

No long-term evidence of hyporesponsiveness after use of pneumococcal conjugate vaccine in children previously immunized with pneumococcal polysaccharide vaccine

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Background: A randomized controlled trial in Fiji examined the immunogenicity and effect on nasopharyngeal carriage after 0, 1, 2, or 3 doses of 7-valent pneumococcal conjugate vaccine (PCV7; Prevnar) in infancy followed by 23-valent pneumococcal polysaccharide vaccine (23vPPV; Pneumovax) at 12 months of age. At 18 months of age, children given 23vPPV exhibited immune hyporesponsiveness to a micro-23vPPV (20%) challenge dose in terms of serotype-specific IgG and opsonophagocytosis, while 23vPPV had no effect on vaccine-type carriage.

Objective: This follow-up study examined the long-term effect of the 12-month 23vPPV dose by evaluating the immune response to 13-valent pneumococcal conjugate vaccine (PCV13) administration 4 to 5 years later.

Methods: Blood samples from 194 children (now 5-7 years old) were taken before and 28 days after PCV13 booster immunization. Nasopharyngeal swabs were taken before PCV13 immunization. We measured levels of serotype-specific IgG to all 13 vaccine serotypes, opsonophagocytosis for 8 vaccine serotypes, and memory B-cell responses for 18 serotypes before and after PCV13 immunization.

Results: Paired samples were obtained from 185 children. There were no significant differences in the serotype-specific IgG, opsonophagocytosis, or memory B-cell response at either time point between children who did or did not receive 23vPPV at 12 months of age. Nasopharyngeal carriage of PCV7 and 23vPPV serotypes was similar among the groups. Priming with 1, 2, or 3 PCV7 doses during infancy did not affect serotype-specific immunity or carriage.

Conclusion: Immune hyporesponsiveness induced by 23vPPV in toddlers does not appear to be sustained among preschool children in this context and does not affect the pneumococcal carriage rate in this age group. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Pneumococcal polysaccharide vaccine, 23vPPV, hyporesponsiveness, memory B cell, opsonophagocytosis, antibody*

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Streptococcus pneumoniae (the pneumococcus) is a gram-positive bacterium that is responsible for a large burden of morbidity and mortality in children less than 5 years of age worldwide.¹ According to the latest World Health Organization (WHO) estimates, pneumococcal diseases, such as pneumonia, meningitis, and septicemia, cause 826,000 child deaths per year, mostly in developing countries.²

Protection against pneumococcal diseases is achieved principally through vaccination, with 2 types of vaccines currently licensed and implemented in many countries. Pneumococcal conjugate vaccines (PCVs), whereby the capsular polysaccharide from up to 13 serotypes is coupled to a protein carrier, have had a remarkable effect on vaccine-type disease in every setting in which they have been used.³ At present, PCVs containing 10 or 13 common disease-causing serotypes³ are available. In comparison, the 23-valent pneumococcal polysaccharide vaccine (23vPPV; Pneumovax; Merck & Co, Whitehouse Station, NJ) is a plain polysaccharide vaccine representing the 23 pneumococcal serotypes that are responsible for more than 90% of disease in

Abbreviations used

CPS:	Cell-wall polysaccharide
FiPP:	Fiji Pneumococcal Project
MOPA:	Multiplexed opsonophagocytic assay
OI:	Opsonization index
OPA:	Opsonophagocytic assay
PBS-T:	PBS containing 0.05% (vol/vol) Tween 20
PCV:	Pneumococcal conjugate vaccine
PCV7:	7-Valent pneumococcal conjugate vaccine
PCV13:	13-Valent pneumococcal conjugate vaccine
23vPPV:	23-Valent pneumococcal polysaccharide vaccine
WHO:	World Health Organization

the United States.⁴ However, 23vPPV is not recommended for use in children less than 2 years of age because of immaturity of their immune system to respond to polysaccharide antigens.⁵ Instead, 23vPPV is administered to older children after a primary series of PCV and in some settings to immunocompromised and healthy adults from the age of 65 years. In Australia indigenous children have a higher rate of pneumococcal disease. Until recently, in addition to PCV in infancy, these children also received 23vPPV as a booster vaccine at 18 months because of its broader serotype coverage.⁶

Vaccine efficacy of 23vPPV for the prevention of invasive pneumococcal disease in adults was found to be 74% in a Cochrane review⁷ based on studies that mainly used older-generation vaccines but has been reported to be much lower in some studies,⁸ whereas protection against pneumonia or death has been difficult to establish.⁷ Early trials of 23vPPV in young children in Papua New Guinea demonstrated a 59% efficacy against acute lower respiratory infections and a significant reduction in mortality.⁹ In the Fiji Pneumococcal Project (FiPP) we set out to evaluate the utility of 23vPPV as a booster vaccine in children primed with various schedules of 7-valent pneumococcal conjugate vaccine (PCV7; Prevnar; Pfizer, New York, NY). We examined the response to a 20% challenge dose of 23vPPV at 17 months of age (to mimic infection) in children previously primed with 0 to 3 doses of PCV7 and randomized to receive or not receive a full dose of 23vPPV at 12 months. This was designed to examine the immunologic effects of 23vPPV at 12 months of age. Although children less than 12 months of age might not be expected to respond to a polysaccharide vaccine, the children given 23vPPV at 12 months of age in this study had good booster antibody responses to the PCV7 types that were higher than those in children who did not receive 23vPPV. However, these 23vPPV-vaccinated children failed to boost these responses further when given a 20% 23vPPV challenge dose at 17 months of age, whereas children who did not receive 23vPPV at 12 months of age produced higher antibody levels to all PCV7 and almost all non-PCV7 serotypes.¹⁰ This has led to safety concerns for these children given the substantial burden of pneumococcal carriage and disease in this population.

We now report the findings of a long-term follow-up investigation of immune hyporesponsiveness in these children. We investigated whether the immune hyporesponsiveness observed at 18 months of age has had a deleterious effect on immune competence, nasopharyngeal carriage of pneumococci, and associated clinical outcomes measured in these children at 5 to 7 years of age.

METHODS**Study population and samples**

The study design and details of FiPP (2003–2008) have been reported previously.^{10–12} Briefly, healthy Fijian infants ($n = 552$) were randomized to receive a primary series of 0, 1, 2, or 3 doses of PCV7, with half the children randomized to receive 23vPPV at 12 months of age. Children given 0 or 1 PCV7 doses during the primary series were given a catch-up PCV7 dose at 2 years of age (Table 1). Between March 2011 and February 2012, families who had participated in FiPP and previously agreed to be contacted for follow-up studies were invited to participate in a follow-up study. After consent, on the first visit, all children had 10 mL of blood drawn into a sodium heparin tube, and a nasopharyngeal swab was taken according to standard methods.¹³ In addition, they were each administered a single dose of 13-valent pneumococcal conjugate vaccine (PCV13). A second blood sample was taken 28 days later.

This study was approved by the Fiji National Research Ethics Review Committee and the Royal Children's Hospital Human Research Ethics Committee in Melbourne, Australia.

Measurement of pneumococcal serotype-specific IgG levels by means of ELISA

Serotype-specific IgG levels to all 23 serotypes were measured by using a previously published modified WHO ELISA developed in our laboratory (see details in the [Methods section](#) in this article's Online Repository at www.jacionline.org).¹⁴

Opsonophagocytosis assay

All opsonophagocytic assays (OPAs) were undertaken in our laboratory based on previously published multiplexed methods (multiplexed opsonophagocytic assay [MOPA]; see details in the [Methods section](#) in this article's Online Repository).¹⁵

Enumeration of pneumococcus-specific memory B cells

We undertook the measurement of pneumococcus-specific memory B cells on site in Fiji using a previously established method (see details in the [Methods section](#) in this article's Online Repository).¹⁶

Measurement of nasopharyngeal carriage

Pneumococcal carriage was measured by using standard culture-based methods similar to the original FiPP study (see details in the [Methods section](#) in this article's Online Repository).^{12,13}

Hospitalization data

The Colonial War Memorial Hospital in Suva, Fiji, uses a computerized system for recording admissions and discharges (Patient Information System). All discharges were entered into a nationwide linked database, and a search for admissions was performed by using demographic identifiers or hospital numbers. In addition to asking parents whether their child has been hospitalized at the time of the first study visit, a thorough search of the computerized discharge system based on the name and date of birth from the commencement of the study up to the present time to identify all hospital admissions involving all children in the original study ($n = 552$) was undertaken. Serious adverse events in children aged greater than 12 months and by receipt or not of the 12-month 23vPPV was assessed. The laboratory results of any lumbar punctures or blood cultures taken were recorded.

Statistical analysis

Serotype-specific IgG and OPA titers were presented as geometric mean concentrations or geometric mean opsonization indices with 95% CIs,

TABLE I. Study design and participant groups

Group	PCV7 primary series	23vPPV	Catch-up PCV7 dose*	Total PCV7 doses
A	3× PCV7	No	No	3
B	3× PCV7	Yes	No	3
C	2× PCV7	No	No	2
D	2× PCV7	Yes	No	2
E	1× PCV7	No	Yes	2
F	1× PCV7	Yes	Yes	2
G	0× PCV7	Yes	Yes	1
H	0× PCV7	No	Yes	1

PCV7, 7-valent pneumococcal conjugate vaccine.

*Catch-up dose at 24 months of age for partially vaccinated children in FiPP.

respectively. Log-transformed data were used for statistical analyses of IgG and OPA data by using 2-tailed paired Student *t* tests. Medians and interquartile ranges were considered for memory B-cell responses, and the Mann-Whitney *U* test was used for analysis. Rates of nasopharyngeal carriage were calculated based on the number of total pneumococcal, PCV7, and 23vPPV vaccine-type or non-vaccine-type isolates in children in each group at each time point divided by the total number of children who had a nasopharyngeal swab taken in each group at each time point. Analyses comparing the proportions of children with nasopharyngeal carriage of any pneumococcus, vaccine-type pneumococci (PCV7 and 23vPPV), and non-vaccine-type pneumococci were performed by using logistic regression in which an interaction between 23vPPV receipt and dose of PCV7 was considered. The McNemar test was used to assess changes in binary outcomes before and after PCV13 vaccination. Linear regression models were used to adjust for the influence of PCV experience on the outcomes measured and also to adjust for the influence of pre-PCV13 levels on post-PCV13 responses. *P* values are presented in 4 decimal places unless *P* values were less than .0001 or greater than .01, the latter of which were considered nonsignificant.

RESULTS

Of the 552 children in the original FiPP study, the families of 437 children gave permission to be contacted for this follow-up study. There were 194 families that were contactable for this follow-up study because the population is mobile and contact details, including telephone numbers, were no longer valid. However, the families of all 194 children, now aged 5 to 7 years, provided written informed consent for this study. A nasopharyngeal swab and paired blood specimens were collected from 185 children who formed the basis of this study. The demographics and breakdown of study participants are summarized in Table II and were similar to those of the original study. Ninety-eight (53%) children had received 23vPPV at 12 months, with each group having approximately equal numbers of children receiving either 0, 1, 2, or 3 prior doses of PCV7 in infancy (Table II). During the follow-up period, there were 31 hospital admissions among children who had been enrolled in FiPP (Table III). There were 7 children who potentially had pneumococcus-related disease, although none were laboratory confirmed. No differences were found between the 23vPPV groups, with 2 cases in the 12-month 23vPPV group and 5 cases in the non-23vPPV group, and all cases had negative culture results. Cerebrospinal fluid was not taken from the child with meningitis, and there was no growth on blood culture. In the 12 months immediately after 23vPPV, there was no difference in hospitalizations between the groups, suggesting no early effect of 23vPPV.

In the study children, who were now aged 5 to 7 years, immunization with a single dose of PCV13 produced strong

TABLE II. Summary data for children (n = 185)* in this follow-up study

Characteristic	No. (%)
Sex	
Male	106 (57.3)
Female	79 (42.7)
Ethnicity	
Fijian (i-Taukei)	135 (73.0)
Indo-Fijian	45 (24.3)
Other	5 (2.7)
Received 23vPPV at 12 mo of age	
Yes	98 (53)
No	87 (47)
Prior number of PCV7 doses	
0	41 (22.2)
1	39 (21.1)
2	52 (28.1)
3	53 (28.6)

*The number represents those with paired pre- and post-PCV13 samples. The actual number of participants is 194, 103 who received 23vPPV and 91 who did not receive 23vPPV.

TABLE III. Hospitalizations during the follow-up period*

Hospitalization	Prior 23vPPV	No prior 23vPPV
Pneumonia	2†	3
Meningitis	0	1‡
<i>Staphylococcus aureus</i> septic arthritis	0	1
Febrile convulsion	1	1
Acute gastroenteritis	2	5
Other§	7	8
Total	12	19

*Hospitalizations reported during the study's follow-up period: 1 year of age until March 2012.

†One positive *Staphylococcus aureus* blood culture.

‡Unknown cause.

§Other includes laceration, superficial abscesses, burns, kerosene ingestion, fractures, skin rash, foreign body inhalation, dehydration, jaundice, and viral illness.

immune responses for all PCV13 vaccine serotypes compared with baseline values (Fig 1). This was observed for serotype-specific IgG and OPA ($P \leq .001$ for all serotypes; Fig 1, A and B). The mean fold increases in serotype-specific IgG levels ranged from 6.1 (serotype 19A) to 48.6 (serotype 4), whereas for OPA, the mean fold increase ranged from 33.6 (serotype 1) to 1025 (serotype 6A). Significant increases in the proportion of children with serotype-specific IgG levels of 0.35 $\mu\text{g/mL}$ or greater and 1.0 $\mu\text{g/mL}$ or greater and an opsonization index (OI) of 8 or greater were also observed for all serotypes (see Fig E1 in this article's Online Repository at www.jacionline.org). Consistent with these findings, there was also a significant increase in the number of pneumococcus-specific memory B cells after immunization for all PCV13 serotypes examined ($P < .001$ for all serotypes; Fig 1, C). Responses to nonvaccine serotypes were negligible after PCV13 immunization, as expected (see Tables E1-E5 in this article's Online Repository at www.jacionline.org).

Fig 2, A-C, shows the pre-PCV13 immune response at 5 to 7 years of age, comparing children who did or did not receive the 12-month 23vPPV dose. Pre-PCV13 levels of serotype-specific IgG and numbers of memory B cells were similar between children who had or had not received 23vPPV for most

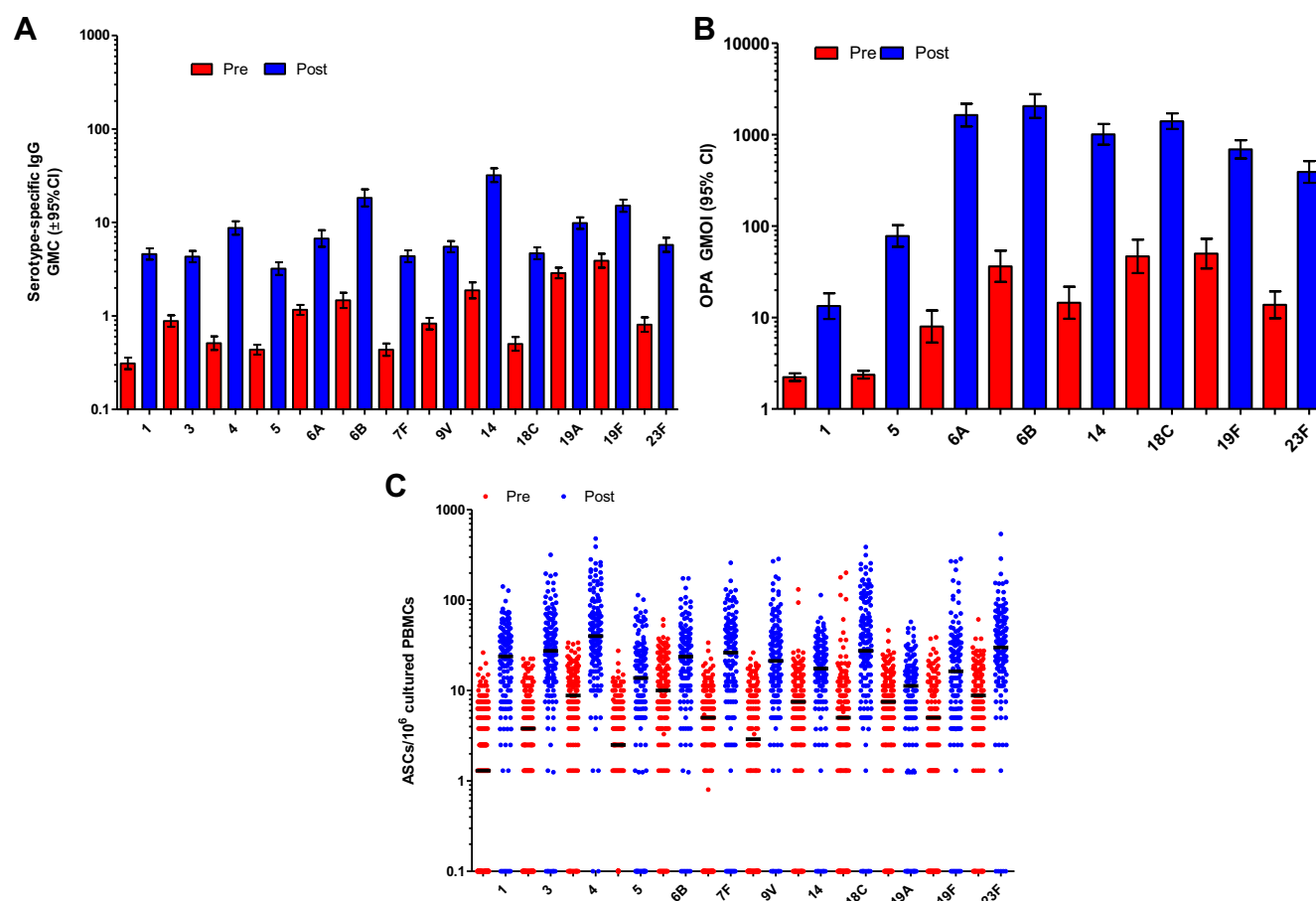


FIG 1. PCV13 induces a strong immune response in children previously vaccinated with 23vPPV at 12 months of age. Results of measurement of serotype-specific IgG levels (in micrograms per milliliter; **A**), opsonophagocytic response (**B**), and memory B-cell response (**C**) in children before and after PCV13 ($n = 185$ paired samples) are shown. ASC, Antibody-secreting cell; GMC, geometric mean concentration; GMOI, geometric mean opsonization index. $P < .0001$ for all comparisons pre- and post-PCV13 responses.

PCV7 and non-PCV7 serotypes, indicating that the titers generated at 17 months of age by children who received 23vPPV had waned by age 5 years (see Table E1). For OPA, responses were also similar in the 2 groups, except for serotype 23F, for which children who had received the 23vPPV had lower OPA titers ($P < .001$; Fig 2, B). There was also a trend toward lower IgG and B-cell responses for serotype 23F in the 23vPPV group. In addition, no differences were observed in the proportions of children with IgG titers of 0.35 $\mu\text{g/mL}$ or greater or 1.0 $\mu\text{g/mL}$ or greater or with an OI of 8 or greater (see Fig E2 and Tables E4-E6 in this article's Online Repository at www.jacionline.org).

After a dose of PCV13, children who had received 23vPPV at 12 months of age responded comparably with those who had not received 23vPPV (Fig 2, C-E). No significant differences in serotype-specific IgG, OPA, or memory B-cell levels were detected, even after adjustment for the number of prior PCV7 doses received and pre-PCV13 levels of pneumococcal immunity (see Fig E3 in this article's Online Repository at www.jacionline.org). Fig 3 demonstrates that, for serotype-specific IgG, all children responded similarly to PCV13 serotypes (with adjustment for the pre-PCV13 IgG level and the effect of the catch-up PCV7 dose at 2 years of age) regardless of 23vPPV receipt at 12 months of age

compared with their responses at 18 months of age (representative data are shown for serotypes 4 and 19A; see Fig E4 in this article's Online Repository at www.jacionline.org for all other serotypes). Similarly, for non-PCV13 serotypes, no children responded to PCV13 immunization (see Tables E1-E5).

Nasopharyngeal carriage was also assessed in these children (Table IV). Overall, 45% ($n = 88$) of children carried pneumococci, with 13 (15%) having a PCV7 type, 37 (42%) having a 23vPPV type (including PCV7 types), and 51 (58%) having nonvaccine types. Overall, 102 isolates were identified, with the most frequently isolated serotypes being 11A (9.7%), 6A (7.8%), and 16F (6.8%) and nontypeable pneumococci accounting for 12.6% of all carriage-positive isolates. For children who received 23vPPV at 12 months of age, there was more carriage of PCV7 (9%) and 23vPPV (21%) serotypes compared with children who had not received 23vPPV (4% and 17%, respectively), whereas overall pneumococcal carriage rates were similar across the 2 groups (40% vs 51%, respectively).

DISCUSSION

This is the first study to comprehensively characterize the long-term effects of immune hyporesponsiveness after 23vPPV

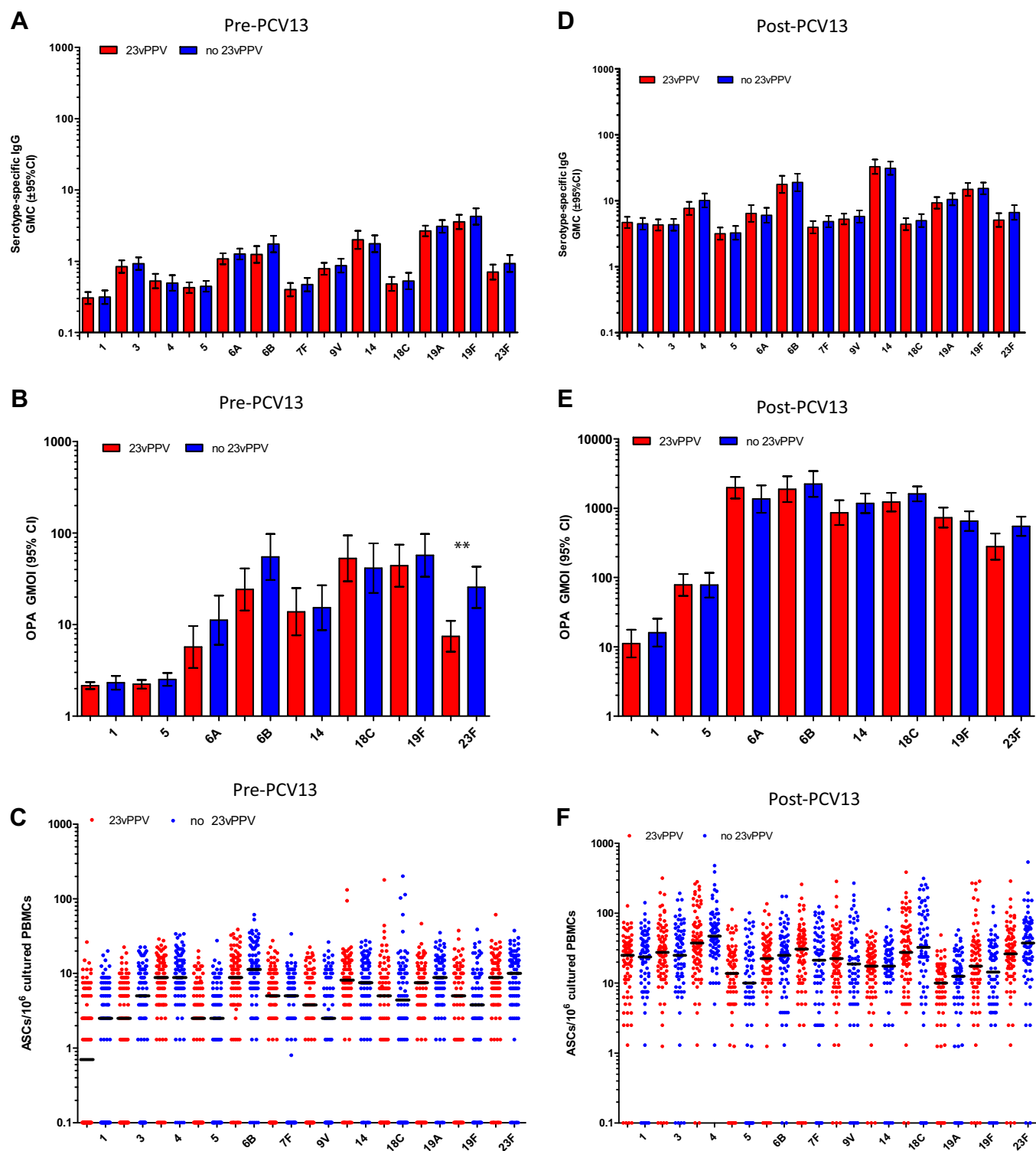


FIG 2. Administration of 23vPPV at 12 months of age did not lead to sustained hypo-responsiveness in children now aged 5 to 7 years. Results of measurement of serotype-specific IgG levels (in micrograms per milliliter), opsonophagocytic response, and memory B-cell response in children who did ($n = 98$) or did not ($n = 87$) receive 23vPPV at 12 months of age before (A-C) and after (D-F) PCV13 are shown ($n = 185$ paired samples). GMC, Geometric mean concentration; GMOI, geometric mean opsonization index. $^{**}P < .001$, comparing children who did or did not receive 23vPPV at 12 months of age.

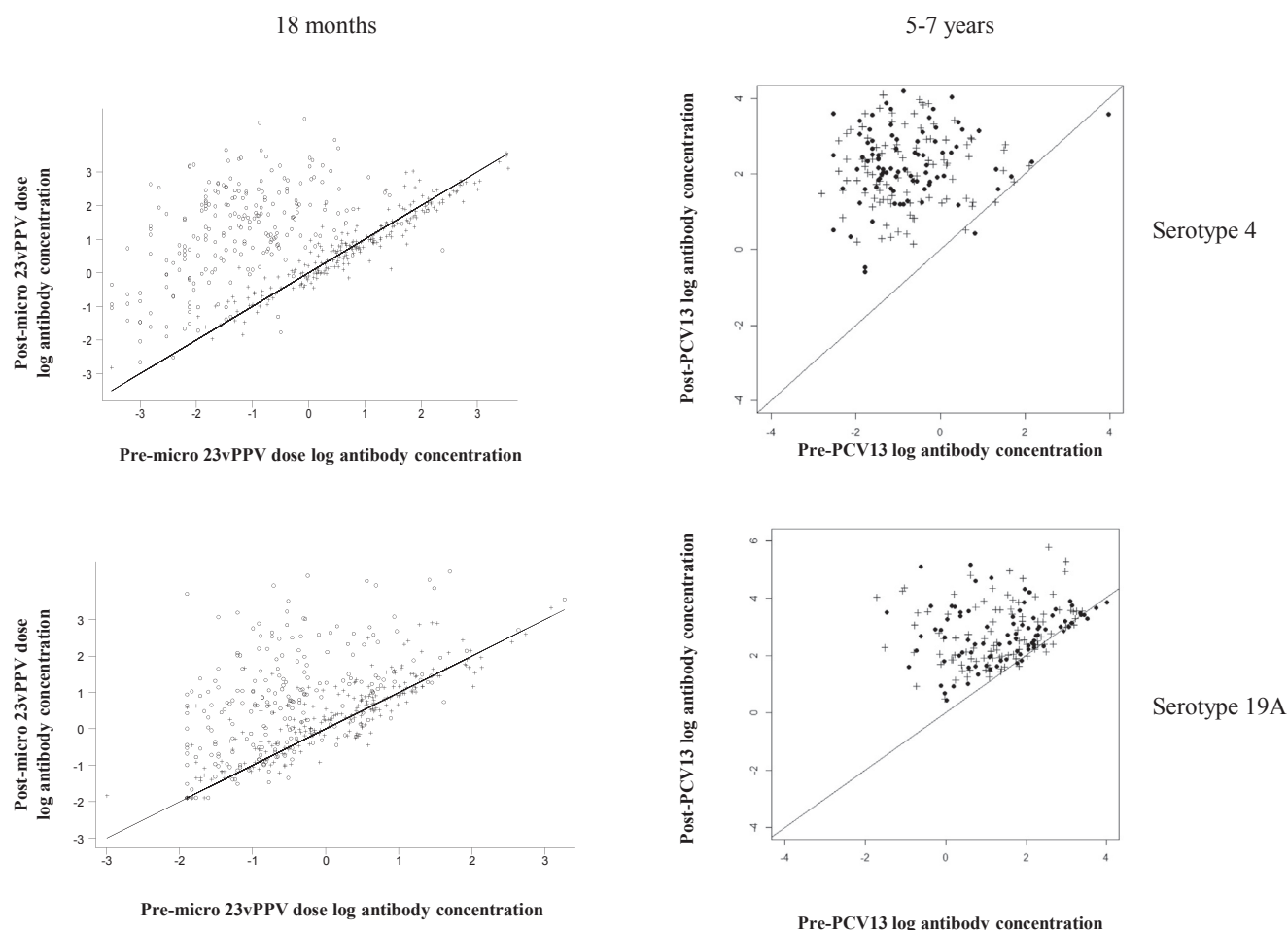


FIG 3. Children responded similarly to all PCV13 serotypes regardless of 23vPPV receipt. The change in serotype-specific IgG levels (in micrograms per milliliter) after PCV13 immunization is shown for serotype 4 and 19A as representative of all serotypes. The *diagonal line* represents no change from pre-PCV13 (baseline) levels. The data at 18 months of age are from Russell et al.¹⁰ and at 5 to 7 years, the data represent the antibody response in children who did (*cross*) or did not (*solid circles*) receive 23vPPV at 12 months of age.

immunization in children at high risk of pneumococcal disease. In a vaccine trial in which 552 Fijian infants were vaccinated with various dose schedules of PCV7 and half were boosted at 12 months with 23vPPV, we demonstrated that 23vPPV recipients at 18 months of age were nonresponsive to a small 20% dose of 23vPPV, whereas children who had not received 23vPPV had good responses. In the present study 194 of the 552 children enrolled in the original trial were followed up 4 to 5 years later. Although pneumococcal carriage rates in the present study were similar in the groups who had or had not received 23vPPV at 12 months (41/102 vs 47/92 in the 23vPPV⁺ and 23vPPV⁻ groups, respectively), carriage of PCV7 serotypes was slightly higher in those who received 23vPPV and had a subsequent very strong response to PCV7.¹¹ We undertook a detailed immunologic investigation of these children, including assessing pneumococcal antibodies to PCV13 serotypes (by means of ELISA and OPA) and pneumococcal B-cell responses before and after a dose of PCV13. No differences between the groups were seen in any of the immunologic measurements before or after PCV13, and responses to PCV13 were normal. Although unknown at the time, the similar response to PCV13 immunization

in infants who did or did not receive 23vPPV at 12 months of age was not surprising given the similar pre-PCV13 levels. Moreover, PCV13 was given to ensure all children were able to respond equally well to vaccination and that the hyporesponsiveness observed at 18 months did not persist. There was no evidence of residual hyporesponsiveness in the 23vPPV-vaccinated children. We also reviewed hospital records for all children in the original study and found no difference in hospitalizations related to pneumococcal disease, although this study was not sufficiently powered for this analysis.

Vaccine-induced hyporesponsiveness was first reported for meningococcal polysaccharide-based vaccines, with prior immunization resulting in reduced antibody titers for both children and adults.^{17,18} For pneumococcal polysaccharide vaccines, studies to detect hyporesponsiveness in adults receiving repeated doses of 23vPPV have produced variable results¹⁹⁻²² while making results difficult to interpret because of issues of possible bias from the nonrandomized study design of a number of these 23vPPV trials. Recent studies have shown that 23vPPV-induced hyporesponsiveness in adults and children was not completely restored by additional doses of PCV7²³ or PCV13,²⁴ suggesting a sustained

TABLE IV. Nasopharyngeal carriage of *Streptococcus pneumoniae* (percentages)

Group	Carriage of any pneumococci	Carriage of PCV7 serotypes	Carriage of 23vPPV serotypes
3 PCV dose group (n = 54)			
23vPPV ⁺ (n = 28)	13 (46)	3 (11)	6 (21)
23vPPV ⁻ (n = 26)	12 (46)	0 (0)	2 (8)
2 PCV dose group (n = 95)			
23vPPV ⁺ (n = 55)	20 (36)	4 (7)	10 (18)
23vPPV ⁻ (n = 40)	21 (53)	2 (5)	8 (20)
1 PCV dose group (n = 45)			
23vPPV ⁺ (n = 19)	8 (42)	2 (11)	5 (26)
23vPPV ⁻ (n = 26)	14 (54)	2 (8)	6 (23)
Total carriage (n = 194)			
23vPPV ⁺ (n = 102)	41 (40)	9 (9)	21 (21)
23vPPV ⁻ (n = 92)	47 (51)	4 (4)	16 (17)

23vPPV⁺, Children who received 23vPPV at 12 months of age; 23vPPV⁻, children who did not receive 23vPPV at 12 months of age.

effect of 23vPPV vaccination. The FiPP study was the first to demonstrate true hyporesponsiveness in infants after the use of 23vPPV. At that time, Australian policy recommended that indigenous children (who are at high risk of pneumococcal disease) who had received PCV in infancy should be boosted with 23vPPV at 18 months of age to provide broader serotype coverage, although only 57% of eligible children were receiving this booster dose.²⁵ An observational study found that children who had received 23vPPV were at higher risk of pneumonia than children who had not, even when controlling for potential confounders.²⁶ After that study and the Fiji study demonstrating hyporesponsiveness, Australian policy was changed, replacing 23vPPV with a fourth dose of PCV for indigenous children. Other countries considering a 23vPPV booster policy for children did not proceed.

Immune hyporesponsiveness is believed to be caused by memory B-cell depletion.^{18,27} Immunization with polysaccharide-based vaccines does not induce memory B-cell formation but triggers activation and recruitment of the memory B-cell compartment to differentiate into antibody-secreting plasma cells in the circulation and secondary lymphoid tissue. Adults immunized with 23vPPV had a reduced memory B-cell frequency associated with decreased B1b cell counts, a critical subset known to protect mice against pneumococcal infection.²⁸ Similarly, reduced numbers of murine splenic and bone marrow-derived memory B cells were observed after a pneumococcal polysaccharide booster,²⁹ whereas for capsular group C meningococcal polysaccharide vaccine, hyporesponsiveness was associated with increased apoptosis of polysaccharide-specific B cells.³⁰ Importantly, despite this effect of 23vPPV, although there has been no direct evidence of any clinical effect of hyporesponsiveness in a large-scale setting, a recent review concluded that 23vPPV booster vaccination should be discouraged in line with WHO recommendations.³¹

In our study we found that there was no immunologic evidence of continuing hyporesponsiveness to PCV13 serotypes 5 years later in children who were demonstrated to be hyporesponsive after 23vPPV vaccination. However, there might still be ongoing hyporesponsiveness to unconjugated serotypes, although we believe this is unlikely. It is likely that the phenomenon is transient, despite the continual exposure of Fijian children to

pneumococcal polysaccharide, because of the high natural carriage rate. Clearly, carriage does not induce or maintain hyporesponsiveness, probably because it does not present pure polysaccharide to the immune system but rather presents polysaccharide along with pneumococcal proteins in a manner similar to conjugate vaccines. However, in a highly vaccinated population with strong herd immunity and no circulating serotypes, it could be speculated that the hyporesponsiveness caused by 23vPPV can persist for a longer period.

To undertake this study, we implemented WHO-recommended pneumococcal immunologic assays (ELISA and OPA), as well as augmenting these methods with a novel B-cell assay. This assay was based on a similar assay developed for meningococcal antigens but was modified to evaluate numbers of memory B cells recognizing pneumococcal serotypes. We were able to demonstrate that this assay identifies immunologic responses after pneumococcal immunization in a manner similar to standard assays. We also believe that it will prove to be a more sensitive marker of long-term immunity, enabling a more precise evaluation of the long-term effect of various pneumococcal vaccination strategies.

The data demonstrating that 23vPPV-vaccinated children have returned to normal immune responses to pneumococcal antigens are compelling and consistent across all serotypes measured and assays used. The carriage data suggest that 23vPPV-vaccinated children might have more susceptibility to carriage of PCV7 vaccine serotypes, although our small sample size makes it difficult to draw conclusions. We have previously shown that the 23vPPV dose at 12 months stimulated very strong antibody responses in PCV7-immunized children¹¹ but that these responses were not associated with any measurable effect on carriage.¹² It is possible that hyporesponsiveness to these serotypes was more prolonged, and although immunologic measures had returned to normal, subtle changes might have persisted, leading to increased susceptibility to carriage. We also did not identify any increased susceptibility to disease in 23vPPV-vaccinated children. Unfortunately, we were only able to identify less than half of the children for the follow-up study, in part because many did not agree to be recontacted and in part because of movement to other areas of Fiji. It is possible that we missed important disease events or even deaths, but Fiji has an excellent health service and very low child mortality; therefore we believe this is very unlikely.

Taken together, our findings have important implications for vaccine policy. The lack of sustained hyporesponsiveness in this population some 5 to 7 years after immunization is reassuring given the serious safety concerns raised by the 12-month 23vPPV dose. However, caution must be applied to the use of 23vPPV vaccines given the demonstrated hyporesponsiveness that occurred within 6 months of vaccination in these children at an age when the risk of pneumococcal disease is greatest. The fact that the Australian government has since replaced the use of 23vPPV with PCV13 as a booster vaccine for all indigenous children and Fiji has introduced a 3+0 PCV10 schedule into their national childhood immunization program in 2012 reinforces the importance of rigorous vaccine programs at a population level to reduce pneumococcal disease burden.

In conclusion, our work has shown that although 23vPPV boosting of Fijian children previously vaccinated with PCV7 resulted in immune hyporesponsiveness, we were not able to identify any evidence of clinical consequences for this, and by

5 years, immune responses to PCV13 were normal. Although our work does not support the use of 23vPPV in children, we have also shown that in those settings in which natural boosting caused by circulating serotypes is high, any negative immunologic effects on vaccinated children are transient, which might be different in a highly vaccinated population.

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Clinical implications: Although this study found no long-term evidence of 23vPPV-induced hyporesponsiveness in children, continued use of 23vPPV in early childhood is not recommended based on the potential increase in disease susceptibility after 23vPPV use.

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METHODS

Study samples

Blood samples were sent to the National Centre for Communicable Disease Reference Laboratory (Mataika House, Suva, Fiji), where they were centrifuged at 1000g for 10 minutes, and the plasma was collected and stored at -80°C until analysis. Freshly isolated PBMCs were collected with Lymphoprep (Axis-Shield, Oslo, Norway) density gradient centrifugation for memory B-cell assays. Nasopharyngeal swabs were placed in 1 mL of skim milk, tryptone, glucose, and glycerol media and then transported to the laboratory in line with WHO recommendations and frozen at -80°C until analysis. All study and laboratory staff were blinded for all assays.

Serotype-specific IgG ELISA

For serotype-specific IgG ELISAs, purified pneumococcal polysaccharides were coated onto medium-binding ELISA plates at 37°C for 5 hours and then stored at 4°C overnight. Serum and control samples were diluted 1:100 in a preabsorption buffer of PBS with 10% (wt/vol) FCS containing cell-wall polysaccharide (CPS; 10 $\mu\text{g/mL}$) and serotype 22F (30 $\mu\text{g/mL}$) to remove nonspecific antibodies and incubated overnight at 4°C . For the serotype 22F ELISA, serum samples were preabsorbed with CPS only. The next day, plates were washed with PBS containing 0.05% (vol/vol) Tween 20 (PBS-T), blocked with PBS/FCS, and incubated at 37°C for 1 hour. The reference standard serum 89-SF (US Food and Drug Administration, Bethesda, Md) was preabsorbed with CPS only because the assigned serotype-specific IgG values are based on ELISA measurement that used preabsorption with CPS but not serotype 22F. After this incubation, serial dilutions of the preabsorbed 89-SF standard, serum samples, and controls were added to the ELISA plates and incubated at 37°C for 2 hours. Plates were then washed with PBS-T, and a horseradish peroxidase-conjugated sheep anti-human IgG was added (1:5000) and incubated at 37°C for 2 hours. After another wash step with PBS-T, the reaction was developed by means of incubation with a 3,3', 5,5'-tetramethylbenzidine substrate solution for 9 minutes and stopped with 1 mol/L phosphoric acid. OD at 450 nm (630-nm reference filter) was measured with a microplate reader. Serotype-specific IgG concentrations for each patient sample were derived from the 89-SF standard values and expressed in micrograms per milliliter.

MOPAs

MOPAs were performed on 8 serotypes (1, 5, 6A, 6B, 14, 18C, 19F, and 23F) on a random sample of 120 children. Serum samples were incubated at 56°C for 30 minutes before making serial dilutions, and 20 μL /well of each dilution was tested in duplicate in round-bottom 96-well plates (Corning, Corning, NY). Eleven 2.3-fold serial dilutions were used for interassay variability experiments, and 8 three-fold dilutions were used for all other experiments. Frozen aliquots of target pneumococci were thawed, washed twice with opsonization buffer B (HBSS with magnesium and calcium, 0.1% gelatin, and 10% FBS), and diluted to approximately 2×10^5 colony-forming units/mL of each serotype for multiplexed assays. For MOPAs, equal volumes of 4 bacterial suspensions in 1 assay group are pooled, and 10 μL of bacterial suspension is added to each well. After 30 minutes of incubation at room temperature, 10 μL of complement and 40 μL of HL60 cells (approximately 4×10^5 cells) were added to each well (HL60 cells washed twice with HBSS before use). Plates were incubated in a tissue culture incubator (37°C in a 5% CO_2 atmosphere) with shaking (mini Orbital Shaker; Bellco Biotechnology, Vineland, NJ) at 100g. After 45 minutes, plates were placed on ice for approximately 12 minutes, and 10 μL of the final reaction mixture was spotted onto 4 different plates of Todd-Hewitt agar supplemented with 0.5% yeast extract. An overlay agar containing one of the 4 antibiotics (selective markers for target bacteria) was added to one of each of the Todd-Hewitt agar plates and incubated overnight at 37°C . The number of bacterial colonies was then enumerated. Results were expressed as opsonization indices (OIs), which

were defined as the interpolated dilutions of serum that kill 50% of bacteria. The lower limit of detection in the assay is 4. The OIs of samples that do not kill 50% of bacteria were reported as 2 for the purposes of analysis. A cutoff of 8 was used as the cutoff for a positive response.

Enumeration of pneumococcus-specific memory B cells

Enumeration of pneumococcus-specific memory B cells was done for 18 of the 23 serotypes in 23vPPV (1, 2, 3, 4, 5, 6B, 7F, 8, 9V, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, and 33F). Freshly isolated PBMCs were resuspended in RPMI-FCS at a concentration of 2×10^6 cells/mL, and 100 μL was added to each well along with 100 μL of an antigen cocktail (*Staphylococcus aureus* Cowan strain—Pansorbin cells [SAC; 1:5000], 2.5 $\mu\text{g/mL}$ CpG, and 83 ng/mL pokeweed mitogen). Plates were incubated at 37°C in a 5% CO_2 atmosphere and 95% humidity for 5 days. At day 5, cells were harvested by means of gentle resuspension and added to a 30-mL tube filled with RPMI-EDTA plus 0.5% FCS (RPMI-FCS). Cells were washed in RPMI-FCS after centrifugation at 800g for 20 minutes and twice after centrifugation at 650g for 15 minutes. After counting with trypan blue, cells were resuspended in RPMI-FCS at a final concentration of 2×10^6 cells/mL for seeding onto antigen-coated ELISpot plates.

Multiscreen hydrophobic polyvinylidene difluoride membrane ELISpot plates were coated with anti-IgG (10 $\mu\text{g/mL}$), tetanus toxoid (5 $\mu\text{g/mL}$), diphtheria toxoid (10 $\mu\text{g/mL}$), or pneumococcal polysaccharides conjugated to methylated human serum albumin at concentrations in the range 10 to 20 $\mu\text{g/mL}$ and were sealed and incubated overnight at 4°C . The following day, ELISpot plates were washed 3 times with PBS and blocked with RPMI-FCS for 30 minutes at 37°C in a 5% CO_2 atmosphere and 95% humidity.

Cultured cells were seeded at 2×10^5 cells/well into the antigen-coated ELISpot plates and incubated overnight at 37°C in a 5% CO_2 atmosphere and 95% humidity. Cells were then washed with PBS-T, and bound IgG was detected after incubation with an alkaline phosphatase-conjugated IgG for 4 hours at room temperature. ELISpot plates were washed with PBS-T 4 times before addition of an alkaline phosphatase substrate solution (nitroblue tetrazolium plus 5-bromo-4-chloro-3-indolylphosphate in dimethyl formamide) to all wells to allow spots to develop. The reaction was stopped with 2 washes in distilled water. Cells were visualized and counted by using an automated ELISpot reader and software. The total frequency of IgG-secreting antibody-forming cells was used as the positive control, and samples with less than 1000 IgG antibody-forming cells/ 10^6 cultured PBMCs were removed from analysis.

Nasopharyngeal carriage measurement

Swab samples were thawed and diluted 1:2 in skim milk, tryptone, glucose, and glycerol media, and 50 μL was inoculated onto Columbia Horse Blood Agar Plates (Oxoid, Thermo Fisher Scientific, Melbourne, Australia) containing 5 $\mu\text{g/mL}$ gentamicin. Plates were incubated at 37°C in a 5% CO_2 atmosphere for 36 to 44 hours. Two randomly selected α -hemolytic colonies plus any additional morphologically distinct α -hemolytic colonies were subcultured, and pneumococcal isolates were identified based on optochin sensitivity, bile solubility testing, and the Phadebact Pneumococcus Test (Boule Diagnostics AB, Huddinge, Sweden). Serotyping was performed by means of latex agglutination, as previously described.^{E1} Ten percent were also serotyped by using a Quellung reaction with specific antisera (Statens Serum Institute, Copenhagen, Denmark). Laboratory staff members were blinded to the group allocation for each isolate.

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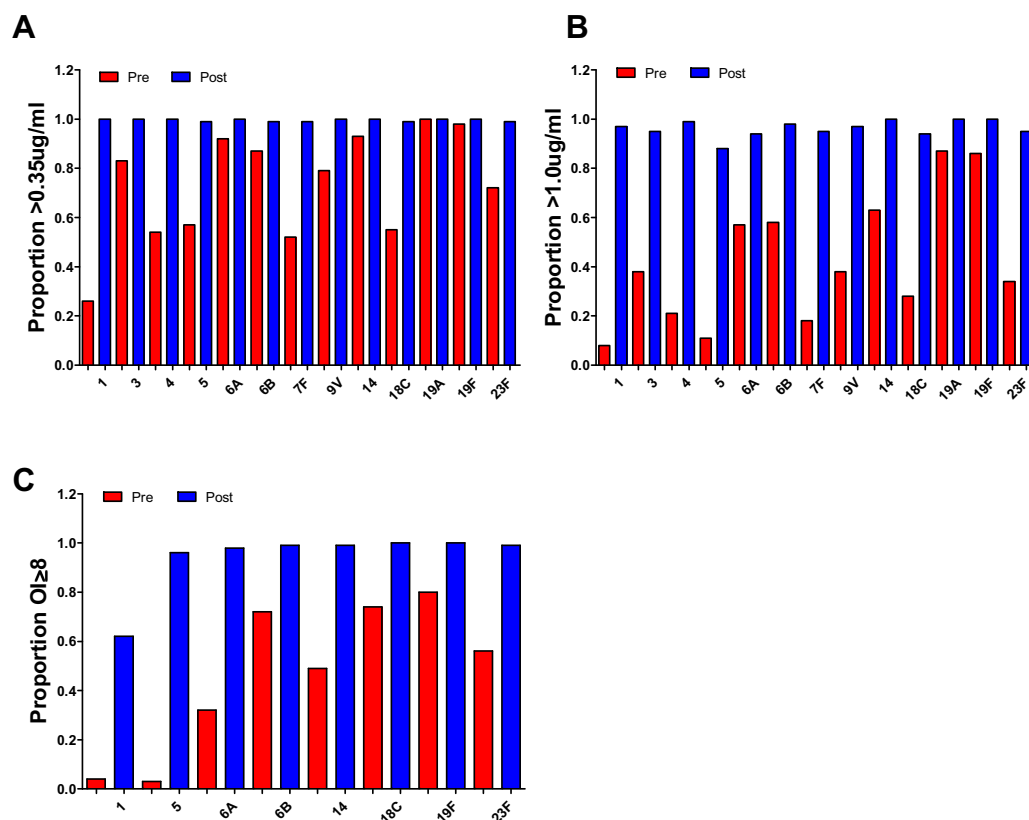


FIG E1. Response to PCV13 immunization in children aged 5 to 7 years. Proportion of children with serotype-specific IgG levels of 0.35 µg/mL or greater (**A**), serotype-specific IgG levels of 1.0 µg/mL or greater (**B**), and opsonophagocytic responses (OIs) of 8 or greater (**C**) are shown before (red bars) and after (blue bars) immunization with PCV13 ($n = 185$ paired subjects). Data were taken from 185 paired samples. **Fig 1, A**, $P < .0001$ for all serotypes except 19A, 19F (both not significant), and 14 ($P = .0002$). **Fig 1, B**, $P < .0001$ for all serotypes except 19A and 19F. **Fig 1, C**, $P < .0001$ for all serotypes.

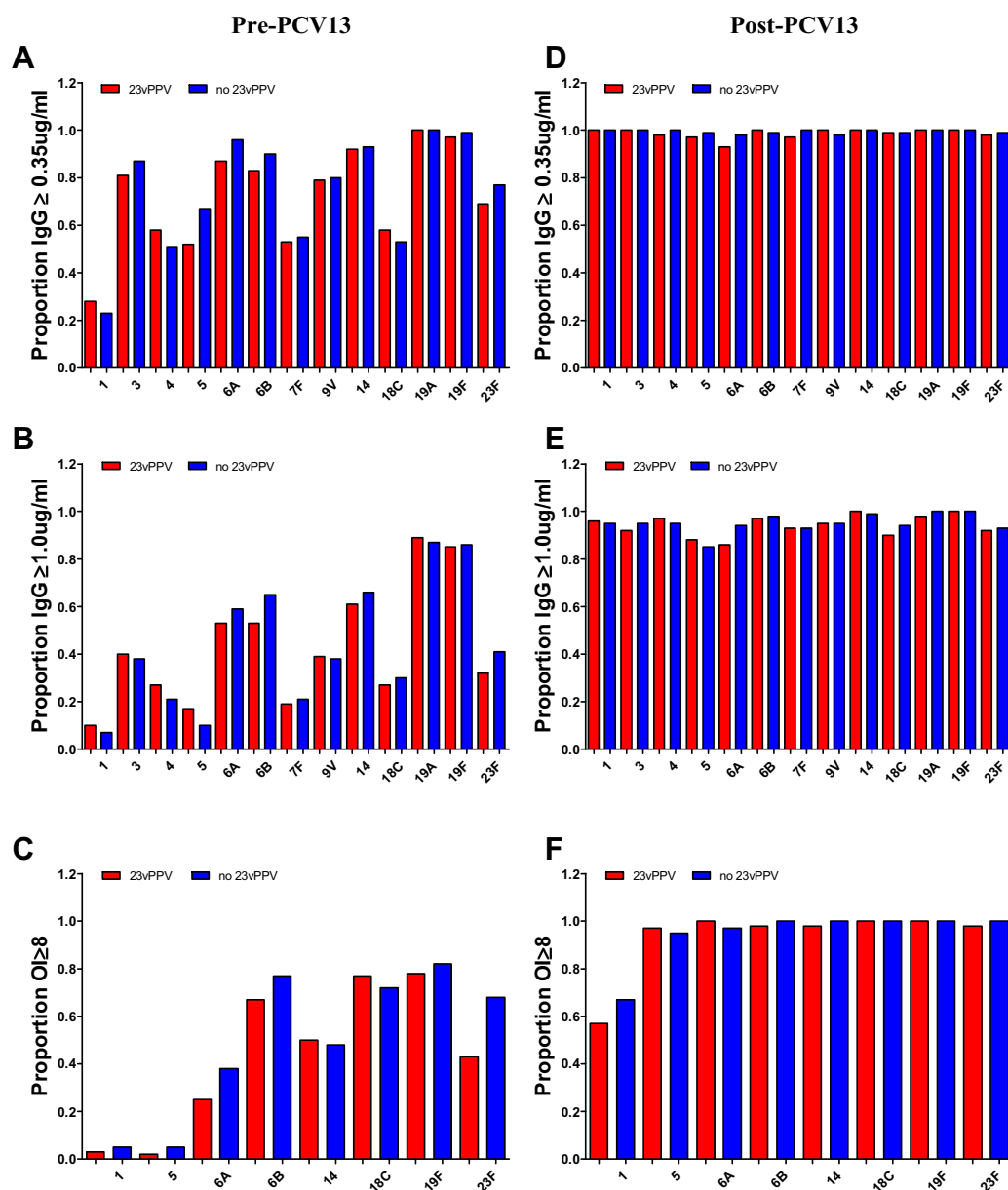


FIG E2. Response to PCV13 immunization in children who did (red bars) or did not (blue bars) receive 23vPPV at 12 months of age. The proportion of children with serotype-specific IgG levels of 0.35 $\mu\text{g/mL}$ or greater, serotype-specific IgG levels of 1.0 $\mu\text{g/mL}$ or greater, and opsonophagocytic responses greater than an OI of 8 are shown before (A-C) and after (D-F) PCV13. For serotype-specific IgG, sample size was 98 children who received 23vPPV and 87 children who did not receive 23vPPV. For opsonophagocytosis, there were 60 children in each group. No significant differences were found, except for serotype 23F in Fig 2, C ($P = .0098$).

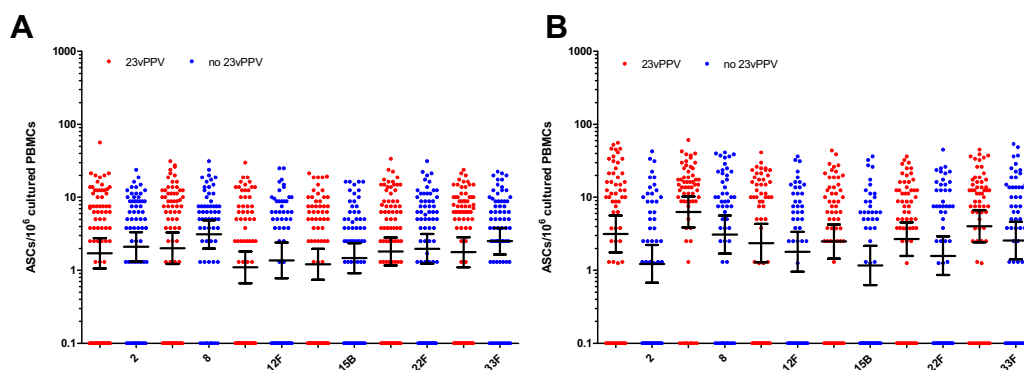


FIG E3. Response to PCV13 immunization in children who did (*red circles*) or did not (*blue circles*) receive 23vPPV at 12 months of age. Enumeration of pneumococcus-specific memory B cells for non-PCV13 serotypes before PCV13 (**A**) and 28 days after PCV13 immunization (**B**). Data are presented as medians $\pm$ interquartile ranges for children who did ($n = 98$) or did not ($n = 87$) receive 23vPPV at 12 months of age. No significant differences were found.

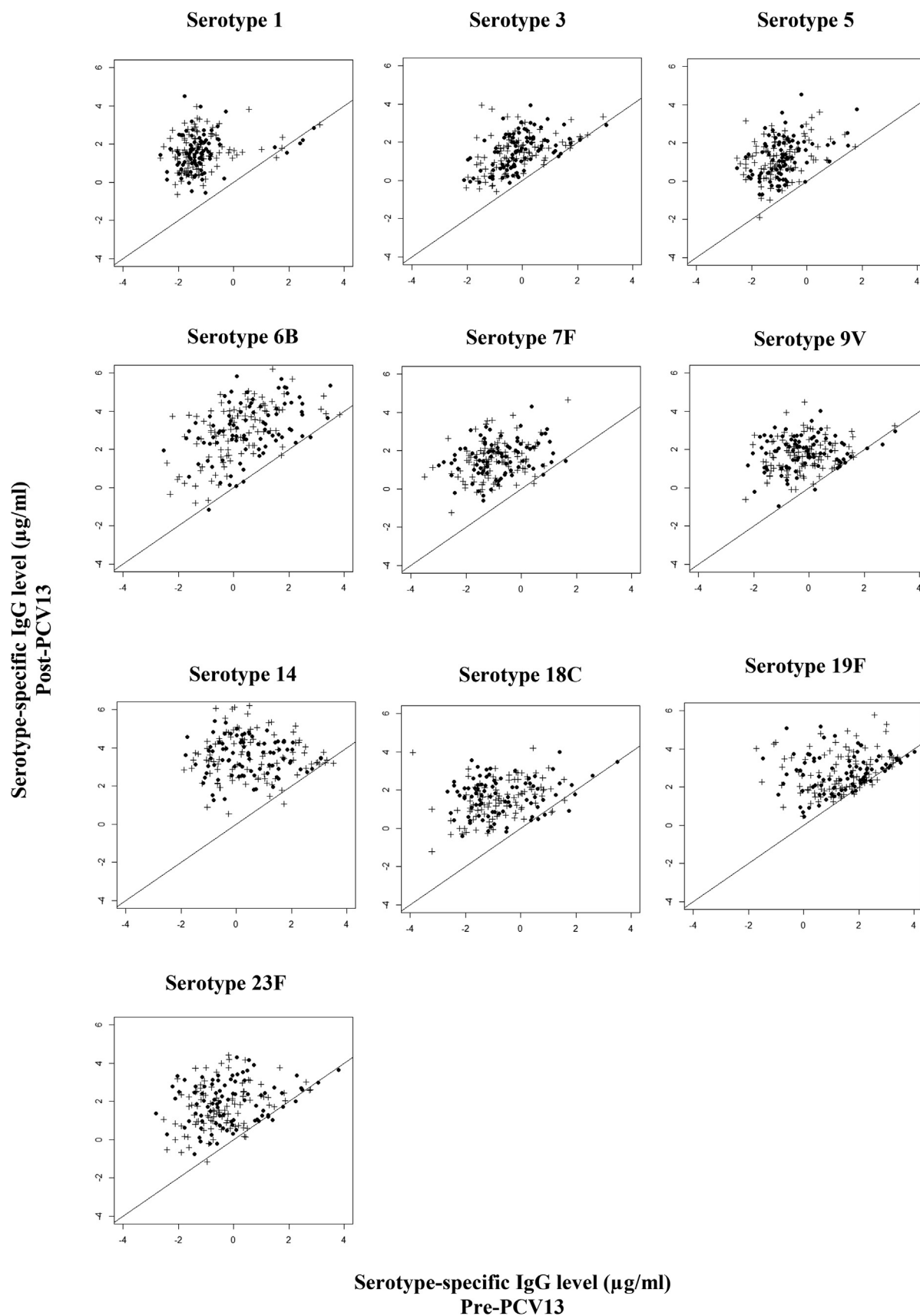


FIG E4. Response to PCV13 immunization in children, now aged 5 to 7 years, who did (*cross*) or did not (*solid circles*) receive 23vPPV at 12 months of age. Children responded similarly to PCV13 serotypes, regardless of 23vPPV receipt. The *diagonal line* represents no change from pre-PCV13 (baseline) levels.

TABLE E1. Serotype-specific IgG levels (in micrograms per milliliter) to all PCV13 and non-PCV13 serotypes included in the 23vPPV vaccine in children who did or did not receive prior 23vPPV at 12 months of age

Serotype	Pre-PCV13		Post-PCV13	
	Prior 23vPPV, GMC (95% CI)	No prior 23vPPV, GMC (95% CI)	Prior 23vPPV, GMC (95% CI)	No prior 23vPPV, GMC (95% CI)
PCV13 serotypes*				
1	0.31 (0.25-0.37)	0.31 (0.25-0.38)	4.80 (3.96-5.83)	4.67 (3.83-5.71)
3	0.83 (0.68-1.02)	0.88 (0.72-1.07)	4.42 (3.65-5.37)	4.48 (3.66-5.47)
4	0.47 (0.38-0.59)	0.45 (0.36-0.57)	8.64 (7.05-10.60)	10.56 (8.43-13.23)
5	0.39 (0.33-0.46)	0.43 (0.36-0.50)	3.39 (2.79-4.13)	3.40 (2.71-4.26)
6B	1.16 (0.90-1.48)	1.72 (1.32-2.24)	18.51 (13.75-24.94)	20.48 (15.14-27.69)
7F	0.37 (0.30-0.45)	0.44 (0.35-0.54)	4.38 (3.58-5.34)	4.96 (4.09-6.02)
9V	0.77 (0.64-0.93)	0.80 (0.64-1.00)	5.51 (4.60-6.61)	6.16 (5.14-7.39)
14	1.85 (1.40-2.44)	1.61 (1.25-2.08)	36.17 (28.44-46.01)	34.33 (28.10-41.93)
18C	0.47 (0.37-0.58)	0.50 (0.38-0.65)	4.61 (3.78-5.63)	5.37 (4.39-6.58)
19A	2.67 (2.23-3.21)	3.03 (2.47-3.73)	9.44 (7.76-11.48)	10.79 (8.85-13.16)
19F	3.55 (2.82-4.48)	4.17 (3.22-5.40)	15.71 (12.69-19.45)	15.79 (12.84-19.40)
23F	0.65 (0.52-0.81)	0.85 (0.65-1.11)	5.65 (4.46-7.15)	6.97 (5.48-8.86)
Non-PCV13 serotypes				
2	0.77 (0.64-0.91)	0.83 (0.69-1.00)	1.10 (0.89-1.37)	1.10 (0.88-1.38)
8	0.61 (0.51-0.75)	0.59 (0.47-0.74)	0.63 (0.51-0.77)	0.64 (0.52-0.80)
9N	0.46 (0.39-0.54)	0.53 (0.43-0.64)	1.74 (1.41-2.15)	1.85 (1.48-2.32)
10A	0.70 (0.56-0.87)	0.78 (0.64-0.96)	0.71 (0.57-0.90)	0.80 (0.66-0.98)
11A	0.51 (0.38-0.69)	0.51 (0.37-0.70)	0.51 (0.38-0.69)	0.55 (0.40-0.77)
12F	0.12 (0.10-0.15)	0.15 (0.12-0.18)	0.13 (0.11-0.16)	0.15 (0.12-0.18)
15B	0.72 (0.60-0.88)	0.72 (0.58-0.88)	1.19 (0.97-1.47)	1.25 (1.02-1.54)
17F	0.30 (0.24-0.39)	0.37 (0.29-0.46)	0.30 (0.23-0.39)	0.38 (0.30-0.47)
20	0.47 (0.36-0.61)	0.47 (0.37-0.59)	0.46 (0.35-0.61)	0.49 (0.38-0.62)
22F	0.79 (0.64-0.98)	0.90 (0.73-1.11)	0.84 (0.68-1.05)	0.95 (0.77-1.18)
33F	0.48 (0.40-0.59)	0.45 (0.37-0.54)	0.51 (0.42-0.63)	0.48 (0.40-0.58)

No significant differences were found for any comparisons.

GMC, Geometric mean concentration.

*Serotype 6A is not included in 23vPPV (n = 98 children who received 23vPPV at 12 months of age and n = 87 children who did not receive 23vPPV at 12 months of age).

TABLE E2. Serotype-specific GMOIs in children who did or did not receive prior 23vPPV at 12 months of age before and after PCV13

Serotype	Pre-PCV13		Post-PCV13	
	Prior 23vPPV, GMOI (95% CI)	No prior 23vPPV, GMOI (95% CI)	Prior 23vPPV, GMOI (95% CI)	No prior 23vPPV, GMOI (95% CI)
1	2.2 (2.0-2.4)	2.3 (2.0-2.8)	11.2 (7.0-17.7)	16.0 (10.1-25.4)
5	2.2 (2.0-2.5)	2.5 (2.1-3.0)	78.3 (54.3-112.8)	77.76 (51.6-117.2)
6A	5.7 (3.4-9.6)	11.2 (6.0-20.7)	1983 (1388-2834)	1359 (863.3-2140)
6B	24.2 (14.2-41.0)	54.8 (30.7-97.7)	1884 (1225-2898)	2240 (1458-3442)
14	13.8 (7.6-25.4)	15.3 (8.7-26.9)	864.2 (573-1303)	1176 (850.9-1626)
18C	52.9 (29.6-94.3)	41.3 (22.2-77.1)	1227 (899.6-1672)	1611 (1257-2065)
19F	44.0 (25.9-74.6)	57.0 (33.3-97.7)	732.5 (525.5-1021)	652.7 (470.3-905.8)
23F	7.4 (5.1-11.0)	25.5 (15.2-42.9)	279.9 (180.6-433.6)	549.8 (399.8-756)

There were 60 children per group who did or did not receive 23vPPV at 12 months of age. No significant differences were found for any comparison.

GMOI, Geometric mean opsonization index.

TABLE E3. Serotype-specific ASCs in children who did or did not receive prior 23vPPV at 12 months of age before and after PCV13 (all serotypes examined)

Serotype	Pre-PCV13		Post-PCV13	
	Prior 23vPPV, median (IQR)	No prior 23vPPV, median (IQR)	Prior 23vPPV, median (IQR)	No prior 23vPPV, median (IQR)
PCV13 serotypes				
1	0.63 (0.43-0.94)	0.93 (0.61-1.41)	15.72 (11.35-21.78)	15.66 (10.63-23.07)
3	1.16 (0.78-1.73)	2.37 (1.56-3.59)	21.14 (15.01-29.77)	19.74 (13.66-28.53)
4	5.15 (3.75-7.07)	7.39 (5.65-9.66)	32.89 (24.76-43.69)	45.86 (36.60-57.45)
5	1.02 (0.67-1.54)	1.18 (0.76-1.82)	9.36 (6.21-14.11)	6.05 (3.44-10.63)
6B	5.81 (4.23-7.97)	8.79 (6.59-11.74)	15.44 (10.86-21.96)	19.18 (13.80-26.64)
7F	1.91 (1.24-2.92)	1.62 (1.03-2.54)	19.35 (13.20-28.36)	12.64 (7.89-20.23)
9V	1.51 (0.97-2.34)	1.40 (0.88-2.22)	17.76 (13.10-24.09)	14.62 (9.34-22.87)
14	4.60 (3.21-6.61)	5.55 (4.08-7.56)	13.52 (10.25-17.82)	12.72 (8.80-18.38)
18C	1.86 (1.19-2.93)	2.37 (1.46-3.85)	25.30 (18.78-34.09)	22.97 (14.13-37.35)
19A	3.21 (2.19-4.71)	5.78 (4.22-7.93)	6.28 (4.41-8.95)	7.65 (5.12-11.43)
19F	2.29 (1.48-3.53)	2.15 (1.36-3.40)	15.46 (10.56-22.65)	10.30 (6.73-15.78)
23F	5.40 (3.96-7.37)	6.24 (4.54-8.58)	21.55 (15.72-29.55)	30.85 (22.25-42.77)
Non-PCV13 serotypes				
2	1.71 (1.06-2.75)	2.09 (1.32-3.32)	3.14 (1.76-5.61)	1.22 (0.67-2.21)
8	2.01 (1.23-3.30)	3.11 (2.00-4.83)	6.31 (3.86-10.29)	3.09 (1.69-5.63)
12F	1.10 (0.66-1.81)	1.37 (0.78-2.40)	2.36 (1.29-4.32)	1.79 (0.96-3.35)
15B	1.21 (0.74-1.96)	1.47 (0.92-2.35)	2.49 (1.45-4.27)	1.16 (0.62-2.15)
22F	1.81 (1.17-2.80)	1.97 (1.23-3.15)	2.68 (1.58-4.53)	1.58 (0.56-2.91)
33F	1.77 (1.10-2.85)	2.50 (1.65-3.80)	4.01 (2.41-6.66)	2.57 (1.42-4.64)

No significant differences were found for all other comparisons.

ASCs, Antibody-secreting cells; *IQR*, interquartile range.

TABLE E4. Proportion of children with serotype-specific IgG levels of 0.35 µg/mL or greater to PCV13 and non-PCV13 serotypes included in the 23vPPV vaccine in children who did or did not receive prior 23vPPV at 12 months of age

Serotype	Pre-PCV13		Post-PCV13	
	Prior 23vPPV, no. (%)	No prior 23vPPV, no. (%)	Prior 23vPPV, no. (%)	No prior 23vPPV, no. (%)
PCV13 serotypes*				
1	28 (29)	21 (24)	98 (100)	87 (100)
3	79 (81)	75 (86)	98 (100)	87 (100)
4	54 (55)	45 (52)	98 (100)	87 (100)
5	49 (50)	57 (66)	97 (99)	87 (100)
6B	82 (84)	79 (91)	98 (100)	86 (99)
7F	50 (51)	46 (53)	97 (99)	87 (100)
9V	79 (81)	68 (78)	98 (100)	87 (100)
14	91 (93)	81 (93)	98 (100)	87 (100)
18C	58 (59)	44 (51)	97 (99)	87 (100)
19A	98 (100)	87 (100)	98 (100)	87 (100)
19F	95 (97)	86 (99)	98 (100)	87 (100)
23F	68 (69)	65 (75)	97 (99)	87 (100)
Non-PCV13 serotypes				
2	81 (83)	73 (84)	87 (89)	76 (87)
8	67 (68)	59 (68)	69 (70)	67 (77)
9N	55 (56)	55 (63)	94 (96)	80 (92)
10A	71 (72)	72 (83)	72 (73)	71 (82)
11A	51 (59)	44 (51)	49 (50)	51 (59)
12F	10 (10)	12 (14)	11 (11)	10 (11)
15B	77 (79)	69 (79)	91 (93)	84 (97)
17F	36 (37)	33 (38)	39 (40)	39 (45)
20	57 (58)	52 (60)	59 (60)	54 (62)
22F	77 (79)	75 (86)	78 (80)	73 (84)
33F	57 (58)	53 (61)	55 (56)	59 (68)

No significant differences were found for any comparison.

*Serotype 6A is not included in 23vPPV (n = 98 children who received 23vPPV and n = 87 children who did not receive 23vPPV).

TABLE E5. Proportion of children with serotype-specific IgG levels of 1.0 µg/mL or greater to PCV13 and non-PCV13 serotypes included in the 23vPPV vaccine in children who did or did not receive prior 23vPPV at 12 months of age

Serotype	Pre-PCV13		Post-PCV13	
	Prior 23vPPV, no. (%)	No prior 23vPPV, no. (%)	Prior 23vPPV, no. (%)	No prior 23vPPV, no. (%)
PCV13 serotypes*				
1	9 (9)	5 (6)	95 (97)	84 (97)
3	38 (39)	32 (37)	91 (93)	84 (97)
4	24 (24)	15 (19)	98 (100)	85 (98)
5	13 (13)	8 (9)	88 (90)	75 (86)
6B	52 (53)	56 (64)	95 (97)	86 (99)
7F	17 (17)	17 (20)	93 (95)	82 (94)
9V	37 (38)	25 (29)	95 (97)	84 (97)
14	60 (61)	56 (64)	98 (100)	87 (100)
18C	26 (27)	25 (29)	89 (91)	84 (97)
19A	85 (87)	76 (87)	98 (100)	87 (100)
19F	84 (86)	75 (86)	98 (100)	87 (100)
23F	30 (31)	33 (38)	92 (94)	83 (95)
Non-PCV13 serotypes				
2	39 (40)	31 (36)	49 (50)	45 (52)
8	27 (28)	22 (25)	27 (28)	23 (26)
9N	14 (14)	33 (38)	67 (68)	63 (72)
10A	30 (31)	36 (41)	37 (38)	36 (41)
11A	32 (33)	26 (30)	29 (30)	28 (32)
12F	3 (3)	3 (3)	4 (4)	3 (3)
15B	33 (34)	29 (33)	53 (54)	45 (52)
17F	17 (17)	15 (19)	18 (18)	16 (18)
20	30 (31)	22 (25)	29 (30)	27 (31)
22F	38 (39)	37 (43)	39 (40)	38 (44)
33F	19 (19)	12 (14)	22 (22)	19 (22)

No significant differences were found for any comparison.

*Serotype 6A is not included in 23vPPV (n = 98 children who received 23vPPV and n = 87 children who did not receive 23vPPV).

TABLE E6. Proportion of children with OIs of 8 or greater in children who did or did not receive prior 23vPPV at 12 months of age before and after PCV13

Serotype	Pre-PCV13		Post-PCV13	
	Prior 23vPPV, no. (%)	No prior 23vPPV, no. (%)	Prior 23vPPV, no. (%)	No prior 23vPPV, no. (%)
1	2 (3)	3 (5)	33 (56)	40 (67)
5	1 (2)	3 (5)	57 (97)	57 (95)
6A	15 (25)	23 (38)	59 (100)	58 (97)
6B	40 (67)	46 (77)	59 (98)	60 (100)
14	30 (50)	29 (48)	59 (100)	60 (100)
18C	46 (77)	43 (72)	59 (100)	60 (100)
19F	47 (78)	49 (82)	59 (100)	60 (100)
23F	26 (43)	41 (68)*	59 (98)	60 (100)

There were 60 children per group who did or did not receive 23vPPV at 12 months of age. No significant differences were found for all other comparisons.

* $P = .0058$ between children who did or did not receive 23vPPV before PCV13.