Effect of Maternal Vaccination on the Immunogenicity of PCV-10 in HIV-exposed Uninfected Infants: A Randomized Study of PCV-10, PPV-23 or Placebo Administered during Pregnancy.

Authors:

Marisa M. Mussi-Pinhata, Shawn Ward, Lauren Laimon, Stephen I. Pelton, David Goldblatt, Jennifer Canniff, Amanda Golner, Frederic Bone, Lassallete Newton, Petronella Muresan, Terence Fenton, Michael J. Johnson, Esau C. João, Breno R. Santos, Jose Pilotto, Elizabeth S. Machado, Jorge A Pinto, Rosa D. Sperhacke, Regis Kreitchmann, Nahida Chakhtoura, Geraldo Duarte, Adriana Weinberg for the NICHD P1091 study team

Affiliation:

Ribeirão Preto Medical School, University of São Paulo, Brazil (G Duarte MD Full Professor, MM Mussi-Pinhata MD Full Professor), **Colorado University Anschutz Medical Campus, Aurora, CO, USA** (A Weinberg MD Full Professor, J Canniff BA, MJ Johnson BS); **Frontier Science Foundation, Brookline, MA, USA** (S Ward MS, P Muresan MS, T Fenton ScD); **Westat, Rockville, MD, USA** (L Laimon MS, L Newton BS); **Boston University School of Medicine, Boston, MA, USA** (SI Pelton MD Full Professor); **University College London, Institute of Child Health, London, UK** (D Goldblatt PhD Full Professor); **Frontier Science Foundation, Buffalo, NY, USA** (A Golner BS, F Bone BS); **Hospital dos Servidores Estaduais, Rio de Janeiro, RJ, Brazil** (EC Joao MD); **Hospital Nossa Senhora da Conceicao, Porto Alegre, RGS, Brazil** (BR Santos MD); **Hospital Geral de Nova Iguacu & Laboratório de AIDS e Imunologia Molecular - Fiocruz, Rio de Janeiro, Brazil** (J Pilotto, PhD), **Instituto de Puericultura e Pediatra Matagão Gesteira, Rio de Janeiro, RJ, Brazil** (ES Machado PhD); **FUNDEP Escola de Medicina, Belo Horizonte, MG, Brazil** (JA Pinto, Full Professor); **Health Research Institute, University of Caxias do Sul, Brazil** (RD Sperhacke, PhD), **Federal University of Health Sciences of Porto Alegre, Brazil** (R Kreitchmann), ***Eunice Kennedy Shriver*** **National Institute of Child Health & Human Development, Bethesda, MD, US** (N Chakhtoura MD).

SUMMARY

Background: Pneumococcal vaccines are administered to pregnant women with HIV without recognizing the effect on the immunogenicity of PCV in their infants.

Methods: The immunogenicity of two doses of PCV-10 was compared in HIV-exposed uninfected infants (HEU) born to mothers randomized 1:1:1 to receive PCV-10, PPV-23 or placebo during pregnancy (NCT02717494). The primary outcomes in this report were infant antibody levels against seven serotypes common to PCV-10 and PPV-23 and seroprotection (antibodies ≥0.35μg/ml) after vaccination. Exploratory objectives included B and T cell memory responses and the effect of infant inflammation on antibody responses to PCV-10.

Findings: After the last dose of PCV-10, infants in the maternal PCV-10 group had significantly lower antibody levels against five serotypes compared to infants in the maternal PPV-23 group; against two serotypes compared to the maternal placebo group; and did not have higher antibody levels against any serotypes. Antibody levels were similar in infants in the maternal PPV-23 and placebo groups. The rate of seroprotection against all seven serotypes was 50% in infants in the maternal PCV-10 compared to 71% in each of the maternal PPV-23 and placebo groups ($p<0.0001$). Increased inflammation in infants was associated with decreased antibody responses.

Interpretation: Antibody responses to PCV-10 in HEU and seroprotection decreased with maternal administration of PCV-10 during pregnancy and infant inflammation. Administration of PPV-23 during pregnancy did not affect the immunogenicity of PCV-10 in HEU, indicating that PPV-23 is preferrable over PCV-10 in pregnancy.

Funding: *Eunice Kennedy Shriver* National Institute of Child Health & Human Development.

INTRODUCTION

Despite successful use of pneumococcal conjugate vaccines in children, *Streptococcus pneumoniae* still represents the leading cause of lower respiratory tract infection morbidity and mortality globally and mainly in less than one-year-old children living in underprivileged populations.[1] Infants born to mothers with HIV, even when they escape infection by this virus, are especially vulnerable to respiratory and other infections [2–4] due to multiple factors such as low levels of protective antibodies acquired transplacentally from their mothers [5], lack of breastfeeding in some areas, intrauterine exposure to HIV and antiretrovirals,[6] increased inflammation,[7] and decreased immune responses to natural infection and/or vaccines.[8,9] Maternal immunization is part of the global efforts to reduce neonatal and young infant infectious morbidity and mortality through increasing pathogen-specific passive immunity in the infant before protection can be acquired through infant immunization.

Before our study, several trials evaluated the 23-valent pneumococcal (PNC) polysaccharide vaccine (PPV-23) and a single study 9-valent PNC conjugate vaccine (PCV-9) in healthy pregnant women.[10] Both vaccines were considered safe and immunogenic, although a definitive role in preventing infant infection was not confirmed.[11] In mothers living with HIV, a single PPV-23 trial was conducted before our study, which showed poor immunogenicity and transplacental transport of maternal antibodies.[12] Importantly, the study was performed before universal use of highly effective antiretroviral therapy (ART) in pregnancy. We conducted a randomized study of PCV-10, PPV-23 and placebo administered to pregnant women with HIV on ART, in which we assessed differences in transplacental transport of maternal antibodies associated with vaccination.[13] We found that infants born to immunized mothers had significantly higher proportions of seroprotective antibody levels against maternal vaccine serotypes than those born to placebo-recipients at birth and eight weeks of life, but no differences between PPV-23 and PCV-10 cohorts.

Although generally protective, maternally-acquired antibodies interfere with infant responses to childhood vaccine, particularly if the maternal antibodies are directed against epitopes in childhood vaccines.[14] The blunting of infant antibody production after vaccination has been ascribed to negative maternal antibody interference based on the association of higher concentration of maternally-derived antibodies at birth with lower antibody concentrations after infant vaccination.[15,16]

In this study we assessed the potential interference of maternal PNC antibodies with vaccine-related anti-capsular antibody responses in HIV-exposed uninfected infants (HEU) whose mothers received PCV-10, PPV-23 or placebo during pregnancy. In an exploratory analysis, we evaluated the effect of inflammatory markers on infant antibody responses to PCV-10 and compared the B-

and T- cell responses in infants born to mothers who received PCV-10, PPV-23 or placebo during pregnancy.

Participants and Methods

Study design: This study addressed secondary objectives of a double-blind, placebo-controlled, randomized trial of the safety, immunogenicity and transplacental transport of maternal antibodies after PNC vaccination of pregnant women with HIV (NCT02717494). In the parent study, 346 women with HIV received PCV-10, PPV-23 or placebo after being randomized at a 1:1:1 ratio between ≥14 and <34 weeks gestational age. The results of the primary objectives, including maternal antibody levels in infants up to 8 weeks of life were published elsewhere.[13]

The outcomes of the current study were the comparison of immune responses to the standard of care administration of two doses of PCV-10 in infants exposed to the three maternal immunization regimens. The study was conducted at eight NICHD clinical trial sites in Brazil under approval of national and local regulatory review boards.

All liveborn infants were eligible to the study. They had interval histories, physical exams including anthropometric data, and diagnoses at birth, 8 weeks of life (≤ 7 days before the 1st dose of PCV-10), 16 weeks of life (≤ 7 days before the 2nd dose of PCV-10), and 24 weeks of life (28 to 117 days after the 2nd dose of PCV-10

Infant blood obtained at 0, 8, 16 and 24 weeks of life was used for anti-PNC antibody quantification. Nasopharyngeal swabs collected in skim milk-tryptone-glucose-glycerin (STGG) broth at 8 weeks and 16 weeks of life in infants were used to measure PNC colonization. Plasma and cryopreserved PBMC were obtained at birth and 24 weeks of life for immunologic assays.

Antibody measurements. Serum antibodies for serotypes 4, 7F, 23F (common to both vaccines) and 33F (PPV-23 only) were measured at the WHO reference laboratory at the University College of London by previously described enzyme-linked immunosorbent assay (ELISA) .[17] Antibodies for serotypes 1, 5, 6B and 14 (common to both vaccines) were measured at the University of Colorado Denver Anschutz Medical Campus using a chemiluminescent multiplex assay (MesoScaleDiscovery) as previously described .[18] Seroprotection against IPD was defined by antibody concentrations ≥0.35 µg/ml.

PNC colonization. STGG was used for PNC isolation. Serotypes were determined by Quellung reaction using serotype-specific antisera (Serum Statens Institute) on pure isolate cultures.[19]

B and T cell testing

B- and T-cells were measured in PBMCs collected from infants at Weeks 0 and 24.

Fluorospots: Cyropreserved PBMC were thawed in RPMI (Corning)/10% FBS (Gemini Bio) plus 50 U/mL Benzonase (Sigma). Cells with viability ≥60% were counted, resuspended in RPMI/10% FBS/PenStrep (Gemini Bio)/HEPES (Corning) complete media and split into two. One half was rested overnight at 37°C. The other half was stimulated with R848 (Mabtech) at 1μg/mL and recombinant human IL-2 (Mabtech) at 100ng/mL and incubated at 37°C for 7 days. Overnight rested cells with viability ≥60% were plated onto IFNγ/IL-17A fluorospot plates (Mabtech Cat# FS-0103) at 250,000 cells for well with media or media plus 500,000 cfu Pn1 culture (courtesy of Dr. Steve Pelton's laboratory), and 100,000 cells for PHA (Sigma) wells. Plates were incubated overnight at 37°C and developed according to the manufacturer's instructions. The 7-day stimulation cells were plated onto IgG/IgA Fluorospot plates (Mabtech cat# FS-05R06G) at 250,000 cells/well coated with 25ng PnPS1 (ATCC) and 50,000 cells/well coated with anti- IgG/IgA monoclonal antibodies. Plates were incubated overnight at 37°C and developed as per manufacturer's instructions. Developed plates were imaged and counted on ImmunoSpot analyzer (C.T.L.). Results were recorded as spot forming cells (SFC)/10^6 PBMC. PNC-specific T cell results were reported as SFC/10^6 PBMC in antigen-stimulated wells after subtraction of unstimulated controls. There were no exclusionary criteria based on responses to PHA. B-cell results for which the companion total IgG was <2 spot-forming cells were flagged as invalid and excluded from analysis.

Soluble factors. These included CCL-2 (MCP-1), CCL-3 (MIP-1a), CCL-4 (MIP-1b), CCL-11 (eotaxin-1), CCL-13 (MCP-4), CCL-17 (TARC), CCL-23 (MDC), CCL-26 (eotaxin-3), CXCL-10 (IP-10), GM-CSF, IFNg, IL-1a, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-8(HA), IL-10, IL-12/p40 IL-12/p70, IL-13, IL-15, IL-16, IL17A, TNFα, TNFβ and VEGF) were measured in plasma using the V-PLEX Human Cytokine 30-plex kits (MesoScale Discovery cat # K15054D). Cytokine Panel 1 plates (GM-CSF, IL-1α, IL-5, IL-7, IL-12/IL-23p40, IL-15, IL-16, IL-17A, TNF-β, and VEGF) were run at 1:2 plasma dilution. Chemokine Panel 1 plates (Eotaxin, MIP-1β, Eotaxin-3, TARC, IP-10, MIP-1α, IL-8, MCP-1, MDC, and MCP-4) were run at 1:4 plasma dilution. Proinflammatory Panel 1 plates (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, il-10, IL-12p70, IL-13 and TNF-α) were run at 1:2 plasma dilution with a 4° overnight incubation on the plate. Plates were read and analyzed on MESO QuickPlex SQ 120 instrument. Results were reported in pg/mL. Three biomarkers (IL-8HA, IL-1α, and IL-1β) were excluded from analysis due to ≥30% of the samples being below the lower level of quantification of the assay. In addition, TNFβ was excluded from the analysis for lack of historical interest. IL-12p40 was excluded due to redundancy with IL-12p70.

Statistical analysis. The study was powered (≥80% with α=0.05) only to detect ≥20% differences in infant seroprotection between any 2 treatment groups. The study was not powered for the

secondary or exploratory analyses. All infants born alive on study were included in the baseline summaries. Analyses of immunology data were restricted to infants with non-missing data and who received PCV-10 vaccinations per protocol. In addition, one infant per four sets of twins was randomly included in the analyses. Anti-PNC antibody concentrations were compared overall among all three treatment arms using a Kruskal-Wallis test. When significant, we examined pairwise comparisons, using a Wilcoxon test.

Proportions of seroprotection, defined by anti-PNC antibody concentrations $\geq$0.35 µg/ml, were calculated to each individual serotype, and overall, to all seven serotypes included in both vaccines (excluding serotype 33F). Overall comparisons among all three treatment arms used a Chi-square test. When significant, we examined pairwise comparisons, using a Fisher's Exact test, due to low counts in many cells. Proportions of infants with nasopharyngeal PNC colonization were calculated and compared between groups in a similar way. We used Spearman correlations to assess relationships between antibodies pre- and post-vaccination. Infants had samples collected for B and T cell testing and soluble biomarkers. Due to poor viability after thawing, only a small number of infants provided adequate samples for these analyses which appeared to have happened randomly across the treatment arms. The resulting set of participants may no longer represent a randomized sample. The $\log_{10}$-transformed inflammatory biomarkers concentrations were further transformed by a principal component analysis (PCA). The first 5 principal components were retained and used, along with infant antibody concentrations at 8 weeks of life and maternal treatment, as explanatory variables in linear regression models with stepwise selection for each antibody serotype. In each model, the antibody concentrations after 2nd dose of PCV-10 were used as the response variable. Statistical analyses were performed using SAS (SAS Institute, Inc., Cary, NC, USA) version 9.4.

RESULTS

Characteristics at birth and PCV-10 vaccination of infants

The study included 347 liveborn neonates born to 346 women who received study treatment antepartum (Figure 1). Birth characteristics did not differ across maternal immunization arm with the exception of prematurity, which was less frequent in the maternal PCV-10 arm compared to PPV-23 or placebo arms [2 (2%) *vs.* 13 (13%) *vs.* 12 (12%), respectively (Table 1)]. Pregnant women received other vaccines as per standard of care: DTPa (40%), Td (10%), Influenza (25%), and Hepatitis B (18%) with similar frequencies among maternal immunization arms (Table S1). Infants included in the analysis of pneumococcal antibody responses received PCV-10 per the Brazilian immunization schedule at 8 (range: 7-16) weeks and 16 (range: 16-35) weeks of life.

Comparison of antibody levels before and after infant vaccination according to the maternal immunization arm

Figure 2 and Table S2 show antibody titers against the pneumococcal polysaccharide serotypes 1, 6B, 14, 5, 7F, 23F, 4, and 33F detected in infants at birth, 8, 16, and 24 weeks of age according to the maternal immunization arm. The distribution of the antibody levels restricted to term infants was similar to that observed for all infants (data not shown). Overall, at 16 weeks, at a median of 61 days (25^{th}-75^{th} percentiles:59-63) after the first PCV-10 dose, antibodies significantly increased for serotypes 1, 5, 7F, and 4 but not for serotypes 6B, 14, and 23F. Nevertheless, at a median of 40 days (25^{th}-75^{th} percentiles:30-51) after the second dose of PCV-10, antibody levels increased for all the seven serotypes common to both vaccines.

Infant antibodies for serotypes 1 and 7F at weeks 16 and 24 were similar across maternal immunization arms. At week 16, antibody levels against serotypes 4, 5, 6B and 23F were higher in infants born to PPV-23- than in those born to PCV-10-recipient mothers, while the antibody levels for the latter group were similar to those in infants of placebo-recipient mothers. Antibodies for serotype 14 had the same trend (PPV-23>PCV-10; PCV-10~Placebo) but did not reach statistical significance. At week 24, the same pattern (PPV-23>PCV-10; PCV-10~Placebo) was observed for serotypes 4, 5 and 23. For serotypes 6B and 14, the antibody levels in infants born to PPV-23- and to placebo-recipients were both higher than those in infants born to PCV-10-recipients.

In general, protective antibody levels (≥0.35µg/mL) were observed for a median of two serotypes before vaccination (week 8) in infants born to immunized mothers and for no serotypes in those born to placebo-recipient mothers. The first dose of PCV-10 vaccination induced protective levels against a median of five to six serotypes, and the second dose to seven serotypes independently of the maternal immunization arm (data not shown). Seroprotection against all seven serotypes common to both vaccines studied was equally uncommon at 8 weeks of life in infants from all maternal treatment arms (0 to 2%) (Table 2). However, at 16 weeks infants born to mothers who received PPV-23 had higher proportion of seroprotection (24%) compared with infants born to PCV-10 recipients (11%) or placebo recipients (17%). Notably, at 24 weeks, only 50% of infants born to PCV-10 recipients achieved seroprotection against the seven vaccines-serotypes studied, compared to 71% in each of the PPV-23 and placebo arms.

Correlations between pre- and post-immunization antibody concentrations

In infants born to mothers immunized with PCV-10, higher antibody levels at week 8 pre-vaccination were associated with lower antibody responses to infant PCV-10 at week 24 for 5 out of 7 serotypes measured in this study (1, 6B, 5, 23F and 4; Table 3). Negative correlations between weeks 8 and

16 antibody levels were also detected for 3 out of 7 serotypes in the maternal PCV-10 cohort (1, 7F, and 4; data not shown). There were no significant correlations between pre- and post-immunization antibody concentrations in infants born to mothers who received PPV-23. In infants born to placebo-recipients, serotype 14 antibody concentrations at weeks 24 and 8 were negatively correlated and for serotype 7F were positively correlated.

Memory B and T cell responses to vaccination

Memory responses against PNC serotypes were measured by FLUOROSpot in PBMCs collected at 24 weeks of age from a subset of 126 infants roughly equally distributed across maternal treatment arms (Table S3). After exclusion of twins, samples with viability <60% or lack of responses in the positive controls (total IgG for B cell memory and PHA stimulation for T cell memory), a total of 27 results were available for IgG and IgA memory B cells and 35 for IFNγ- and IL17-secreting T cells (Table 4). Overall, memory responses were very low or undetected and did not show discernible differences by maternal treatment group. Due to the low responses, potential correlations between memory B and T cells and antibody responses were not investigated.

Effect of inflammatory factors on antibody responses to PCV-10

We measured the plasma levels of 30 biomarkers associated with inflammation in a subset of 113 infants at 24 weeks of life. The demographic characteristics of the infant subset did not appreciably differ from those of the entire cohort (Table S4). After exclusion of redundant biomarkers or those with >30% of results below the level of detection of the assay, 25 biomarkers remained in the statistical analysis (Table S5). To decrease the dimensionality of the dataset, remaining biomarkers were grouped using PCA (Table 5). The scores of the first 5 components, which explained 57% of the variance in the dataset, were used in regression analyses in which the outcome measures were infant antibody concentrations to each of the seven PNC vaccine serotypes after the second dose of PCV-10 (Table 6). The regression analyses also included maternal vaccination arm and infant antibody concentrations at the time of administration of the 1st dose of PCV-10 (8 weeks of life), which were shown above to significantly affect the PCV-10 immunogenicity in infants. The results showed that high scores in components 2 and 5, which included high loadings for CXCL-10, GM-CSF, IL-2, IL-5, IL-6, IL-10, IL-15, IFNγ and TNFα, were associated with low antibody concentrations for serotypes 4, 5, 6B and/or 7F; high antibody levels pre-vaccination (week 8) were associated with low antibody levels for serotypes 6B, 14 and 23F; and maternal receipt of PPV-23 was associated with higher serotype 4 antibody levels.

Pneumococcus colonization and disease

Few (46/324, 14%) infants were colonized at week 8 before the first PCV-10 dose or at week 16 (57/320, 18%) after this dose (Table S6), independently of the maternal immunization arm. Among colonized infants at week 8, 17% had serotypes included in both vaccines, 39% were only in PPV-23 and 43% were in neither vaccine. At 16 weeks, corresponding numbers were 9%, 26% and 65%, respectively. Although there were no significant differences in overall colonization according to maternal vaccination regimen, infants born to mothers who received PPV-23 during pregnancy tended to have lower level of colonization with PPV-23 serotypes compared with the PCV-10 group (p of 0.047 at 8 weeks and 0.13 at 16 weeks) and compared with the placebo group (p of 0.11 at 8 weeks and 0.09 at 16 weeks).

During the follow up period, 59 (17.4%) infants were diagnosed with an infection or infestation, but none of these were documented pneumococcal infections. Among syndromic diagnoses of interest, there were 10 neonatal sepsis, 2 bacterial sepsis, 10 bacterial pneumonia, 21 bronchiolitis, and 1 acute sinusitis. These episodes were similarly distributed across the maternal treatment arms.

DISCUSSION

As part of the first randomized, double-blind, placebo-controlled study comparing PCV-10 with PPV-23 and placebo in women living with HIV, we investigated the effect of maternal immunization on the immunogenicity of PCV in HEU infants. We determined that infants born to PCV-10 recipient mothers had lower vaccine-related serotype anti-capsular antibody levels and seroprotection rates after the second dose of PCV-10 than those whose mothers received PPV-23 or placebo. The blunting effect of maternal PCV on infant's antibody response to PCV was associated with a consistent negative correlation between pre- and post-immunization antibody concentrations. Importantly, similar correlations were not detected in infants born to PPV-23 or placebo recipient mothers even for serotypes for which pre-vaccination antibody levels did not differ between infants born to PCV-10 and PPV-23 recipient mothers (three of the seven studied serotypes) or were higher in PPV-23 compared with PCV-10 group (one serotype). This suggests that the mechanism underlying the negative interference of maternal antibodies generated by PCV-10 with the infant antibody responses to PCV-10 are not exclusively a function of antibody concentrations. It is conceivable that some mother-infant pairs may share the antigenic dominance in their antibody responses against PCV-10, which contributes to the negative effect of maternal antibodies. However, this still fails to explain the persistence of the negative interference on the second dose of PCV-10, when maternal antibodies were very low or undetected.

Negative interference of maternal vaccination on infant responses to vaccines have been identified for almost all routinely used vaccines in infancy, [14,20,21] including pertussis, tetanus and influenza . For pertussis, the negative interference was shown to affect the infant response to the 1-year booster dose.[22] This tampering has been demonstrated when similar antigens or carrier proteins are included in maternal and infant vaccines,[15,16] including Influenza vaccination in HIV-infected mother-infant pairs.[23] Several mechanisms for the molecular and cellular basis for this interference have been proposed, although it remains incompletely understood. The main point would be the presence of maternal antibodies with characteristics that could mask epitopes and inhibit B cell responses through interactions with cell receptors, induce removal of vaccine antigens by macrophages or neutralize vaccine virus, among others.[24] Also, difference in IgG subclasses or other structural characteristics in induced antibodies by different vaccines may affect their interference with the biological mechanisms and should be additionally studied.

Compared with the available immunogenicity data after a 3-dose course of PCV-10 primary vaccination in HEU born to unvaccinated mothers,[25] the antibody levels and seroprotection rates for specific serotypes in this study tended to be lower in HEU infants born to PCV recipient mothers. In contrast, the values in infants born to PPV and placebo-recipient mothers were comparable to those obtained in HEU infants born to unvaccinated mothers and in infants born to unvaccinated mothers without HIV, who received 2 doses of PCV-10 using the same schedule of immunization and post-vaccine evaluation timing that we used in our study .[26,27]

Altered immune responses to the conventional pneumococcal vaccination in infancy have been reported in babies born to healthy mothers who received PCV-9.[28] To our knowledge, no other data on PCV response on infants of pneumococcal immunized mothers living with HIV is available. It has been shown that maternal antibodies induced by tetanus-diphtheria-acellular pertussis (TdaP) immunization during pregnancy also inhibit the infant PCV vaccine responses,[15] possibly mediated by antibodies against the same carrier protein of the pneumococcal conjugate vaccine or other vaccine epitope-masking antibodies. [24] Approximately half of the mothers participating in our study received other vaccines per standard of care, such as TdaP, Influenza, tetanus/diphtheria, and hepatitis B. However, the randomized study allocation balanced their distribution among groups, supporting the notion that the infant response's blunting effect was solely attributable to the maternal PCV-10 vaccination.

Inflammatory markers have been associated with decreased responses to vaccines in older adults and in adults living with HIV. We have previously shown HEU infants have enhanced activation and expression of proinflammatory cytokines, and oxidative stress.[7,29] To explore the potential associations of inflammatory biomarkers on infant responses to vaccination, we measured cytokines and chemokine levels using a panel of 25 soluble biomarkers grouped in 5 principal components.

The regression analysis including the prevaccination antibody levels and maternal immunization group showed that high levels of CXCL-10, GM-CSF, IL-2, IL-5, IL-6, IL-10, IL-15, IFNγ and TNFα had a negative effect on the infants' response to two PCV-10 doses suggesting, for the first time, that this might be an essential mechanism involved in the altered response to vaccines in HEU as compared to HIV-unexposed infants.[9]

In a previous study, a decade ago, in HUE infants <6 months of age from the same population, we found increasing rates of colonization with age (26%, 37%, and 48% at weeks 8, 16, and 24, respectively) ,[30] which were twice more frequent than those found in the current study. Since *S. pneumoniae* nasopharyngeal carriage varies mainly due to environmental factors, this decline might result from the widespread implementation of PCV-10 vaccination in infancy in the country during the last ten years and its indirect effect on PNC carriage in the population. In the present study, while most colonized infants carried a PNC serotype not present in either of the maternal vaccines, likely due to the few numbers of colonized infants, we could not show an effect of the maternal immunization on the overall or vaccine serotype-related PNC carriage. However, we detected a trend of a lower level of colonization with PPV-23 serotypes in infants born to PPV-23 recipient mothers as compared with the PCV-10 or placebo groups. Previously, only a study in healthy mothers vaccinated with PPV-23 demonstrated lower infant colonization frequency than those born to unvaccinated ones. [31]

Limitations of this study included the fact that participants were from a single geographic location, and the low representation of Asians and American Indians. We also did not verify if the negative effect of maternal PCV-10 immunization on infant antibody responses persisted even after a booster dose.

In conclusion, administration of PCV-10 to pregnant women with HIV decreased the infant antibody responses to the full primary immunization regimen with PCV-10, whereas administration of PPV-23 did not. Additionally, considering that PPV-23 provides broader serotype coverage, these data support preferential administration of PPV-23 to women with HIV during pregnancy. The biological impact of PPV-23 maternal vaccination of mothers living with HIV for preventing infant pneumococcal infection warrants further investigation.

Contributions: MMMP designed the study, oversaw the clinical performance, participated in data analysis and wrote the manuscript. AW designed the study, oversaw the clinical and laboratory performance, participated in the data analysis and reviewed the manuscript. PM contributed to the study design, performed the statistical analyses and wrote the manuscript., GD and NC contributed to the study design, oversaw the clinical performance and contributed to the manuscript preparation.

SW performed statistical analyses and contributed to the manuscript preparation. TF contributed to the study design and statistical analyses. SIP and DG oversaw laboratory assays and contributed to the manuscript preparation. AG and FB managed the study database. LL and LN organized the study. ECJ, BRS, JP, ESM, JAP, RS and RK led clinical site study teams. JC and MJJ performed laboratory assays and contributed to manuscript preparation. All authors reviewed and approved the manuscript.

Declaration of interests. MMMP receives grants from NIH, CNPq, GSK and Janssen**,** AW receives grants from NIH, GSK, Janssen and Merck. NC is employed by NIH. SIP receives personal fees from Merck, Sanofi and Pfizer and receives grants from NIH and Pfizer. DG receives personal fees from Merck and grants from GSK and Merck.

Acknowledgments: This study was funded by the *Eunice Kennedy Shriver* National Institute of Child Health & Human Development through contract HHSN275201800001I. We thank Dr. Helena Keiko Sato from the Centro de Vigilância Epidemiologica da Secretaria da Saúde do Estado de Sao Paulo for donating the PCV-10 doses used for maternal immunization; Drs. George Siberry, Heather Watts and Elizabeth Smith, and the IMPAACT network for their support in the early development of this study; and Ms. Amanda Golner for data management. Participating clinical sites and personnel were the following:

Site 5071: Instituto de Puericultura e Pediatria Martagao Gesteria
Principal Investigator: Ricardo Hugo Oliveira, M.D.
Study Coordinator: Maria Chermont Sapia, M.D.
Site 5072: Hospital Federal dos Servidores (Rio de Janeiro)
Principal Investigator: Esau Custodio Joao, M.D
Study Coordinator: Leon Claude Sidi, M.D.
Site 5073: FUNDEP (Belo Horizonte) Escola de Medicina
Principal Investigator: Jorge Pinto, M.D.
Study Coordinator: Flavia Faleiro, M.D.
Site 5074: University of São Paulo Brazil- Ribeirão Preto Medical School
Principal Investigator: Marisa M. Mussi-Pinhata, M.D.
Study Coordinator: Conrado Milani Coutinho, M.D.
Site 5084: Universidade de Caxias do Sul
Principal Investigator: Rosa Dea Sperhacke, PharmD, Ph.D.
Study Coordinator: Silva Mariani Costamilan, B.S., R.N.
Site 5097: Hospital Geral de Nova Iguaçu
Principal Investigator: José Henrique da Silva Pilotto, M.D.
Study Coordinator: Gisely Gonçalves Falco, R.N
Site 5098: Hospital Santa Casa Porto Alegre Brazil

Principal Investigator: Regis Kreitchmann, M.D.

Study Coordinator: Maria Fernanda Severini, PharmB, M.S.

Site 5117: Hospital Nossa Senhora da Conceicao

Principal Investigator: Breno Riegel Santos, M.D.

Study Coordinator: Rita Lira, M.D.

Westat

Clinical Research Associate/Pharmacist: Adriana Ferreira, PharmD, M.B.A., C.C.R.A.

REFERENCES

1 Troeger C, Blacker B, Khalil IA, *et al.* Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis* 2018; **18**: 1191–210.

2 Taron-Brocard C, Chenadec J Le, Faye A, *et al.* Increased risk of serious bacterial infections due to maternal immunosuppression in HIV-exposed uninfected infants in a European country. *Clin Infect Dis* 2014. DOI:10.1093/cid/ciu586.

3 Mussi-Pinhata MM, Motta F, Freimanis-Hance L, *et al.* Lower respiratory tract infections among human immunodeficiency virus-exposed, uninfected infants. *Int J Infect Dis* 2010; **14**. DOI:10.1016/j.ijid.2010.01.006.

4 Brennan AT, Bonawitz R, Gill CJ, *et al.* A Meta-analysis Assessing Diarrhea and Pneumonia in HIV-Exposed Uninfected Compared with HIV-Unexposed Uninfected Infants and Children. J. Acquir. Immune Defic. Syndr. 2019. DOI:10.1097/QAI.0000000000002097.

5 DR M, Y F, SH L, *et al.* Fc Characteristics Mediate Selective Placental Transfer of IgG in HIV-Infected Women. *Cell* 2019.

6 Dzanibe S, Jaspan HB, Zulu MZ, Kiravu A, Gray CM. Impact of maternal HIV exposure, feeding status, and microbiome on infant cellular immunity. *J Leukoc Biol* 2019; **105**: 281–9.

7 Dirajlal-Fargo S, Mussi-Pinhata MM, Weinberg A, *et al.* HIV-exposed-uninfected infants have increased inflammation and monocyte activation. *Aids* 2019; **33**: 845–53.

8 Jones CE, Naidoo S, De Beer C, Esser M, Kampmann B, Hesseling AC. Maternal HIV infection and antibody responses against vaccine-preventable diseases in uninfected infants. *JAMA - J Am Med Assoc* 2011. DOI:10.1001/jama.2011.100.

9 Falconer O, Newell M-L, Jones CE. The Effect of Human Immunodeficiency Virus and Cytomegalovirus Infection on Infant Responses to Vaccines: A Review. *Front Immunol* 2018; **9**: 328.

10 Amir, Weber, Beard, Bomyea T. Maternal Immunization with Pneumococcal 9-valent Conjugate Vaccine and Early Infant Otitis Media. *Bone* 2008; **23**: 1–7.

11 Chaithongwongwatthana S, Yamasmit W, Limpongsanurak S, Lumbiganon P, Tolosa JE. Pneumococcal vaccination during pregnancy for preventing infant infection. *Cochrane Database Syst Rev* 2015; **2015**. DOI:10.1002/14651858.CD004903.pub4.

12 Almeida VDC, Mussi-Pinhata MM, De Souza CBS, *et al.* Immunogenicity of 23-valent

pneumococcal polysaccharide vaccine in HIV-infected pregnant women and kinetics of passively acquired antibodies in young infants. *Vaccine* 2009; **27**: 3856–61.

13 Weinberg A, Muresan P, Laimon L, *et al.* Safety, Immunogenicity and Transplacental Antibody Transport of Conjugated and Polysaccharide Pneumococcal Vaccines Administered to Pregnant Women with HIV: A Multicenter Randomized Controlled Trial. *Lancet HIV* 2021.

14 Siegrist CA, Córdova M, Brandt C, *et al.* Determinants of infant responses to vaccines in presence of maternal antibodies. *Vaccine* 1998; **16**: 1409–14.

15 Zimmermann P, Perrett KP, Messina NL, *et al.* The Effect of Maternal Immunisation During Pregnancy on Infant Vaccine Responses. *EClinicalMedicine* 2019; **13**: 21–30.

16 Barug D, Berbers GAM, van Houten MA, *et al.* Infant antibody levels following 10-valent pneumococcal-protein D conjugate and DTaP-Hib vaccinations in the first year of life after maternal Tdap vaccination: An open-label, parallel, randomised controlled trial. *Vaccine* 2020; **38**: 4632–9.

17 Burton RL, Antonello J, Cooper D, *et al.* Assignment of opsonic values to pneumococcal reference serum 007sp for use in opsonophagocytic assays for 13 serotypes. *Clin Vaccine Immunol* 2017. DOI:10.1128/CVI.00457-16.

18 Marchese RD, Puchalski D, Miller P, *et al.* Optimization and validation of a multiplex, electrochemiluminescence-based detection assay for the quantitation of immunoglobulin G serotype-specific antipneumococcal antibodies in human serum. *Clin Vaccine Immunol* 2009. DOI:10.1128/CVI.00415-08.

19 Finkelstein JA, Huang SS, Daniel J, *et al.* Antibiotic-resistant Streptococcus pneumoniae in the heptavalent pneumococcal conjugate vaccine era: Predictors of carriage in a multicommunity sample. *Pediatrics* 2003. DOI:10.1542/peds.112.4.862.

20 Jones C, Pollock L, Barnett SM, Battersby A, Kampmann B. The relationship between concentration of specific antibody at birth and subsequent response to primary immunization. *Vaccine* 2014; **32**: 996–1002.

21 Voysey M, Kelly DF, Fanshawe TR, *et al.* The influence of maternally derived antibody and infant age at vaccination on infant vaccine responses: An individual participant meta-analysis. *JAMA Pediatr* 2017; **171**: 637–46.

22 Maertens K, Caboré RN, Huygen K, *et al.* Pertussis vaccination during pregnancy in Belgium: Follow-up of infants until 1 month after the fourth infant pertussis vaccination at 15 months of age. Vaccine. 2016; **34**: 3613–9.

23 Nunes MC, Cutland CL, Dighero B, *et al.* Kinetics of hemagglutination-inhibiting antibodies following maternal influenza vaccination among mothers with and those without HIV infection and their infants. *J Infect Dis* 2015. DOI:10.1093/infdis/jiv339.

24 Niewiesk S. Maternal antibodies: Clinical significance, mechanism of interference with immune responses, and possible vaccination strategies. Front. Immunol. 2014. DOI:10.3389/fimmu.2014.00446.

25 Madhi SA, Koen A, Jose L, *et al.* Vaccination with 10-valent pneumococcal conjugate vaccine in infants according to HIV status. *Med (United States)* 2017; **96**. DOI:10.1097/MD.0000000000005881.

26 de Los Santos AM, Rodríguez-Weber MA, Sánchez-Márquez P, *et al.* Can two different

pneumococcal conjugate vaccines be used to complete the infant vaccination series? A randomized trial exploring interchangeability of the 13-valent pneumococcal conjugate vaccine and the pneumococcal non-typeable Haemophilus influenzae p. *Expert Rev Vaccines* 2020; **19**: 995–1010.

27 Temple B, Toan NT, Dai VTT, *et al.* Immunogenicity and reactogenicity of ten-valent versus 13-valent pneumococcal conjugate vaccines among infants in Ho Chi Minh City, Vietnam: a randomised controlled trial. *Lancet Infect Dis* 2019; **19**: 497–509.

28 Daly KA, Scott Giebink G, Lindgren BR, *et al.* Maternal immunization with pneumococcal 9-valent conjugate vaccine and early infant otitis media. *Vaccine* 2014; **32**: 6948–55.

29 Abu-Raya B, Kollmann TR, Marchant A, MacGillivray DM. The immune system of HIV-exposed uninfected infants. *Front Immunol* 2016; **7**: 1–10.

30 De Carvalho Almeida V, Negrini BVM, Cervi MC, Isaac MDL, Mussi-Pinhata MM. Pneumococcal nasopharyngeal carriage among infants born to human immunodeficiency virus-infected mothers immunized with pneumococcal polysaccharide vaccine during gestation. *Pediatr Infect Dis J* 2011; **30**: 466–70.

31 Munoz FM, Englund JA, Cheesman CC, *et al.* Maternal immunization with pneumococcal polysaccharide vaccine in the third trimester of gestation. In: Vaccine. 2001. DOI:10.1016/S0264-410X(01)00397-8.

Figure legends

Figure 1. Consort diagram

Figure 2. Kinetics of the anti-PNC antibody serum concentrations from birth to 24 weeks of age in infants born to vaccine and placebo recipient-mothers.

Geometric mean titers (μg/mL) and 95% confidence intervals of pneumococcal antibody levels according to infants age (at birth=week 0; before PCV10 vaccination=week8; after the 1st dose of PCV-10=week16; and after the 2nd dose of PCV10=week24) are shown for each of eight pneumococcal polysaccharide serotypes. The abscissa shows the time points of each measurement. The ordinate shows antibody concentrations in μg/ml.

Table 1- Birth characteristics of 347 infants born to HIV-infected mothers according to the maternal immunization arm.

Birth Characteristics	Maternal immunization arm		
	PCV-10 (N=116)	**PPV-23 (N=112)**	**Placebo (N=119)**
Gestational age (wks)			
Median (range)	39 (36, 41)	39 (32, 41)	38 (33, 42)
N missing	18	14	16
Prematurity*			
Yes	2 (2%)	13 (13%)	12 (12%)
No	96 (98%)	85 (87%)	91 (88%)
Small for gestational age			
Yes	7 (6%)	10 (10%)	14 (13%)
No	102 (94%)	95 (90%)	97 (87%)
Birth weight (g)			
Median (range)	3115 (2180, 4290)	3150 (1005, 4390)	2980 (1455, 4240)
N missing	1	0	0

PCV-10: 10-valent pneumococcal conjugate vaccine, PPV-23: 23-valent pneumococcal polysaccharide vaccine;
*χsquare test: overall, p=0.01; PPV-23 *vs.* PCV-10 p=0.003; PCV-10 *vs* Placebo p=0.008; PPV-23 *vs*. Placebo p=0.73

Table 2. Seroprotection (antibody levels ≥ 0.35 μg/ml) before and after PCV-10 vaccination in infants according to serotype and the maternal vaccination arm.

	Maternal Vaccination Arm						
	PCV-10		PPV-23		Placebo		P value**
Infants with pneumococcal antibody values ≥ 0.35 mg/ml	**N**	**Percent (95%CI)**	**N**	**Percent (95%CI)**	**N**	**Percent (95%CI)**	
At week 8 (before PCV-10 vaccination)							
All seven serotypes	108	0 (0,3)	104	2 (0,7)	109	0 (0,3)	0.10
Serotype 1	108	21 (14,30)	104	38 (28,48)	109	2 (0,6)	**<.0001**#£$
Serotype 4	108	41 (31,51)	104	15 (9,24)	109	1 (0,5)	**<.0001**#£$
Serotype 5	108	22 (15,31)	104	19 (12,28)	109	5 (2,10)	**0.0002**£$
Serotype 6B	108	47 (38,57)	104	51 (41,61)	109	6 (3,13)	**<.0001**£$
Serotype 7F	107	24 (17,34)	104	38 (28,48)	109	4 (1,9)	**<.0001**£$
Serotype 14	108	75 (66, 83)	104	69 (59,78)	109	39 (29,48)	**<.0001**£$
Serotype 23F	106	36 (27,46)	104	35 (26,45)	109	4 (1,9)	**<.0001**£$
At week 16 (after PCV-10 first dose)							
All seven serotypes	100	11 (6,19)	100	24(16,34)	101	17 (10,26)	**0.001**#
Serotype 1	100	78 (69,86)	100	89 (81,94)	101	82 (73,89)	0.10
Serotype 4	98	78 (68,85)	100	93 (86,97)	101	87 (79,93)	**0.007**#
Serotype 5	100	62 (52,72)	100	81 (72,88)	101	62 (52,72)	**0.004**#$
Serotype 6B	100	41 (31,51)	100	58 (48,68)	101	36 (26,46)	**0.004**#$
Serotype 7F	97	82 (73,89)	100	88 (80,94)	101	83 (74,90)	0.51
Serotype 14	100	84 (75,91)	100	87 (79,93)	101	85 (77,91)	0.84
Serotype 23F	91	31 (22,41)	91	49 (39,60)	97	37 (28,48)	**0.03**#
At week 24 (after PCV-10 second dose)							
All seven serotypes	103	50 (40-60)	102	71 (61-79)	103	71 (61-79)	**<.0001**#£
Serotype 1	103	91 (84,96)	102	94 (88-98)	101	95 (89,98)	0.59
Serotype 4	102	92 (85,97)	101	100 (96,100)	103	96 (90,99)	**0.007**#
Serotype 5	103	87 (79,93)	101	95 (89,98)	101	95 (89,98)	0.08
Serotype 6B	103	79 (69,86)	102	92 (85,97)	101	87 (79,93)	**0.02**#
Serotype 7F	102	95 (89,98)	101	99 (95,100)	103	95 (89,98)	0.24
Serotype 14	103	90 (83,95)	102	98 (93,100)	101	96 (90,99)	0.05#
Serotype 23F	97	84 (75,90)	92	90 (82,95)	100	91 (84,96)	0.23

***** Data were derived from 7 serotypes common to PCV-10 and PPV-23; ** Fisher's Exact Test comparing all three treatment arms. Significance comes from PPV vs. PCV (#), PPV vs placebo ($) and/or PCV vs placebo (£).

Table 3 – Correlations between pneumococcal IgG antibodies measured at weeks 8 (before vaccination) and 24 (after two doses of PCV-10) according to serotype.

Serotype	Maternal vaccination arm	Spearman Correlation coefficient	P value	N
1	**PCV-10**	**-0.214**	**0.03136**	101
	Placebo	0.071	0.49274	96
	PPV-23	-0.042	0.67936	97
4	**PCV-10**	**-0.357**	**0.00027**	100
	Placebo	-0.089	0.38605	98
	PPV-23	-0.143	0.16544	96
5	**PCV-10**	**-0.255**	**0.00998**	101
	Placebo	-0.069	0.50376	96
	PPV-23	-0.083	0.41838	97
6B	**PCV-10**	**-0.199**	**0.04567**	101
	Placebo	-0.012	0.90696	96
	PPV-23	-0.165	0.10533	97
7F	PCV-10	-0.114	0.26145	99
	Placebo	**0.199**	**0.0491**	98
	PPV-23	-0.108	0.29664	96
14	PCV-10	-0.170	0.08948	101
	Placebo	**-0.290**	**0.00414**	96
	PPV-23	-0.147	0.15121	97
23F	**PCV-10**	**-0.349**	**0.00056**	94
	Placebo	-0.071	0.49468	95
	PPV-23	-0.202	0.06117	87

Table 4. Infant B and T cell memory responses to pneumococcal serotype 1 after the second dose of PCV10.

Cell panel	Analyte	Maternal vaccination arm	N	Median (Q1,Q3)*
B cells	IgA	PCV-10	6	1 (0,4)
		PPV-23	8	1 (0,3)
		Placebo	13	0 (0,2)
		Overall	**27**	**0 (0,2)**
	IgG	PCV-10	6	1 (0,4)
		PPV-23	8	2 (0,2)
		Placebo	13	0 (0,2)
		Overall	**27**	**0 (0,2)**
T cells	IL17	PCV-10	7	0 (0,0)
		PPV-23	11	0 (0,2)
		Placebo	17	0 (0,2)
		Overall	**35**	**0 (0,2)**
	IFNg	PCV-10	7	0 (0,2)
		PPV-23	11	2 (0,2)
		Placebo	17	0 (0,2)
		Overall	**35**	**0 (0,2)**

Numbers represent mean spot forming cells (SFC)/10^6 PBMC in antigen-treated wells after subtraction of SFC in media control wells.

Table 5 – Inflammatory Biomarkers Principal Components Weights

	Principal Component				
Biomarker (pg/ml)	**1**	**2**	**3**	**4**	**5**
CCL-2 (MCP-1)	**0.2843**	0.0774	0.1886	**-0.2302**	0.1170
CCL-3 (MIP-1α)	**0.2555**	0.0438	-0.1451	**-0.2211**	**-0.2044**
CCL-4 (MIP-1β)	**0.2679**	-0.1122	-0.1080	**-0.2245**	-0.0986
CCL-11 (eotaxin-1)	**0.2161**	**-0.2572**	**0.3700**	-0.0768	0.0313
CCL-13 (MCP-4)	**0.2232**	**-0.3006**	**0.3132**	-0.0302	0.0737
CCL-17 (TARC)	0.1940	**-0.2957**	-0.1942	-0.1598	0.0953
CCL-23 (MDC)	0.1610	-0.1931	-0.1494	0.0075	**0.2055**
CCL-26 (eotaxin-3)	0.1855	**-0.2805**	**0.4128**	0.0437	-0.0038
CXCL-10 (IP-10)	0.1832	**0.3675**	**0.2073**	0.0076	-0.1146
GM-CSF	**0.2081**	-0.0496	-0.0346	**0.2928**	0.1634
IFNγ	**0.2168**	**0.3387**	**0.2196**	0.0293	-0.0416
IL-2	0.1085	0.1332	0.1092	-0.0107	**0.4137**
IL-4	0.1227	-0.0647	0.0773	**0.3329**	-0.0110
IL-5	0.1487	-0.0161	-0.0319	**0.3867**	**0.2202**
IL-6	0.1583	**0.2695**	0.0124	-0.1352	-0.0370
IL-7	**0.2328**	-0.0587	**-0.2032**	-0.0402	0.1753
IL-8	**0.2330**	-0.0990	-0.1780	**-0.2662**	**-0.3637**
IL-10	**0.2249**	**0.2940**	0.0484	0.0984	-0.1368
IL-12/p70	0.1029	-0.0312	0.0790	**0.4755**	**-0.3508**
IL-13	0.1982	-0.0920	-0.0353	**0.2030**	**-0.4065**
IL-15	0.1757	**0.2517**	0.0737	-0.1384	**0.2956**
IL-16	0.1220	0.0534	**-0.3157**	0.1332	0.1097
IL-17a	0.1577	0.0839	**-0.2279**	**0.2426**	0.1974
TNFα	**0.2419**	**0.2283**	**-0.2058**	0.0030	-0.0683
VEGF-A	**0.2273**	-0.1952	**-0.2695**	0.0519	0.0366

Note: Component weights with magnitude ≥0.2 are shown in bold.

Table 6- Effect of inflammation on infant antibody responses after the 2nd dose of PCV-10 (Linear regression models results for each serotype)

Serotype	N	Variable	Coefficient	p-value
1	103	-		
4	103	Component 5	-0.08	0.017
		PCV-10	-0.04	0.664
		PPV-23	0.24	0.014
5	103	Component 5	-0.08	0.012
6B	103	Week 8 antibody level	-0.15	0.022
		Component 2	-0.06	0.031
7F	103	Component 5	-0.06	0.021
14	103	Week 8 antibody level	-0.16	0.009
23F	95	Week 8 antibody level	-0.27	0.028