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Evaluation of intermittent preventive treatment of malaria against group B *Streptococcus* colonization in pregnant women: a nested analysis of a randomized controlled clinical trial of sulfadoxine/pyrimethamine versus mefloquine

Mesküre Capan-Melser[1–3], Ghyslain Mombo Ngoma[1,2,4], Daisy Akerey-Diop[1,2], Arti Basra[1,2], Heike Würbel[1,2], Mirjam Groger[1,3], Jean R. Mackanga[1,2], Rella Zoleko-Manego[2,5], Ulla Schipulle[1,2], Julia Schwing[1,2], Felix Lötsch[1,3], Khalid Rehman[3], Pierre-Blaise Matsiegui[5], Selidji T. Agnandji[1,2], Ayôla A. Adegnika[1,2], Sabine Bélard[1,2,6], Raquel González[7], Peter G. Kremsner[1,2], Clara Menendez[7] and Michael Ramharter[1–3]*

[1]*Centre de Recherches Médicales de Lambaréné, Hôpital Albert Schweitzer, Lambaréné, Gabon;* [2]*Institut für Tropenmedizin, Universität Tübingen, Tübingen, Germany;* [3]*Department of Medicine I, Division of Infectious Diseases and Tropical Medicine, Medical University of Vienna, Vienna, Austria;* [4]*Département de Parasitologie, Université des Sciences de la Santé, Libreville, Gabon;* [5]*Ngounie Medical Research Centre, Fougamou, Gabon;* [6]*Department of Pediatric Pneumology and Immunology, Charité-Universitätsmedizin Berlin, Berlin, Germany;* [7]*Barcelona Centre for International Health Research (CRESIB, Hospital Clínic-Universitat de Barcelona), IS Global, Barcelona, Spain*

*Corresponding author. Department of Medicine I, Division of Infectious Diseases and Tropical Medicine, Medical University of Vienna, Währinger Gürtel 18–20, 1190 Vienna, Austria. Tel: +43-1-40400-44400; Fax: +43-1-40400-44180; E-mail: michael.ramharter@meduniwien.ac.at

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Objectives: *Streptococcus agalactiae* constitutes an important cause of neonatal infections in sub-Saharan Africa. Sulfadoxine/pyrimethamine—the current intermittent preventive treatment of malaria in pregnancy (IPTp)—has proven *in vitro* activity against group B *Streptococcus* (GBS). Because of specific drug resistance to sulfadoxine/pyrimethamine, mefloquine—an antimalarial without *in vitro* activity against GBS—was evaluated as a potential alternative. This study assessed the potential of sulfadoxine/pyrimethamine-IPTp to reduce the prevalence of GBS colonization in pregnant women in Gabon when compared with the inactive control mefloquine-IPTp.

Methods: Pregnant women participating in a randomized controlled clinical trial evaluating mefloquine-IPTp versus sulfadoxine/pyrimethamine-IPTp were invited to participate and recto-vaginal swabs were collected at delivery for detection of GBS colonization. Prevalence of recto-vaginal GBS colonization was compared between IPTp regimens and risk factor and birth outcome analyses were computed.

Results: Among 549 participants, 106 were positive for GBS colonization at delivery (19%; 95% CI = 16%–23%). Prevalence of maternal GBS colonization showed no significant difference between the two IPTp regimens (mefloquine-IPTp: 67 of 366 women = 18%; 95% CI = 14%–22%; sulfadoxine/pyrimethamine-IPTp: 39 of 183 women = 21%; 95% CI = 15%–27%). Risk factor analysis for GBS colonization demonstrated a significant association with illiteracy (adjusted OR = 2.03; 95% CI = 1.25–3.30). GBS colonization had no impact on birth outcome, anaemia at delivery, gestational age and birth weight.

Conclusions: Sulfadoxine/pyrimethamine did not reduce colonization rates when used as the IPTp drug during pregnancy. Illiteracy was associated with GBS colonization.

Keywords: *Streptococcus agalactiae*, maternal GBS colonization, intermittent preventive treatment in pregnancy, sub-Saharan Africa

Introduction

Streptococcus agalactiae—commonly referred to as group B *Streptococcus* (GBS)—is a Gram-positive bacterium that has been identified as a human pathogen since the early 1900s. Recto-vaginal colonization of pregnant women is common in industrialized countries as well as in sub-Saharan Africa, ranging from 10% to 32% of all pregnant women.[1–5] GBS colonization during pregnancy increases the risk of miscarriage and invasive bacterial infections in newborns leading to GBS-associated sepsis,

pneumonia and meningitis.[5] Prenatal screening in pregnant women and administration of intrapartum antibiotics reduce the incidence of invasive disease.[6,7] However, targeted strategies for the prevention of invasive neonatal GBS infections are currently not implemented in most sub-Saharan African regions due to limitations in resources and infrastructure.

Sulfadoxine/pyrimethamine is recommended by the WHO for use as an intermittent preventive treatment of malaria in pregnancy (IPTp).[8] The concept of IPTp is to repeatedly administer a curative dose of an antimalarial drug to all asymptomatic pregnant women for the prevention of malaria-related adverse pregnancy outcomes. Due to increasing drug resistance of *Plasmodium falciparum* to sulfadoxine/pyrimethamine, mefloquine has recently been evaluated as a potential alternative drug for IPTp.[9,10]

Sulfadoxine/pyrimethamine is an antifolate antibiotic with known activity against Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus pneumoniae* and *S. agalactiae*).[11] Mefloquine has important activity against *Schistosoma haematobium* and *in vitro* activity against *S. pneumoniae*.[11,12] However, mefloquine has no important activity against other bacterial pathogens including GBS.[11,13–15] We therefore hypothesized that sulfadoxine/pyrimethamine-IPTp would be associated with a reduction in GBS colonization prevalence at delivery. To test this hypothesis and to assess potential risk factors for GBS colonization, we performed a nested assessment of GBS colonization at delivery in a randomized controlled clinical trial evaluating sulfadoxine/pyrimethamine-IPTp versus mefloquine-IPTp.

Methods

HIV-negative pregnant women participating at the Gabonese study sites of a multicentre randomized controlled clinical trial comparing the safety and efficacy of mefloquine and sulfadoxine/pyrimethamine as IPTp in sub-Saharan Africa (MIPPAD trial; NCT 00811421) were invited to participate in this study.[9,16] IPTp drugs were administered twice during pregnancy with the first dose administered between the 13th and 28th week of gestation and the second dose ≥1 month later. Mefloquine was administered either as a single full dose (15 mg/kg bodyweight) or as a split dose of 7.5 mg/kg each on two consecutive days. Sulfadoxine/pyrimethamine was given as single-dose treatment following current WHO recommendations (three tablets of 500 mg/25 mg of sulfadoxine/pyrimethamine). Pregnant women were followed up until 1 month after delivery and their infants were repeatedly assessed during their first year of life.

This study was conducted at the Centre des Recherches Médicales de Lambaréné, Hôpital Albert Schweitzer and the Ngounié Medical Research Centre in Fougamou between April 2010 and January 2012.[16] During the study period, 557 pregnant women participating in the MIPPAD trial consented to participate in this substudy. Data for analysis were available from 549 participants. The study was approved by the institutional ethics review board, was performed following international regulations including the Declaration of Helsinki and written informed consent was obtained from HIV-negative pregnant women permanently residing in the study area with a gestational age at the first visit of ≤28 weeks. Impartial witnesses signed the consent form in the case of inability to read or write after oral consent was obtained from the participant. Written informed consent was sought from a legal representative in addition to the participant in the case of age <18 years. Demographic characteristics were assessed at inclusion and outcome variables were evaluated at delivery. Literacy, defined as the ability to read and write, was assessed during the informed consent process. Low birth weight was defined as weight at delivery <2500 g and anaemia was defined as haemoglobin <110 g/L.

Bacterial swabs were taken from the vagina and rectum at presentation for delivery. All samples were collected using nylon flocked swabs, which were submerged into 1 mL of liquid Amies transport medium (eSwab, Copan Diagnostics, Brescia, Italy). A total of 200 μL of the transport medium of each recto-vaginal swab was inoculated into 5 mL of Lim Broth (Todd–Hewitt broth, 1% yeast extract, 15 μg/mL nalidixic acid and 10 μg/mL colistin) (Becton Dickinson),[17] which is selective for Gram-positive bacteria. This culture was incubated under aerobic conditions at 37°C for 24 h and then subcultured for 24 h on Granada agar (Becton Dickinson)[18] at 37°C in an anaerobic chamber (BugBox, LedTechno, Heusden-Zolder, Belgium). Granada agar was examined for yellow-orange-pigmented colonies confirming growth of GBS.

Assuming a 20% prevalence of GBS colonization in the inactive control group at delivery and a 30% reduction in the active intervention group, sample size calculation yielded a required sample size of 350 and 175 participants (in a 1:2 randomization) to detect a statistical difference with a power of $\beta = 0.8$ at $\alpha = 0.05$. In this unblinded trial, participants were randomly allocated to treatment groups following a computer-generated randomization list with opaque sealed envelopes produced by MIPPAD's sponsor institution biostatistician.[9] Statistical analysis was performed using a commercial software package (Stata/IC 13.1, StataCorp, College Station, TX, USA). Descriptive data were depicted as proportions. Unadjusted bivariable and adjusted multivariable logistic regression analysis was performed to provide ORs for the outcome GBS colonization at delivery. The covariables IPTp regimen, maternal age (stratified in age groups), literacy, parity and anaemia at baseline were included in the logistic regression model for the evaluation of their influence on GBS colonization. Adjusted and unadjusted ORs were computed and depicted with their respective 95% CIs and *P* values.

Results

Characteristics of the study population and GBS colonization rates in sulfadoxine/pyrimethamine-IPTp and mefloquine-IPTp

A total of 557 pregnant women consented to participate in this study and outcome data were available for 549 women (Figure 1 and Table 1). Characteristics of the participants including maternal age, literacy, parity and baseline anaemia were comparable between groups and are outlined in Table 1. Considering all

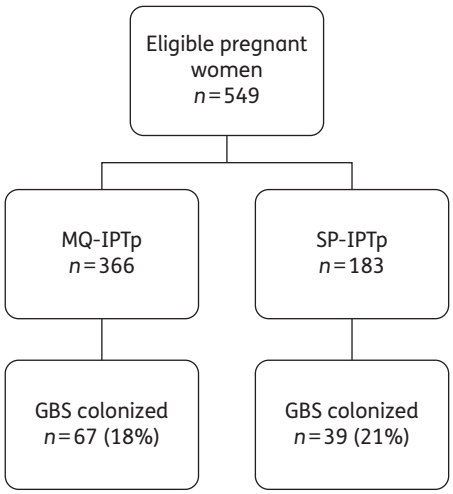

Figure 1. Study flow. MQ, mefloquine; SP, sulfadoxine/pyrimethamine.

participants, the prevalence of GBS colonization was 19% at delivery (95% CI=16%–23%; 106 of 549 participants). A total of 366 participants received mefloquine-IPTp, among whom GBS colonization was detected in 67 women (18%; 95% CI=14%–22%). Similarly, 39 out of 183 participants (21%; 95% CI=15%–27%) were GBS positive in the sulfadoxine/pyrimethamine-IPTp group (see Figure 1). No significant differences in the proportion of women with GBS colonization were detected between sulfadoxine/pyrimethamine-IPTp and mefloquine-IPTp treatment groups as assessed by crude and adjusted ORs: OR=1.21 (95% CI=0.78–1.80) and adjusted OR=1.22 (95% CI=0.78–1.92) (Table 2).

Table 1. Baseline characteristics of the study population (n=549)

	Mefloquine (n=366), n (%)	Sulfadoxine/pyrimethamine (n=183), n (%)
Maternal age (years)		
<20	115 (31)	58 (32)
20–30	177 (48)	89 (49)
>30	74 (20)	36 (20)
Literacy		
yes	295 (81)	143 (78)
no	71 (19)	40 (22)
Parity[a]		
nulliparous	99 (27)	56 (31)
pauciparous	127 (35)	63 (34)
multiparous	140 (38)	64 (35)
Anaemia at baseline[b]		
yes	260 (71)	136 (74)
no	106 (29)	47 (26)

[a]Pauciparous defined as one to two previous pregnancies and multiparous defined as more than two previous pregnancies.
[b]Anaemia defined as haemoglobin <110 g/L.

Risk factor analysis for GBS colonization and birth outcome analysis

Risk factor analysis showed a significant association between GBS colonization and illiteracy of pregnant women (Table 2). The prevalence was 16% in literate participants and 31% in illiterate participants (OR=2.25; 95% CI=1.39–3.61) with an associated P value of 0.001. This statistically significant difference remained unaltered after adjustment for IPTp regimen, maternal age and parity (adjusted OR=2.03; 95% CI=1.25–3.30; P=0.004). Maternal age, parity and anaemia at baseline were not independent risk factors for GBS colonization. Data of the birth outcome analysis are shown in Table 3. GBS colonization had no impact on birth outcome, anaemia at delivery, gestational age and birth weight (Table 3).

Discussion

The proportion of women colonized with GBS in this Gabonese study population is comparable to reports from other regions of Africa, Europe and North America.[1,4,5] Interestingly, illiteracy was a strong predictor for GBS colonization at delivery. Again, this finding is supported by previous reports from other geographical settings.[19–21] It is unclear whether differences in access to healthcare, differences in antimicrobial drug usage or more

Table 2. Participants' characteristics and GBS colonization

	n	GBS+, n (%)	OR (95% CI)	P	Adjusted OR[a] (95% CI)	P
IPTp						
mefloquine	366	67 (18)	1		1	
sulfadoxine/pyrimethamine	183	39 (21)	1.21 (0.78–1.80)	0.40	1.22 (0.78–1.92)	0.386
Maternal age (years)						
<20	173	26 (15)	1		1	
20–30	266	56 (21)	1.51 (0.91–2.51)	0.115	1.01 (0.53–1.94)	0.977
>30	110	24 (22)	1.58 (0.85–2.92)	0.146	0.83 (0.35–1.97)	0.664
Literacy						
yes	438	72 (16)	1		1	
no	111	34 (31)	2.25 (1.39–3.61)	0.001	2.03 (1.25–3.30)	0.004
Parity						
nulliparous	155	20 (13)	1		1	
pauciparous	190	36 (19)	1.58 (0.87–2,86)	0.132	1.50 (0.75–2.97)	0.251
multiparous	204	50 (25)	2.19 (1.24–3.87)	0.007	2.17 (0.97–4.85)	0.059
Anaemia at baseline						
no	153	32 (21)	1			
yes	396	74 (19)	0.87 (0.55–1.38)	0.553		

[a]Adjusted for IPTp regimen, maternal age, literacy and parity.

Table 3. Pregnancy outcomes and GBS colonization

	n	GBS+, *n* (%)	OR (95% CI)	*P*
Delivery outcome				
live birth	535	104 (19)	1	
stillbirth	14	2 (14)	0.69 (0.15–3.13)	0.63
Type of delivery				
vaginal	525	102 (19)	1	
Caesarean	22	4 (18)	0.92 (0.31–2.78)	0.89
Anaemia at delivery[a]				
no anaemia	296	48 (16)	1	
anaemia	247	56 (23)	1.52 (0.97–2.33)	0.06
Gestational age				
term delivery (≥37 weeks)	332	63 (19)	1	
preterm delivery (<37 weeks)	204	41 (20)	0.93 (0.60–1.44)	0.75
Birth weight[b]				
normal	447	90 (20)	1	
low	84	13 (15)	0.73 (0.39–1.37)	0.32

[a]Anaemia defined as haemoglobin <110 g/L.
[b]Low birth weight defined as <2500 g.

general differences in living conditions account for this difference.[19–21] Contrary to this, other demographic characteristics including maternal age, parity and anaemia at baseline were not associated with GBS carriage.

There was no difference in GBS colonization at delivery between the sulfadoxine/pyrimethamine-IPTp and mefloquine-IPTp groups. Sulfadoxine/pyrimethamine-IPTp has been shown to exert *in vitro* activity against GBS, whereas mefloquine does not confer any inhibitory effect.[11] Despite these experimental data, this clinical study demonstrated that the *in vitro* activity of sulfadoxine/pyrimethamine against GBS does not translate into effective *in vivo* clearance of recto-vaginal GBS colonization at delivery when administered as two-dose IPTp. Reasons for this apparent discrepancy may include inadequate target site concentrations of sulfadoxine/pyrimethamine, repeated reinfections during pregnancy or inadequate dosing regimens for achieving clearance of GBS.[22] It may therefore not be excluded that IPTp regimens with three or more doses of sulfadoxine/pyrimethamine may lead to higher activity against GBS at delivery.

The aim of this study was to evaluate whether routine sulfadoxine/pyrimethamine-IPTp may confer a reduction in GBS colonization at delivery. It may not be excluded that sulfadoxine/pyrimethamine may indeed lead to a reduction in GBS colonization after drug intake but was unable to prevent frequent reinfections by partners. Likewise, there was no systematic collection of data on concomitant use of antibiotics, which may have confounded our findings. Finally, this study was not powered to detect any differences in neonatal infections and focused primarily on maternal GBS colonization.

Given the programmatic advantages of interventions protecting against multiple infectious pathogens, the high neonatal morbidity and mortality in sub-Saharan Africa and the absence of prenatal screening programmes for GBS colonization, the evaluation of IPTp regimens with collateral activity against GBS colonization and indeed other common infections during pregnancy including intestinal helminths or urogenital schistosomiasis seems warranted.[12,23,24]

Birth outcome analysis did not show any correlation between preterm delivery and GBS colonization. These data are in line with a systematic review demonstrating no association between GBS status and preterm delivery.[25] However, in the case of preterm delivery, GBS colonization is a known risk factor for early neonatal sepsis—a risk that can be reverted by adequate intrapartum antibiotic prophylaxis.[26] Previous reports also demonstrated a significantly higher prevalence of GBS colonization in pregnant women with adverse pregnancy outcome.[27]

In conclusion, two-dose sulfadoxine/pyrimethamine-IPTp is not efficacious in reducing GBS colonization in pregnant women in Gabon. The clinical evaluation of more frequent dosing regimens or alternative drugs for the concomitant prevention of malaria and other highly prevalent infectious diseases is therefore still of high public health importance.

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Transparency declarations

None to declare.

Author contributions

M. C.-M., G. M.-N., D. A.-D., A. B., H. W., M. G., J. R. M., R. Z.-M., U. S., J. S., F. L., K. R., P.-B. M., S. T. A. and A. A. A. contributed to the design and conduct of the study and participated in analysing data and drafting the manuscript. S. B., R. G., P. G. K., C. M. and M. R. contributed to trial design, analysis of data and drafting of the manuscript.

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