multiple confounders including previous gravidities, use of ITNs and IRS, and gestational age at first ANC visit and delivery, our results could be biased by unmeasured variables, such as nutritional habits which affect hemoglobin levels. However, we did not observe any difference in parasitological outcomes at first ANC visit between both groups of women, allowing us to discard a beneficial effect of IPTp3+ due to lower risk of malaria infection among women of higher socio-economic status who are expected to use ANC consultations more frequently. Finally, we did not collect information on the dates of IPTp-SP uptakes and therefore we cannot discard that a shorter period since last IPTp dose explains the apparent parasitological effect observed at delivery in women receiving three or more doses.

In conclusion, this study shows how surveillance at maternity wards offer a unique opportunity to continuously monitor the effectiveness of IPTp against important health outcomes.³⁰ Three or more doses of IPTp-SP are effective in improving parasitological and clinical pregnancy outcomes (new-born birthweight) in the Maputo province in southern Mozambique, an area with high prevalence of molecular markers of SP-resistance and low malaria burden. While IPTp-SP may exert its beneficial impact on LBW through a mechanism that is unrelated to malaria,¹⁴ this study suggest that SP still retains a protective effect on *P. falciparum* infections, that could contribute to the overall effectiveness of the intervention. Therefore, the use of SP for IPTp should remain as a fundamental component in the strategy to protect pregnant women from malaria and its adverse effects in areas of high molecular markers of SP-resistance³¹ until more effective alternatives for malaria chemoprevention are found. Strengthening operational capacities for delivering chemoprevention in pregnancy and continuing research in this field is required for an optimal use of the limited arsenal of tools in the fight against malaria.

Funding

This work was funded by the National Institute of Health (1R01AI123050), the Bill and Melinda Gates Foundation (INV-019032), the Departament d'Universitats i Recerca de la Generalitat de Catalunya (AGAUR; 2021 SGR 01517), Ministerio de Ciencia e Innovación (MCIN/AEI/10.13039/501100011033), “la Caixa” Foundation (ID 100010434, fellowship code LCF/BQ/DI20/11780016, N.B) and the Fundação Calouste Gulbenkian (fellowship ID 270139, G.M.). We also acknowledge support from the Spanish Ministry of Science and Innovation and State Research Agency through the “Centro de Excelencia Severo Ochoa 2019–2023” program (CEX2018–000806-S), and support from the Generalitat de Catalunya through the CERCA Program. This research is part of the ISGlobal's Program on the Molecular Mechanisms of Malaria which is partially supported by the Fundación Ramón Areces. CISM is supported by the Government of Mozambique and the Spanish Agency for International Development (AECID). This publication uses data from the MalariaGEN SpotMalaria project as described in “Jacob CG et al.; Genetic surveillance in the Greater Mekong Subregion and South Asia to support malaria control and elimination; eLife 2021;10:e62997 <https://doi.org/10.7554/eLife.62997>.” The project is coordinated by the MalariaGEN Resource Center with funding from Wellcome (206194, 090770). The authors would like to thank the staff of Wellcome Sanger Institute Sample Management, Genotyping, Sequencing and Informatics teams for their contribution. The funders had no role in the study design, data collection, data analysis, data interpretation, or writing of this report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of Competing Interest

All authors reported no conflicts of interest.

Acknowledgments

The authors are grateful to the mothers who agreed to participate in the study, the clinical teams at the Magude Health Center, the Ilha Josina Health center and the Manhiça District Hospital, and the personnel at CISM and ISGlobal (Laboratory team and Maternal, Infant and Reproductive Health Team). We are also thankful to Dr. Deepak Gaur from Jawaharlal Nehru University, New Delhi, India; deceased in 2021, who offered very kindly recombinant proteins produced in his lab.

Author contributions

Alfredo Mayor and Eusebio Macete designed the study. Sónia Maculuve, Henriques Mbeve, Gizela Bambo recruited study participants. Boaventura Cuna, Cardoso Melembe and Nelo Ndimande managed the data and samples. Glória Matambisso, Nanna Brokhattingen, Pau Cisteró and Anna Escoda analysed the samples. Kevin Teth, Benoit Gamain, Chris Drakeley, Chetan Chitnis and Virander Chauhan produced recombinant antigens for serological determinations. Alfredo Mayor and Glória Matambisso analysed and interpreted the data. Nanna Brokhattingen and Llorenç Quintó contributed to the interpretation of data. Alfredo Mayor and Glória Matambisso drafted the manuscript. All authors critically revised the manuscript, had full access to all the data in the study and accept responsibility to submit for publication.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jinf.2024.106144](https://doi.org/10.1016/j.jinf.2024.106144).

References

- Greenwood B, Schellenberg D. *Chemoprevention for the populations of Malaria Endemic Africa*. *Diseases* 2022;**10**(4):101–10.
- Menendez C, Sautte F, Greenwood B, Schellenberg D, R. S, P. L. A. *Avoiding another lost decade in reducing malaria burden in African infants and young children*. *Lancet Glob Health* 2022;**10**:1385–6.
- Plowe CV. *Malaria chemoprevention and drug resistance: a review of the literature and policy implications*. *Malar J* 2022;**21**:1–25.
- Rogerson SJ, Desai M, Mayor A, Sicuri E, Taylor SM, van Eijk AM, et al. *Burden, pathology, and costs of malaria in pregnancy: new developments for an old problem*. *Lancet Infect. Dis.* 2018;**3099**.
- World Health Organization. *WHO Guidelines for malaria - 3 June 2022*. (<http://apps.who.int/bookorders>). (2022).
- Kublin JG, Dzinjalimala FK, Kamwendo DD, Malkin EM, Cortese JF, Martino LM, et al. *Molecular markers for failure of sulfadoxine-pyrimethamine and chlorproguanil-dapsone treatment of Plasmodium falciparum malaria*. *J Infect Dis* 2002;**185**:380–8.
- van Eijk AM, Larsen DA, Kayentao K, Koshy G, Slaughter DEC, Roper C, et al. *Effect of Plasmodium falciparum sulfadoxine-pyrimethamine resistance on the effectiveness of intermittent preventive therapy for malaria in pregnancy in Africa: a systematic review and meta-analysis*. *Lancet Infect Dis* 2019;**19**:546–56.
- Braun V, Rempis E, Schnack A, Decker S, Rubaihayo J, Tumwesigye NM, et al. *Lack of effect of intermittent preventive treatment for malaria in pregnancy and intense drug resistance in western Uganda*. *Malar J* 2015;**14**:1–10.
- Chico RM, Cano J, Ariti C, Collier TJ, Chandramohan D, Roper C, et al. *Influence of malaria transmission intensity and the 581G mutation on the efficacy of intermittent preventive treatment in pregnancy: systematic review and meta-analysis*. *Trop Med Int Health* 2015;**20**:1621–33.
- Minja DTR, Schmiegelow C, Mmbando B, Boström S, Oesterholt M, Magistrado P, et al. *Plasmodium falciparum mutant haplotype infection during pregnancy associated with reduced birthweight, Tanzania*. *Emerg Infect Dis* 2013;**19**:1446–54.
- Kamau A, Musau M, Mwakio S, Amadi D, Nyaguara A, Bejon P, et al. *Impact of intermittent presumptive treatment for Malaria in pregnancy on hospital birth outcomes on the Kenyan Coast*. *Clin Infect Dis* 2023;**76**:875–83.
- Desai M, Julie G, Steve MT, Ryan EW, Carole K, Kassoum K, et al. *Impact of sulfadoxine-pyrimethamine resistance on effectiveness of intermittent preventive therapy for Malaria in pregnancy at clearing infections and preventing low birth weigh*. *Clin Infect Dis* 2016;**62**(3):323–33.
- Mlugu EM, Minzi O, Asghar M, Färnert A, Kamuhabwa AAR, Aklillu E, et al. *Effectiveness of sulfadoxine-pyrimethamine for intermittent preventive treatment of malaria and adverse birth outcomes in pregnant women*. *Pathogens* 2020;**9**:1–19.
- Roh ME, Kuile FO, Rerolle F, Glymour MM, Shiboski S, Gosling R, et al. *Overall, anti-malarial, and non-malarial effect of intermittent preventive treatment during pregnancy with sulfadoxine-pyrimethamine on birthweight: a mediation analysis*. *Lancet Glob Health* 2020;**8**:942–53.
- Chico RM, Chaponda EB, Ariti C, Chandramohan D. *Sulfadoxine-pyrimethamine exhibits dose-response protection against adverse birth outcomes related to malaria and sexually transmitted and reproductive tract infections*. *Clin Infect Dis* 2017;**64**:1043–51.
- Menéndez C, Bardaji A, Sigauque B, Sanz S, Aponte JJ, Mabunda S, et al. *Malaria prevention with IPTp during pregnancy reduces neonatal mortality*. *PLoS One* 2010;**5**:1–6.
- Kajubi R, Ochieng T, Kakuru A, Jagannathan P, Nakalembe M, Ruel T, et al. *Monthly sulfadoxine-pyrimethamine versus dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in pregnancy: a double-blind, randomised, controlled, superiority trial*. *Lancet* 2019;**393**:1428–39.
- Matambisso G, Brokhattingen N, Maculuve S, Cisteró P, Mbeve H, Escoda A, et al. *Gravidity and malaria trends interact to modify P. falciparum densities and detectability in pregnancy: a 3-year prospective multi-site observational study*. *BMC Med* 2022;**20**:1–12.
- Fonseca AM, González R, Bardaji A, Jairoce C, Rupérez M, Jiménez A, et al. *VAR2CSA serology to detect plasmodium falciparum transmission patterns in pregnancy*. *Emerg Infect Dis* 2019;**25**:1851–60.
- Simpson SH, Eurich DT, Majumdar SR, Padwal RS, Tsuyuki RT, Varney J, et al. *A meta-analysis of the association between adherence to drug therapy and mortality*. *BMJ* 2006;**333**:15.
- Arnaldo P, Rovira-Vallbona E, Langa JS, Salvador C, Guetens P, Chiheb D, et al. *Uptake of intermittent preventive treatment and pregnancy outcomes: health facilities and community surveys in Chókwè district, southern Mozambique*. *Malar J* 2018;**17**:1–13.
- Galatas B, Guinovart C, Bassat Q, Aponte JJ, Nhamussua L, Macete E, et al. *A prospective cohort study to assess the micro - epidemiology of Plasmodium falciparum clinical malaria in Ilha Josina Machel Manhiça*. *Malar J* 2016:1–12.
- Galatas B, Saúte F, Martí-Soler H, Guinovart C, Nhamussua L, Simone W, et al. *A multiphase program for malaria elimination in southern Mozambique (the Magude project): a before-after study*. *PLoS Med* 2020;**17**:1–23.
- Ministerio da saude Programa Nacional de Controlo da Malaria, Normas de Manejo dos casos de Malária em Moçambique, 2006.
- Oyola SO, Ariani CV, Hamilton WL, Kekre M, Amenga-Etego LN, Ghansah A, et al. *Whole genome sequencing of Plasmodium falciparum from dried blood spots using selective whole genome amplification*. *Malar J* 2016;**15**:1–12.
- World Health Organization. *WHO recommendation on antenatal care for a positive pregnancy experience: summary*. *Lancet* 2018;**387**:1–10.
- Instituto Nacional de Saúde (INS) e ICF. *Inquérito Nacional sobre Indicadores de Malária em Moçambique 2018*. Maputo, Moçambique (2019).
- Mama A, Ahiabor C, Torniyigah B, Frempong NA, Kusi KA, Adu B, et al. *Intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine and parasite resistance: cross-sectional surveys from antenatal care visit and delivery in rural Ghana*. *Malar J* 2022;**21**:1–9.
- Ruizendaal E, Tahita MC, Geskus RB, Versteeg I, Scott S, D'Alessandro U, et al. *Increase in the prevalence of mutations associated with sulfadoxine-pyrimethamine resistance in Plasmodium falciparum isolates collected from early to late pregnancy in Nanoro, Burkina Faso*. *Malar J* 2017;**16**:1–15.
- Brabin BJ, Wasame M, Uddenfeldt-Wort U, Dellicour S, Hill J, Gies S. *Monitoring and evaluation of malaria in pregnancy—developing a rational basis for control*. *Malar J* 2008;**7**:1–12.
- Walker PGT, Floyd J, ter Kuile F, Cairns M. *Estimated impact on birth weight of scaling up intermittent preventive treatment of malaria in pregnancy given sulphadoxine-pyrimethamine resistance in Africa: a mathematical model*. *PLoS Med* 2017;**14**:1–19.