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Streptococcus pneumoniae colonization in pneumococcal vaccine-naïve human immunodeficiency virus-exposed infected and -uninfected South African children

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Abstract

Pneumococcal nasopharyngeal colonization is a pre-requisite for pneumococcal disease; the risk for pneumococcal disease is high in children born to women living with human immunodeficiency virus (HIV). We investigated pneumococcal colonization, serotype distribution and antibiotic susceptibility of *Streptococcus pneumoniae* isolates carried by perinatal HIV-infected and HIV-exposed-uninfected (HEU) children.

Serial nasopharyngeal swabs were collected from 331 HIV-infected and 491 HEU children, at up to 6 scheduled timepoints, between median ages of 25 to 181 weeks. Pneumococcus was identified by culture; serotyping and antibiotic susceptibility testing were done by conventional methods. No pneumococcal vaccine was given.

HIV-infected children were less likely to be colonized with 7-valent pneumococcal conjugate vaccine 7 serotypes than HEU at a median of 25 weeks of age (23% vs 36%; $P < .001$); however, no differences in colonization between the 2 groups were observed at subsequent study-visits. Over the 36-months study-period pneumococcal colonization increased in both HIV-infected (from 45% to 77%) and HEU (from 57% to 61%) children. Over the study-period, pneumococcal isolates non-susceptible to cotrimoxazole decreased from 92% to 57% and had a similar trend to penicillin (from 65% to 42%) in HIV-infected children. Similarly, pneumococcal nonsusceptible to cotrimoxazole decreased from 93% to 57% and to penicillin from 69% to 37% in HEU children.

Vaccine serotype colonization was common in this population and similar rates were observed in HIV-infected and HEU children. The prevalence of pneumococcal isolates non-susceptible to cotrimoxazole and penicillin decreased with age.

Editor: Shih-Min Wang.

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Overall support for the International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT) was provided by the National Institute of Allergy and Infectious Diseases (NIAID) with co-funding from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) and the National Institute of Mental Health (NIMH), all components of the National Institutes of Health (NIH), under Award Numbers UM1AI068632 (IMPAACT LOC), UM1AI068616 (IMPAACT SDMC) and UM1AI106716 (IMPAACT LC), and by NICHD contract number HHSN275201800001I. The study was also partially supported by a grant from Secure The Future Fund (a philanthropy program sponsored by Bristol-Myers Squibb) and from the Department of Science and Technology and National Research Foundation: South African Research Chair Initiative in Vaccine Preventable Diseases; and the Medical Research Council: Respiratory and Meningeal Pathogens Research Unit. The content is solely the responsibility of the authors and does not necessarily represent the official views of the sponsors.

SAM has received honoraria for serving on speaker bureaus for GSK and Pfizer and has also been on advisory boards related to pneumococcal vaccines for GSK, Pfizer, Novartis and MERCK. MCN has received honoraria from Pfizer and travel support from Sanofi-Pasteur.

The other authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

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How to cite this article: Nunes MC, Kim S, Zeldow B, Violari A, Violari A, Dittmer S, Cassim H, Thomas T, van Niekerk N, Cotton MF, Mitchell C, Adrian PV, Madhi SA. Streptococcus pneumoniae colonization in pneumococcal vaccine-naïve human immunodeficiency virus-exposed infected and -uninfected South African children. Medicine 2020;99:9(e19353).

Received: 3 October 2019 / Received in final form: 12 January 2020 / Accepted: 29 January 2020

http://dx.doi.org/10.1097/MD.0000000000019353

Abbreviations: GEE = generalized estimating equation, ART = antiretroviral therapy, HEU = HIV-exposed-uninfected, HIV = human immunodeficiency virus, NPS = nasopharyngeal swabs, PCR = polymerase chain reaction, PCV = pneumococcal conjugate vaccine.

Keywords: carriage, colonization, HIV, *Streptococcus pneumoniae*

1. Introduction

Streptococcus pneumoniae (pneumococcus) is a leading cause of childhood morbidity and mortality worldwide.[1] Pneumococcal colonization of the human nasopharynx begins in the first months of life.[2,3] Although typically asymptomatic, nasopharyngeal carriage is considered a prerequisite for disease.[4] Children born to mothers with human immunodeficiency virus (HIV) infection experience high rates of respiratory illness and invasive pneumococcal disease (IPD).[5] Although this is partly due to HIV-infection, children exposed to HIV in utero but not infected with it (HIV-exposed uninfected [HEU]) also have higher risk of IPD mortality and hospitalization than HIV-unexposed children.[6–8] Nonetheless, studies consistently report higher morbidity in HIV-infected than HEU children.[6,9]

There are conflicting reports on whether the increase in IPD in HIV-infected children is related to higher rates of nasopharyngeal colonization.[10,11] We have shown no difference in pneumococcal acquisition in vaccine-naïve children on the basis of in utero HIV exposure.[12] In that study; however, all children were HEU; therefore, it is important to compare pneumococcal colonization between HIV-infected and HEU children.

Since 2000, the World Health Organization has recommended trimethoprim-sulfamethoxazole (cotrimoxazole) prophylaxis for immunosuppressed adults and children born to women living with HIV starting at 4 to 6 weeks of age and continued until HIV infection can be excluded.[13] Implementing widespread and prolonged use of cotrimoxazole prophylaxis could; however, be associated with drug resistance in common colonizing potentially pathogenic organisms.

In this longitudinal study in South Africa, we evaluated pneumococcal colonization prevalence during the first 3 years of life in children born to HIV-infected mothers, as well as antibiotic susceptibility to the main antimicrobials.

2. Materials and methods

2.1. Study participants, sample collection, and processing

This study addressed a tertiary objective of IMPAACT P1041 (Clinical Trials.gov NCT00080119), which was a Phase II/III, randomized, double-blind, placebo-controlled trial of HEU and HIV-infected children in whom isoniazid prophylaxis against tuberculosis was evaluated.[14] Participants enrolled at Chris Hani Baragwanath Academic Hospital, Johannesburg, South Africa were asked to participate in the pneumococcal colonization sub-study. Infants born to women living with HIV were identified through programs for the prevention of mother-to-child transmission of HIV. Participants were enrolled between December 2004 and June 2008, before the introduction of pneumococcal conjugate vaccine (PCV) into the South African public immunization program. All children in this study were naïve for pneumococcal vaccines. See supplemental text, http://links.lww.com/MD/D878 for detailed study inclusion and exclusion criteria. When the trial was prematurely stopped for futility, HEU participants had completed more visits than HIV-infected participants.

The HIV-infection status of the infants was determined by HIV-1 DNA polymerase chain reaction (PCR) testing. Participants were randomly assigned to receive daily isoniazid or placebo. Additionally, open-label daily cotrimoxazole chemoprophylaxis was provided to all HIV-infected children until at least 12-months of age with continuation thereafter if World Health Organization guidelines for continuation were met, and to HEU until their HIV-negative status was confirmed by a repeat HIV-1 DNA PCR and if no longer at risk for acquiring HIV from breastfeeding. Most HIV-infected children began combination antiretroviral therapy (ART) at or after enrolment.

Participants were enrolled at 3 to 4 month of age (Visit-0) and nasopharyngeal swabs (NPS) were collected on 6 occasions spanning a median of 156 weeks; Visit-1 through Visit-6, which occurred at mean ages of 25, 37, 49, 85, 133, and 181 weeks. Dacron-tipped swabs on a flexible aluminium shaft (Wiltshire, England) were inserted gently though a nostril and then inoculated into skim milk tryptone-glucose-glycerol transport media and stored at −70°C until processing as described previously.[15] Standard microbiologic tests were used for culture and identification of *S pneumoniae*,[15] a sweep of the growth was used for serotyping. If more than 1 serotype was identified, the individual colonies representative of each morphology were sub-cultured and serotyped. Serotyping was performed with the Quellung method (Statens Serum Institute, Copenhagen, Denmark).

S pneumoniae isolates were tested by disc diffusion susceptibility to clindamycin, erythromycin, penicillin, tetracycline, chloramphenicol, and rifampicin (Mast Diagnostics, Merseyside, United Kingdom), and minimal inhibitory concentrations were determined by Etest (AB Biodisk, Solna, Sweden) for cotrimoxazole and ceftriaxone. The results were interpreted according to Clinical and Laboratory Standards Institute guidelines and breakpoint of penicillin non-susceptibility was accordingly meningitis criteria.[16]

2.2. Statistical analysis

Serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F were categorized as 7-valent PCV (PCV7) serotypes and these plus 1, 3, 5, 6A, 7F, 19A were categorized as PCV13-serotypes. To compare characteristics between HIV-infected and HEU participants, Wilcoxon test was used for quantitative variables and Chi-square test or in the case of small cell counts Fisher exact test for binary variables. Separately within HIV status groups, colonization levels were compared by randomized treatment (isoniazid vs placebo) at each study-visit before combining treatment groups. The Kaplan–Meier method was used to calculate the cumulative prevalence by visit, which was defined as the probability of pneumococcal colonization up to and including that visit, censoring at last follow-up if the participant had discontinued the study before the visit. Cumulative prevalence was estimated

with any pneumococci and grouped into PCV7, PCV13, nonvaccine serotypes, not-typeable, and for 6A and 19A stratified by HIV status, and log-rank tests were used to compare HIV-infected and HEU children.

A generalized estimating equation (GEE) approach was used to fit logistic regression models for prevalence of pneumococci at all visits using an exchangeable working correlation structure. Adjusted models included covariates for current (time-updated) cotrimoxazole use, sex, being breastfed at study entry, visit as a categorical variable, and for HIV-infected children, current (time-updated) ART use. A GEE approach was also used to fit ordinal logistic models for antibiotic resistance outcomes using an independent working correlation structure using data from all visits. For both logistic and ordinal logistic models, empirical standard errors were used for confidence intervals and score tests. All *P*-values are 2-sided at the nominal 5% significance level.

2.3. Ethics

IMPAACT P1041 was approved by the institutional review boards of the participating sites including the Medicines Control Council, Pretoria, South Africa, and the Division of AIDS at National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA; and was registered at ClinicalTrials.gov (NCT00080119). Written informed consent for participation was obtained from legal guardians before any study procedure and from participants in Johannesburg enrolled into this sub-study.

3. Results

Overall, of 879 children enrolled in the P1041 study in Johannesburg, 822 (94%) children had NPS results and thus contributed data to this sub-study, including 331 HIV-infected and 491 HEU children. Overall, 405 (49%) were randomized to isoniazid and 417 (51%) to placebo, Table 1. The baseline characteristics were generally similar between both groups although fewer HIV-infected children were male (42% vs 51%, $P=.01$) and a higher proportion were breastfed at enrolment (12% vs 7%, $P=.02$). The mean age at the time of NPS collection for each visit did not differ between groups. The proportion of children receiving cotrimoxazole prophylaxis was, expectedly, higher in the HIV-infected group at all visits. ART was being taken by 68% of HIV-infected children at Visit-1 increasing to 92% by Visit-6, Table 1.

3.1. Pneumococcal colonization

HIV-infected children contributed 1085 NPS and HEU contributed 2136 NPS. The percentage of children with pneumococcal colonization was similar between the isoniazid and placebo treatment at all study-visits in both groups; except at Visit-6, based on results from 26 participants, where a lower percentage of HIV-infected children in the isoniazid group were colonized with non-PCV13 serotypes (7%, 1 of 15) compared to children in the placebo group (55%, 6 of 11; $P=.02$).

Overall pneumococcal colonization prevalence was lower at Visit-1 in HIV-infected than HEU (45% vs 57%, $P=.001$) children. This difference was due to lower colonization prevalence by PCV7-serotypes in HIV-infected children (23%) than in HEU (36%), Figures 1 and 2.

Because of a significant interaction effect of HIV status and study-visit in PCV7-serotype colonization, PCV13-serotypes, and any pneumococci in models adjusted for sex, cotrimoxazole use, and being breastfed at study entry (all $P \leq .02$), separate models were developed by HIV status and are shown in Table 2. Unadjusted and adjusted logistic regression models showed that being on cotrimoxazole prophylaxis was not associated with PCV7- or PCV13-serotype colonization in both HIV status groups. Prevalence of colonization with any pneumococci was statistically significantly lower while participants were on cotrimoxazole only in the unadjusted model, Table 2. Among HIV-infected children, pneumococcal colonization prevalence generally increased with study-visit for PCV7-serotypes, PCV13-serotypes and any pneumococci in unadjusted and adjusted models (all $P<.001$). ART use was associate with lower prevalence of PCV7-serotype colonization in the adjusted model ($P=.04$), but not for PCV13-serotypes or any pneumococci. Among HEU children, pneumococcal colonization differed by study-visit (significantly in both unadjusted and adjusted models for each pneumococcus grouping, all $P \leq .05$), but increasing initially and then either plateauing or decreasing, Table 2. Randomization treatment did not predict colonization of PCV7, PCV13, or any pneumococci serotypes or substantially change the associations of other characteristics with prevalence (not shown).

During the study-period, HEU children had PCV7-serotypes cultured at earlier time-points than HIV-infected ($P=.008$), Figure 2 and Supplemental Table 1, http://links.lww.com/MD/D878. This was largely driven by the higher prevalence of PCV7-serotypes in HEU at the Visit-1. The estimated cumulative prevalence of colonization with grouped PCV13-serotypes, any PCV13-serotype not in PCV7 or any pneumococci were, however, similar between HIV-infected and HEU children, Figure 2 and Supplemental Table 1, http://links.lww.com/MD/D878. We estimated that all HIV-infected children and 96% of HEU children were colonized with at least 1 pneumococcus serotype during the study-period, while 81% each, had at least 1 PCV7-serotype, and 93% and 90%, respectively, had at least 1 PCV13-serotype, Supplemental Table 1, http://links.lww.com/MD/D878.

3.2. Antibiotic susceptibility

Of samples positive for pneumococcus (629 of 1085 in HIV-infected and 1435 of 2316 in HEU children), antibiotic susceptibility testing was done for cotrimoxazole in 89%, penicillin in 51%, erythromycin in 77%, tetracycline in 87%, clindamycin in 78%, chloramphenicol in 93%, rifampicin in 92% and ceftriaxone in 92%.

The prevalence of isolates non-susceptible (intermediate or resistant) to cotrimoxazole at Visit-1, when >98% of colonized children were receiving cotrimoxazole, was very high (HIV-infected 92% and HEU 93%). In contrast, at Visit-6 when only 1 (0.7%) HEU and 1 (5%) HIV-infected child was on cotrimoxazole, the prevalence of nonsusceptibility had reduced to 57% in both groups, Table 3. Pneumococcus isolated during visits when HIV-infected children were receiving cotrimoxazole were more likely to be nonsusceptible to cotrimoxazole than when children were not receiving cotrimoxazole; however, the odds ratio was attenuated and statistical significance disappeared after adjusting for sex and study-visit, Table 3. Pneumococcus isolated during visits where HEU children received cotrimoxazole showed a similar pattern in unadjusted analysis, with odds ratios attenuated and non-significant after adjusting for sex and visit.

Table 1

Demographic and clinical characteristics of study participants by HIV status.

Characteristic	Total N = 822	HIV-infected N = 331	HIV-uninfected N = 491	*P*-value
Age in weeks at study visit, N, median (range)				
Visit-1/study wk 12	797, 25 (22, 26)	310, 25 (22, 36)	487, 25 (22, 34)	.93
Visit-2/study wk 24	748, 37 (34, 37)	283, 37 (35, 45)	465, 37 (34, 47)	.83
Visit-3/study wk 36	717, 49 (46, 62)	267, 49 (46, 62)	450, 49 (47, 57)	.45
Visit-4/study wk 72	519, 85 (82, 106)	157, 85 (82, 93)	362, 85 (83, 106)	.31
Visit-5/study wk 120	350, 133 (129, 167)	42, 134 (130, 140)	308, 133 (129, 167)	.79
Visit-6/study wk 168	270, 181 (178, 199)	26, 181 (180, 199)	244, 181 (178, 196)	.61
Treatment group, N (%)				
Isoniazid	405 (49%)	164 (50%)	241 (49%)	.90
Placebo	417 (51%)	167 (50%)	250 (51%)	
Receiving cotrimoxazole, N (%)*				
Visit-1	785 (98%)	299 (96%)	486 (>99%)	<.001
Visit-2	731 (98%)	270 (95%)	461 (99%)	.002
Visit-3	594 (83%)	244 (91%)	350 (78%)	<.001
Visit-4	75 (14%)	73 (46%)	2 (0.6%)	<.001
Visit-5	11 (3%)	9 (21%)	2 (0.6%)	<.001
Visit-6	3 (1%)	2 (8%)	1 (0.4%)	.03
Male, N (%)	389 (47%)	139 (42%)	250 (51%)	.01
Race/ethnicity, N (%)				
Black	820 (99.8%)	330 (99.7%)	490 (99.8%)	1.00
Mixed	2 (0.2%)	1 (0.3%)	1 (0.2%)	
Housing type, N (%)				
Formal (brick) house	569 (69%)	230 (69%)	339 (69%)	.89
Informal (shack/wooden)	253 (31%)	101 (31%)	152 (31%)	
Number living in home, median (IQR)	5 (3, 6)	5 (3, 6)	5 (3, 7)	.50
Breastfed at study baseline (%)	73 (9%)	39 (12%)	34 (7%)	.02
CDC HIV classification, N (%)				
A		46 (14%)	n/a	
B		15 (5%)	n/a	
C		2 (1%)	n/a	
N		268 (81%)	n/a	
Absolute CD4+ cell count, Median (IQR)		1826 (1277, 2400)	n/a	
Percent CD4+ cell, Median (IQR)		30.10 (24.6, 36.6)	n/a	
HIV-1 RNA (copies/ml), Median (IQR)		387,000 (11,000, ≥750,000)	n/a	
On ART, N (%)*				
Visit-1		211 (68%)	n/a	
Visit-2		238 (84%)	n/a	
Visit-3		231 (87%)	n/a	
Visit-4		143 (91%)	n/a	
Visit-5		39 (93%)	n/a	
Visit-6		24 (92%)	n/a	

ART = combination antiretroviral therapy defined as 3 or more antiretroviral drugs from 2 or more classes, IQR = interquartile range, SD = standard deviation.
* For the denominator see age in weeks at study-visit.

The prevalence of isolates non-susceptible to penicillin also generally decreased at later study-visits. Among HIV-infected children, prevalence of penicillin nonsusceptibility was not associated with cotrimoxazole use, and while it decreased with study-visit (65% at Visit-1 to 42% at Visit-6), it was not statistically significant in unadjusted or adjusted models, Table 3. Pneumococcus isolated during visits when HEU children were receiving cotrimoxazole were more frequently nonsusceptible to penicillin (64%) than when not receiving cotrimoxazole (55%, $P = .03$) in unadjusted analysis, although when adjusting for sex and study-visit statistical significance was lost, Table 3.

The prevalence of isolates non-susceptible to erythromycin did not differ significantly by visit in HIV-infected or HEU children but varied between 15% and 23% for HIV-infected children and 14% to 25% for HEU children, Table 3. There was no difference in the prevalence of non-susceptibility to erythromycin among HIV-infected and HEU children, by receipt of cotrimoxazole.

Nonsusceptibility to tetracycline was identified in 31% of all isolates. HIV-infected children when receiving cotrimoxazole were more likely to have isolates less resistant to tetracycline (unadjusted, $P = .03$ and adjusted for sex and visit, $P = .02$), Supplemental Table 2, http://links.lww.com/MD/D878. Non-susceptibility to clindamycin was observed in 5% of all isolates. The use of cotrimoxazole had no impact on the prevalence of nonsusceptible isolates to clindamycin in both groups, but this analysis may be underpowered, Supplemental Table 2, http://links.lww.com/MD/D878. For the other antibiotics tested, of all isolates <7%, <1%, and 1% were nonsusceptible to chloramphenicol, ceftriaxone, and rifampicin, respectively. No differences in prevalence of nonsusceptible isolates to these antibiotics

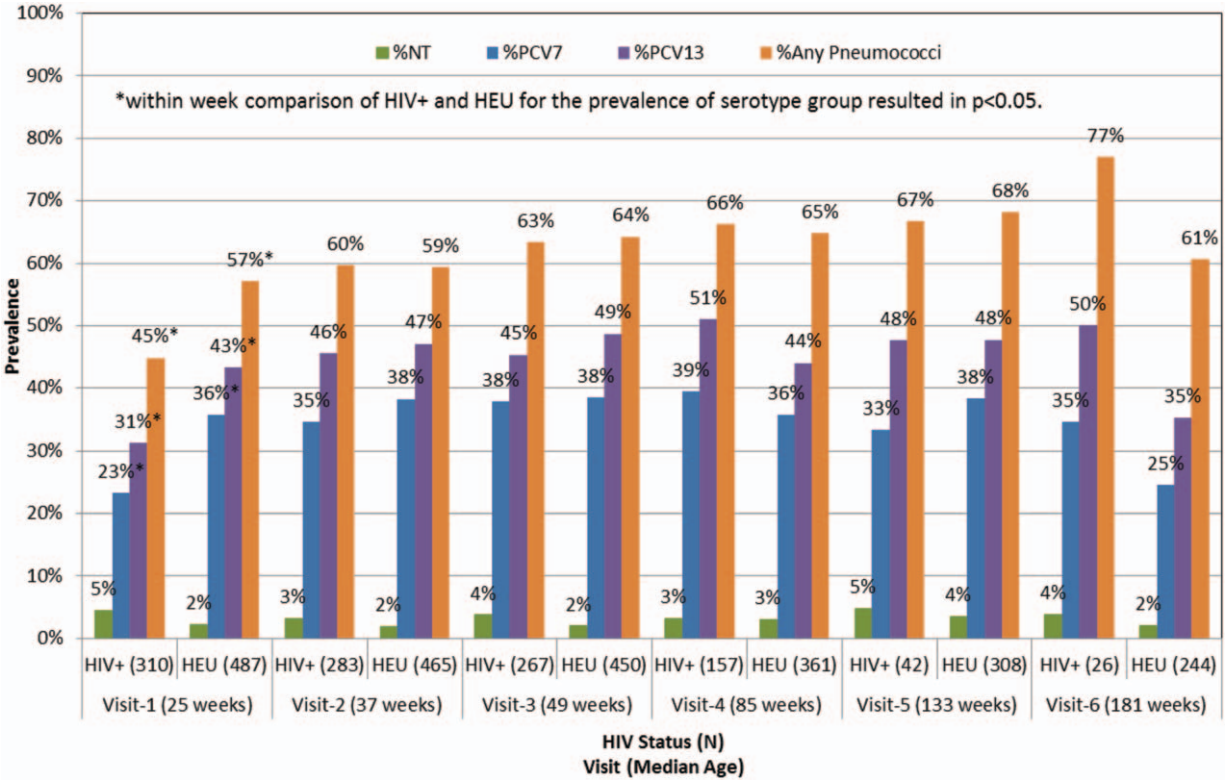

Figure 1. Prevalence of *Streptococcus pneumoniae* colonization. Number in parenthesis reflect the number of children assessed at each visit. $^{*}P < .05$ for within week comparison of HIV-infected and HEU children prevalence of serotype group. NT = nontypeable isolates, PCV7 = serotypes included in the 7-valent pneumococcal conjugate vaccine (ie, 4, 6B, 9V, 14, 18C, 19F, 23F), PCV13 = serotypes included in the 13-valent pneumococcal conjugate vaccine (ie, PCV7-serotypes plus 1, 3, 5, 7F).

were detected by cotrimoxazole usage and study-visit (data not shown).

4. Discussion

In the present study PCV-naïve HIV-infected and HEU children had high prevalence of pneumococcal colonization from 25-weeks to 3.5-years of age. While we observed some variation by study-visit, we detected similar prevalence of overall colonization and vaccine serotype colonization in both study groups. In previous studies we have shown that PCV-naïve HEU children were colonized at a similar frequency and by the same pneumococcal serotypes as HIV-unexposed children,[12] and we demonstrated that PCV7-immunized HIV-infected and HEU children had similar prevalence of PCV7-serotype colonization to HIV-unexposed children.[17] A longitudinal study from Zambia reported; however, that infants born to HIV-infected mothers were at increased risk of pneumococcal colonization compared to HIV-unexposed infants.[10,18]

After global implementation of programs for prevention of mother-to-child transmission of HIV, most children born to women living with HIV are now HEU. Nevertheless, these HEU children are at higher risk of morbidity and mortality than children born to HIV-uninfected mothers.[8,19,20] Although children with HIV experience higher rates of IPD and mortality, our study did not demonstrate that this group had differential nasal pneumococcal colonization from that of HEU children; but they might be at increased risk of acquiring invasive serotypes that are very seldom carried in the nasopharynx.[9] On the other hand, antibody functional assays on the context of PCV have shown that HIV-exposed children require higher antibody concentrations for effectiveness against certain pneumococcal serotypes.[21]

PCV was not used in South Africa during this study. Unsurprisingly, a high colonization prevalence of PCV-serotypes was detected in these children. Since 2009, PCV7, and from 2011, PCV13, are in the national immunization program in South Africa and a gradual reduction in PCV-serotype colonization at the population level has been documented in HIV-infected and uninfected children, most of the latter being HIV-unexposed.[22]

Cotrimoxazole is recommended in patients with documented or suspected HIV infection to prevent opportunistic bacterial infections including *Pneumocystis jirovecii* pneumonia.[23] A significant correlation has been reported between regional consumption of cotrimoxazole and pneumococci resistance in the following year.[24,25] Exposure to sulfonamides is a main factor driving emergence of cotrimoxazole resistant pneumococcus and might induce cross-resistance to other antibiotics.[26] While there are concerns that high prevalence of pathogens resistant to cotrimoxazole will reduce the benefits of this intervention, the efficacy of this prophylaxis has not been affected by the background prevalence of cotrimoxazole resistance and appears similar in settings with high and low background prevalence of cotrimoxazole resistance.[13,27,28]

In our study cotrimoxazole and penicillin resistance was extremely common, by contrast non-susceptibility to clindamy-

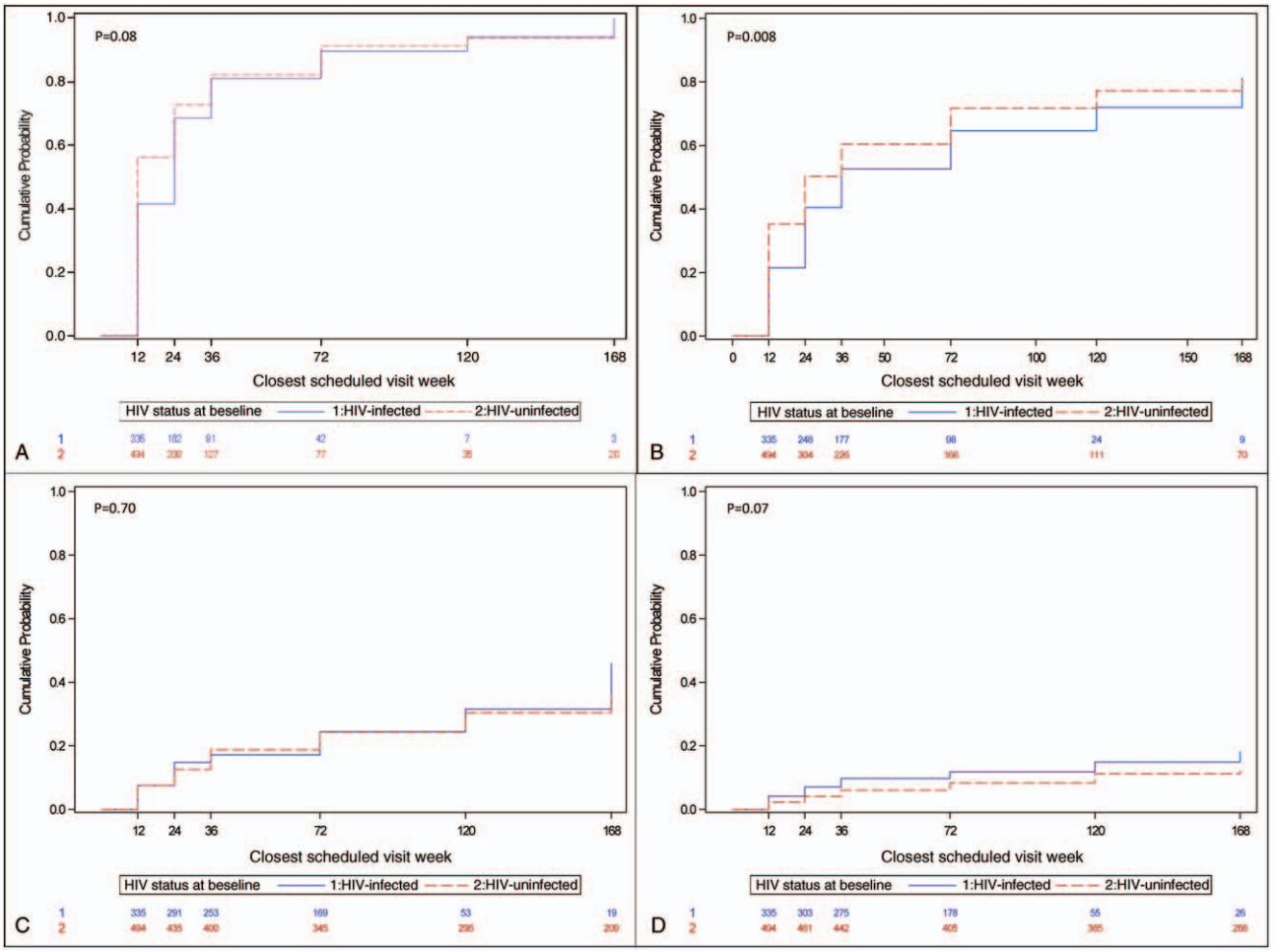

Figure 2. Kaplan–Meier estimates of cumulative proportion with Pneumococcal serotype grouping. (A) Kaplan–Meier estimate of cumulative probability of any pneumococci; (B) Kaplan–Meier estimate of cumulative probability of any PCV7-serotypes; (C) Kaplan–Meier estimate of cumulative probability of PCV13-serotypes not included in PCV7; (D) Kaplan–Meier estimate of cumulative probability of nontypeable pneumococcus. PCV = pneumococcal conjugate vaccine.

cin, ceftriaxone, chloramphenicol, and rifampicin was uncommon. Cotrimoxazole exposure was not associated with cross-resistance to other classes of antibiotics, and resistance level to tetracycline was slightly lower in isolates exposed to cotrimoxazole. A study in South Africa on IPD isolates collected from 2003 to 2007 reported that patients on cotrimoxazole prophylaxis were more likely to have a pneumococcal isolates nonsusceptible to cotrimoxazole (84% vs 54%), penicillin (49% vs 30%), and rifampicin (11% vs 3%) and having multidrug resistance (27% vs 16%).[29] In contrast to our study a report from the Zambia among HIV-exposed infants (14% were HIV-infected), found that cotrimoxazole prophylaxis increased pneumococcal resistance to clindamycin, yet had also no effect on resistance to penicillin, tetracycline, erythromycin, and chloramphenicol.[18] Although limited by inclusion of few studies (8 of high quality) a systematic-review on whether cotrimoxazole prophylaxis in HIV-infected and/or exposed individuals increased bacterial resistance to other antibiotics suggested that cotrimoxazole usage protected against developing resistance to other antibiotic classes. The authors noted, however that more carefully designed studies are needed to answer the question conclusively.[30]

Our study had limited power to look at differences in colonization by individual serotypes but was better powered to detect PCV-serotypes as a group. We included in our analysis the PCV7-serotypes as these have been described as the "paediatric-serotypes" and the most dominant colonizers in pre-vaccine era.[31] Other limitations include that we used cumulative prevalence to describe the level of pneumococcal colonization of over a period of time. Cumulative prevalence is not a well-defined quantity and combines prevalence at single point in time and incidence. Because this study was conducted in children starting at approximately 6 months of age, cumulative prevalence in this context may, however approximate cumulative incidence. Nevertheless, because pneumococcus can be cleared between visits, the estimates presented may underestimate the true values. Another limitation is that only 26 HIV-infected children attended the last study-visit.

In this study up to 3.5-years of age, HIV-infected and HEU children had similarly high pneumococcus colonization. Pneumococcal colonization and serotype distribution were also not influenced by cotrimoxazole exposure, suggesting that the need for pneumococcal vaccines is unchanged with the use cotrimoxazole prophylaxis.

Table 2

Modeling nasal pneumococcal colonization by serotype group by HIV status.[*]

HIV-infected covariate	N	PCV7 serotype					PCV13 serotype					Any pneumococci				
		%[†]	Unadjusted		Adjusted[‡]		%[†]	Unadjusted		Adjusted[‡]		%[†]	Unadjusted		Adjusted[‡]	
			OR (95%CI)	*P*	OR (95%CI)	*P*		OR (95%CI)	*P*	OR (95%CI)	*P*		OR (95%CI)	*P*	OR (95%CI)	*P*
Taking COT				.66		.17			.35		.10			.01		.69
Yes	897	33%	0.92 (0.63, 1.34)		1.35 (0.89, 2.04)		42%	0.85 (0.60, 1.19)		1.42 (0.94, 2.14)		57%	0.65 (0.46, 0.92)		1.09 (0.71, 1.68)	
No	188	34%	ref.		ref.		44%	ref.		ref.		65%	ref.		ref.	
Sex				.33		.32			.18		.16			.11		.12
Female	621	34%	1.16 (0.85, 1.59)		1.17 (0.85, 1.61)		44%	1.22 (0.91, 1.63)		1.24 (0.92, 1.68)		60%	1.26 (0.95, 1.68)		1.26 (0.94, 1.70)	
Male	464	31%	ref.		ref.		40%	ref.		ref.		55%	ref.		ref.	
ART				.43		.04			.83		.17			0.37		.45
Yes	886	32%	0.86 (0.61, 1.23)		0.65 (0.44, 0.95)		42%	1.04 (0.74, 1.45)		0.77 (0.54, 1.10)		58%	1.18 (0.82, 1.71)		0.86 (0.58, 1.27)	
No	199	36%	ref.		ref.		42%	ref.		ref.		56%	ref.		ref.	
Breastfed[§]				.44		.33			.44		.29			.95		.78
Yes	134	30%	0.84 (0.52, 1.33)		0.80 (0.50, 1.27)		40%	0.86 (0.58, 1.27)		0.81 (0.54, 1.20)		57%	0.99 (0.65, 1.49)		0.94 (0.62, 1.44)	
No	951	33%	ref.		ref.		43%	ref.		ref.		58%	ref.		ref.	
Study Visit				<.001		<.001			<.001		<.001			<.001		<.001
Visit-1	310	23%	ref.		ref.		31%	ref.		ref.		45%	ref.		ref.	
Visit-2	283	35%	1.71 (1.26, 2.32)		1.85 (1.35, 2.54)		46%	1.79 (1.34, 2.41)		1.89 (1.40, 2.55)		60%	1.78 (1.33, 2.38)		1.83 (1.36, 2.47)	
Visit-3	267	38%	1.99 (1.46, 2.72)		2.22 (1.60, 3.08)		45%	1.80 (1.34, 2.41)		1.93 (1.42, 2.62)		63%	2.10 (1.57, 2.81)		2.18 (1.62, 2.93)	
Visit-4	157	39%	2.13 (1.40, 3.25)		2.80 (1.78, 4.41)		51%	2.39 (1.63, 3.51)		3.07 (1.98, 4.77)		66%	2.55 (1.73, 3.77)		2.79 (1.75, 4.43)	
Visit-5	42	33%	1.70 (0.85, 3.40)		2.40 (1.18, 4.88)		48%	2.10 (1.08, 4.07)		2.93 (1.44, 6.00)		67%	2.70 (1.39, 5.25)		3.00 (1.46, 6.15)	
Visit-6	26	35%	1.84 (0.79, 4.31)		2.82 (1.17, 6.82)		50%	2.40 (1.04, 5.55)		3.57 (1.40, 9.07)		77%	4.81 (1.60, 14.4)		5.46 (1.63, 18.3)	
Taking COT				.15		.79			.16		.55			.03		.35
Yes	1302	37%	1.15 (0.95, 1.38)		0.94 (0.62, 1.44)		46%	1.14 (0.95, 1.35)		1.13 (0.76, 1.68)		60%	0.83 (0.70, 0.98)		1.23 (0.81, 1.86)	
No	1014	34%	ref.		ref.		43%	ref.		ref.		64%	ref.		ref.	
Sex				.54		.48			.94		.93					.99
Female	1136	35%	0.94 (0.76, 1.16)		0.93 (0.75, 1.15)		45%	0.99 (0.80, 1.23)		0.99 (0.80, 1.22)		62%	1.00 (0.80, 1.25)	.99	1.00 (0.80, 1.25)	
Male	1180	37%	ref.		ref.		45%	ref.		ref.		62%	ref.		ref.	
Breastfed[§]				.79		.80			.66					.76		.71
Yes	166	37%	1.06 (0.68, 1.67)		1.06 (0.67, 1.67)		42%	0.90 (0.58, 1.41)		0.89 (0.57, 1.40)	.62	60%	0.94 (0.61, 1.43)	.76	0.92 (0.60, 1.42)	
No	2150	36%	ref.		ref.		45%	ref.		ref.		62%	ref.		ref.	
Study Visit				.002		.003			.006		.01			.01		.05
Visit-1	487	36%	ref.		ref.		43%	ref.		ref.		57%	ref.		ref.	
Visit-2	465	38%	1.11 (0.90, 1.36)		1.11 (0.90, 1.36)		47%	1.16 (0.95, 1.41)		1.16 (0.96, 1.41)		59%	1.10 (0.90, 1.35)		1.10 (0.90, 1.35)	
Visit-3	450	38%	1.12 (0.88, 1.42)		1.10 (0.85, 1.43)		49%	1.23 (0.98, 1.53)		1.26 (0.99, 1.60)		64%	1.35 (1.08, 1.69)		1.41 (1.11, 1.80)	
Visit-4	362	36%	1.00 (0.75, 1.33)		0.94 (0.56, 1.59)		44%	1.03 (0.78, 1.35)		1.16 (0.71, 1.89)		65%	1.40 (1.07, 1.84)		1.72 (1.03, 2.86)	
Visit-5	308	38%	1.11 (0.84, 1.47)		1.05 (0.63, 1.75)		48%	1.19 (0.91, 1.55)		1.34 (0.82, 2.18)		68%	1.62 (1.22, 2.15)		1.98 (1.16, 3.38)	
Visit-6	244	25%	0.58 (0.41, 0.81)		0.54 (0.31, 0.95)		35%	0.70 (0.52, 0.94)		0.79 (0.48, 1.31)		61%	1.17 (0.86, 1.58)		1.43 (0.85, 2.43)	

CI = confidence interval, COT = cotrimoxazole, N = number of participant visits, OR = odds ratio, *P* = *P*-value, ref. = reference group.

[*] Generalized estimating equations used to fit logistic regression models. Adjusted models have current cotrimoxazole usage, sex, ever breastfed evaluated at study entry, visit, and for HIV+ participants, current ART use, as covariates.

[†] percentage of participant visits colonized.

[‡] Adjusted models fit separately for HIV-infected and HIV-uninfected participants containing covariates shown in table.

[§] Breastfed evaluated at study entry; very few children were breastfed on study.

Table 3

Unadjusted and adjusted models of resistance to cotrimoxazole, penicillin and erythromycin separately by participant's HIV status*.

		Cotrimoxazole (N = 570)								Penicillin (N = 299)						
						Unadjusted		Adjusted‡					Unadjusted		Adjusted‡	
HIV-infected covariate	Pneumococci isolated N (%)†	N	S	I	R	OR (95%CI)	P	OR (95%CI)	P	N	S	No-S	OR (95%CI)	P	OR (95%CI)	P
Visit Week							<.001		.007					.39		.40
12	139 (44.8%)	135	8.1%	39.3%	52.6%	ref.		ref.		46	34.8%	65.2%	ref.		ref.	
24	169 (59.7%)	153	7.8%	27.5%	64.7%	1.5 (1.0, 2.4)		1.6 (1.0, 2.4)		61	47.5%	52.5%	0.6 (0.3, 1.3)		0.6 (0.3, 1.3)	
36	169 (59.7%)	157	12.1%	37.6%	50.3%	0.9 (0.6, 1.3)		0.9 (0.6, 1.3)		104	36.5%	63.5%	0.9 (0.4, 1.9)		0.9 (0.5, 1.9)	
72	104 (66.2%)	86	22.1%	40.7%	37.2%	0.5 (0.3, 0.8)		0.6 (0.3, 1.1)		56	33.9%	66.1%	1.0 (0.5, 2.3)		1.1 (0.4, 2.8)	
120	28 (66.7%)	25	36.0%	36.0%	28.0%	0.3 (0.1, 0.6)		0.3 (0.1, 0.8)		20	45.0%	55.0%	0.7 (0.2, 1.9)		0.7 (0.2, 2.6)	
168	20 (75.9%)	14	42.9%	21.4%	35.7%	0.3 (0.1, 0.9)		0.3 (0.1, 1.0)		12	58.3%	41.7%	0.4 (0.1, 1.3)		0.4 (0.1, 1.8)	
Taking COT							<.001		.30					.60		.80
Yes	507 (56.5%)	468	10.5%	34.8%	54.7%	2.4 (1.6, 3.7)		1.4 (0.8, 2.5)		228	38.6%	61.4%	1.2 (0.7, 2.0)		1.1 (0.5, 2.5)	
No	122 (64.9%)	102	26.5%	37.3%	36.3%	ref.		ref.		71	42.3%	57.7%	ref.		ref.	
ART							.42							.94		
Yes	517 (58.4%)	467	13.7%	35.8%	50.5%	0.8 (0.5, 1.3)				249	39.4%	60.6%	1.0 (0.5, 2.0)			
No	112 (56.3%)	103	11.7%	33.0%	55.3%	ref.				50	40.0%	60.0%	ref.			
Sex							.001		<.001					.80		.92
Male	255 (55.0%)	230	14.3%	44.3%	41.3%	0.6 (0.4, 0.8)		0.5 (0.4, 0.8)		127	38.6%	61.4%	1.1 (0.6, 1.8)		1.0 (0.6, 1.7)	
Female	374 (60.2%)	340	12.6%	29.1%	58.2%	ref.		ref.		172	40.1%	59.9%	ref.		ref.	

		Cotrimoxazole (N = 1276)								Penicillin (N = 755)						
						Unadjusted		Adjusted‡					Unadjusted		Adjusted‡	
HIV-uninfected covariate	Pneumococci Isolated N (%)†	N	S	I	R	OR (95%CI)	P	OR (95%CI)	P	N	S	No-S	OR (95%CI)	P	OR (95%CI)	P
Visit Week							<.001		<.001					.002		.002
12	278 (57.1%)	273	7.0%	17.9%	75.1%	ref.		ref.		156	31.4%	68.6%	ref.		ref.	
24	276 (59.4%)	256	3.9%	16.8%	79.3%	1.3 (0.9, 1.9)		1.3 (0.9, 1.9)		105	36.2%	63.8%	0.8 (0.5, 1.3)		0.8 (0.5, 1.3)	
36	289 (64.2%)	254	18.1%	22.8%	59.1%	0.4 (0.3, 0.6)		0.5 (0.3, 0.7)		143	38.5%	61.5%	0.7 (0.5, 1.1)		0.6 (0.4, 1.0)	
72	234 (64.6%)	224	31.3%	37.5%	31.3%	0.2 (0.1, 0.2)		0.3 (0.1, 0.5)		155	38.7%	61.3%	0.7 (0.5, 1.1)		0.4 (0.1, 0.9)	
120	210 (68.2%)	170	27.1%	31.8%	41.2%	0.2 (0.2, 0.3)		0.3 (0.2, 0.7)		113	46.0%	54.0%	0.5 (0.3, 0.9)		0.3 (0.1, 0.7)	
168	148 (60.7%)	99	43.4%	23.2%	33.3%	0.1 (0.1, 0.2)		0.2 (0.1, 0.4)		83	62.7%	37.3%	0.3 (0.2, 0.5)		0.1 (0.0, 0.4)	
Taking COT							<.001		.09					.03		.09
Yes	784 (60.2%)	731	9.4%	17.5%	73.1%	4.6 (3.7, 5.8)		1.5 (0.9, 2.6)		375	36.0%	64.0%	1.5 (1.0, 2.0)		0.5 (0.2, 1.2	
No	651 (64.2%)	545	30.3%	33.6%	36.1%	ref.		ref.		380	45.0%	55.0%	ref.		ref.	
Sex							.56		.89					.24		.25
Male	731 (61.9%)	646	19.2%	22.0%	58.8%	1.1 (0.9, 1.3)		1.0 (0.8, 1.3)		376	38.3%	61.7%	1.2 (0.9, 1.6)		1.2 (0.9, 1.7)	
Female	704 (62.0%)	630	17.5%	26.8%	55.7%	ref.		ref.		379	42.7%	57.3%	ref.			

	Erythromycin (N = 479)							
					Unadjusted		Adjusted‡	
HIV-infected covariate	N	S	I	R	OR (95%CI)	P	OR (95%CI)	P
Visit Week	106	84.9%	2.8%	12.3%		.48		.56
12	131	85.5%	2.3%	12.2%	ref.		ref.	
24	133	77.4%	5.3%	17.3%	1.0 (0.5, 1.9)		1.0 (0.5, 1.9)	
36	75	80.0%	2.7%	17.3%	1.6 (0.9, 3.0)		1.6 (0.8, 3.1)	
72	22	77.3%	4.5%	18.2%	1.4 (0.6, 3.2)		1.2 (0.5, 2.9)	
120	12	83.3%	8.3%	8.3%	1.6 (0.5, 5.2)		1.4 (0.3, 5.6)	
168					1.1 (0.2, 5.1)		0.9 (0.1, 5.7)	
Taking COT	395	82.5%	3.5%	13.9%		.43		.54
Yes	84	78.6%	3.6%	17.9%	0.8 (0.4, 1.4)		0.8 (0.3, 1.7)	
No					ref.		ref.	
ART	389	81.2%	3.6%	15.2%		.46		
Yes	90	84.4%	3.3%	12.2%	1.3 (0.7, 2.4)			
No					ref.			
Sex	201	80.1%	4.5%	15.4%		.45		.39
Male	278	83.1%	2.9%	14.0%	1.2 (0.7, 2.0)		1.2 (0.8, 2.0)	
Female	106	84.9%	2.8%	12.3%	ref.		ref.	

	Erythromycin (N = 1114)							
					Unadjusted		Adjusted‡	
HIV-uninfected covariate	N	S	I	R	OR (95%CI)	P	OR (95%CI)	P
Visit Week						.15		.12
12	222	86.5%	1.4%	12.2%	ref.		ref.	
24	225	83.1%	4.9%	12.0%	1.3 (0.8, 1.9)		1.3 (0.8, 1.9)	
36	243	83.5%	4.1%	12.3%	1.2 (0.8, 1.9)		1.3 (0.8, 2.1)	
72	193	86.0%	2.6%	11.4%	1.0 (0.6, 1.8)		1.4 (0.5, 3.9)	
120	140	75.0%	4.3%	20.7%	2.1 (1.2, 3.7)		2.8 (1.0, 7.9)	
168	91	85.7%	7.7%	6.6%	1.0 (0.5, 2.0)		1.3 (0.4, 4.0)	
Taking COT	97	86.6%	7.2%	6.2%		.59		.52
Yes	637	84.1%	3.6%	12.2%	0.9 (0.6, 1.3)		1.3 (0.5, 3.3)	
No	477	82.8%	4.0%	13.2%	ref.		ref.	
Sex						.77		.74
Male	555	84.0%	3.6%	12.4%	0.9 (0.7, 1.4)		0.9 (0.6, 1.4)	
Female	559	83.2%	3.9%	12.9%	ref.			

CI = confidence interval, COT = cotrimoxazole, I = intermediate, N = number of participant visits, No-S = nonsusceptible, *P* = *P*-value, R = resistant, ref. = reference group, S = susceptible.

* Generalized estimating equations used to fit cumulative logit models for HIV-infected and HIV-uninfected groups separately.

‡ Adjusted models have cotrimoxazole usage, sex, and visit as covariates.

† N (%): Number of participant visits with any pneumococci isolated (percentage of total participant visits with results reported).

Acknowledgments

The study team thank all the parents/legal guardians for allowing their children to participate in the study. We also thank all the health care workers providing care to the study participants and thank other members of the P1041 team. Grace Montepiedra is acknowledged for her critical review of the manuscript.

Author contributions

AV, MFC, CM and SAM designed the study. SD, HC, TT, NvN, PVA and SAM acquired the data. MCN, SK, BZ, NvN and PVA analysed the data. All authors participated in the interpretation of the data. All reviewed and revised the manuscript.
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