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Opsonophagocytic Activity Following a Reduced Dose 7-valent Pneumococcal Conjugate Vaccine Infant Primary Series and 23-valent Pneumococcal Polysaccharide Vaccine at 12 Months of Age

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Abstract

Opsonophagocytic activity (OPA) was measured following reduced infant doses of 7-valent pneumococcal conjugate vaccine (PCV-7) with or without 23-valent pneumococcal polysaccharide vaccine (PPV-23) at 12 months, and subsequent re-exposure to a small dose of pneumococcal polysaccharide antigens (mPPS) at 17 months. Fijian infants were randomized to receive 0, 1, 2, or 3 PCV-7 doses. Half received PPV-23 at 12 months and all received mPPS at 17 months. OPA was performed on up to 14 serotypes. Three and 2 PCV-7 doses resulted in similar OPA for most PCV-7 serotypes up to 9 months and for half of the serotypes at 12 months. A single dose improved OPA compared with the unvaccinated group. PPV-23 significantly improved OPA for all serotypes tested but in general, was associated with diminished responses following re-challenge.

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Keywords

pneumococcal vaccination; opsonophagocytic activity

1. Introduction

Streptococcus pneumoniae is the leading vaccine preventable cause of serious infection in infants [1]. A recent review estimated that over 14 million episodes of serious pneumococcal disease occurred worldwide in the year 2000, with over 800,000 deaths in children under 5 years or age [2]. Pneumococcal capsular polysaccharide is a major virulence factor of *S. pneumoniae* and this capsule resists phagocytosis by the host immune cells [3,4]. The primary mechanism of protection in the host against invasive pneumococcal disease (IPD) is mediated by the presence of opsonophagocytic antibodies [5]. Antibodies to pneumococcal polysaccharide offer protection by opsonizing pneumococci and activating complement thereby promoting phagocytosis of pneumococci. The ability of anti-pneumococcal antibodies to induce phagocytosis and killing is believed to be a surrogate for protection. As both antibody concentration and avidity contribute to opsonophagocytic activity (OPA) and confer protection in a mice challenge models [6–10], OPA is considered to be the best marker of vaccine efficacy. While it is recommended that antibody concentration, as measured by a standardized enzyme immunoassay (ELISA), should be the primary measure when licensing new pneumococcal conjugate vaccines [9], OPA has been accepted as a necessary additional measure and the reference method for measuring the protective capacity of pneumococcal antibodies [11]. A recent study has shown that only OPA can be a surrogate for immune protection for the vaccine related serotype 19A [12]. Moreover, OPA levels have been found to be more meaningful than ELISA titers with respect to the prediction of protective efficacy against acute otitis media (AOM) [13].

The aim of this study in Fiji was to find an optimal pneumococcal vaccination strategy for resource poor countries in terms of serotype coverage, flexibility, and affordability. We undertook a Phase II vaccine trial in Fiji to document the safety, immunogenicity and impact on pneumococcal carriage of various pneumococcal vaccination regimens combining one, 2, or 3 doses of PCV-7 in infancy. To broaden serotype coverage, the additional benefit of a booster of 23-valent pneumococcal polysaccharide vaccine (PPV-23) at 12 months of age was also assessed. To address the concerns of immunological hyporesponsiveness to PPV-23 in young children following re-challenge, the immunological response at 17 months of age to a small challenge dose of 20% of the PPV-23 (mPPS) was evaluated.

Our findings to date include evidence of similar immunogenicity following either a 2 or 3 dose PCV-7 primary series, as measured by IgG antibody concentrations to capsular polysaccharides by ELISA [14]. We also found that a single PCV-7 dose would offer protection in the first 12 months of life for many serotypes [14]. The one or 2 dose PCV-7 schedules induced immunologic memory [15]. There were significant responses for the non-PCV-7 serotypes included in PPV-23 booster which persisted for at least 5 months following vaccination [15]. Memory responses following PPV-23 were most profound for children who had received only a single dose of PCV-7 previously, compared with the 2 or 3 dose groups [15]. However despite higher antibody concentrations at 17 months in children who had received PPV-23 at 12 months, the response to a re-challenge was poor to all 23 serotypes compared to children who did not receive the 12 month PPV-23 indicating immunological hyporesponsiveness [16]. In this manuscript we present the functional antibody responses to these different pneumococcal vaccination schedules, as measured by OPA.

2. Materials and Methods

2.1 Study participants

The study was a single blind, open-label randomized Phase II vaccine trial undertaken in Suva, Fiji. Healthy infants aged between 6 and 8 weeks were eligible for enrolment. Details of the selection criteria and the randomization procedure have been reported elsewhere [14–16].

The study was conducted and monitored according to Good Clinical Practice. Monitoring was undertaken by Pharmaceutical Product Development Inc. It was approved by the Fiji National Research Ethics Review Committee and the University of Melbourne Human Research Ethics Committee.

2.2 Study procedures and vaccines

Infants were stratified by ethnicity and randomized into one of 8 groups to receive 0, 1, 2 or 3 doses of PCV-7 in infancy with or without a PPV-23 booster at 12 months of age. Infants received the 7-valent CRM₁₉₇ protein-polysaccharide conjugate vaccine containing polysaccharide antigen from pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F, 23F (*Prevenar*TM, Wyeth Vaccines). The vaccine contains 2 µg per serotype, except serotype 6B which contains 4 µg. The 3 dose group received PCV-7 at 6 weeks, 10 weeks, and 14 weeks of age. The 2 dose group received PCV-7 at 6 and 14 weeks of age and the single dose group received PCV-7 at 14 weeks of age. Routine vaccines, with combined diphtheria-tetanus-pertussis-Hepatitis B and *Hemophilus influenzae* type b (*Hiberix*TM mixed with *Tritanrix*TM-*HepB*TM, GlaxoSmithKline) and oral polio were given at 6, 10, and 14 weeks of age. Half the children in all primary series groups were randomized to receive a full dose (0.5mL) of PPV-23 (*Pneumovax*TM, Merck & Co., Inc., which consists of a purified mixture of 25µg of capsular polysaccharide from 23 pneumococcal serotypes: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33F) at 12 months of age in the left upper deltoid muscle. All children received Measles-Rubella vaccine at 12 months of age in the right upper deltoid muscle. All children received 0.1mL of the PPV-23 (mPPS) at 17 months of age. The children randomized to receive 0 or one PCV-7 dose in infancy, had a single dose of PCV-7 administered at 2 years of age.

2.3 Laboratory assays

All children except the group randomized to receive no PCV-7 or PPV-23 in infancy had blood drawn at 18 weeks and 12 months of age. Those children who were randomized to not receive the 12 month PPV-23 additionally had blood drawn at 9 months of age. Children who received the 12 month PPV-23 additionally had blood drawn 14 days post-PPV-23. All children had blood drawn before and 4 weeks following mPPS. Serum was separated from blood by centrifugation at the health centre, kept chilled, and transported to the Colonial War Memorial Hospital laboratory, Suva, where it was divided into aliquots and stored at –70°C on the same day, until transported to the Pneumococcal Laboratory, Murdoch Childrens Research Institute, Melbourne on dry ice for analysis, then to the Bacterial Respiratory Pathogen Reference Laboratory, University of Alabama at Birmingham, USA for OPA analysis.

Opsonophagocytic assays were performed for 8 serotypes (serotypes 1, 4, 5, 6B, 9V, 14, 18C, and 23F) at each blood draw and on 6 additional serotypes (3, 6A, 6C, 7F, 19A, 19F) for the pre- and post-mPPS blood draws using a fourfold multiplexed OPA (MOPA) [17]. In brief, all serum samples were incubated at 56°C for 30 min before serial dilutions. Frozen aliquots of target pneumococci were thawed, washed twice with opsonization buffer B (Hanks' balanced salt solution [HBSS] with Mg and Ca, 0.1% gelatin, and 10% FBS) by

centrifugation (12,000 X g, 2 min), and diluted to the proper bacterial density ($\sim 5 \times 10^4$ CFU/ml for each of the 4 serotypes). Ten microliters of bacterial suspension was added to each well. After 30 min of incubation at room temperature, 10 μ l of complement and 40 μ l of HL60 cells were added to each well. HL60 cells were washed twice before use with HBSS by centrifugation (350 X g, 5 min), and 4×10^5 cells were added to each well. Plates were incubated in a tissue culture incubator (37°C, 5% CO₂) with shaking (mini orbital shaker; Bellco Biotechnology, Vineland, NJ) at 700 rpm. After a 45-min incubation, plates were placed on ice for 10 to 15 min and an aliquot of the final reaction mixture (10 μ l) was spotted onto four different Todd-Hewitt broth–yeast extract (0.5%) agar plates [18]. After application of an overlay agar containing one of the four antibiotics to each Todd-Hewitt broth–yeast extract (0.5%) agar plate and overnight incubation at 37°C, the number of bacterial colonies in the agar plates was enumerated [18]. The MOPA results are expressed as opsonization indices (OIs). An OI is defined as the interpolated dilution of serum that killed 50% of bacteria.

Serum anti-pneumococcal antibody concentrations to each of the serotypes were measured by the third generation ELISA which included a pre-absorption step with 22F polysaccharide to increase the specificity of the assay. The details have been described elsewhere [14–16].

2.4 Statistical analysis

The results presented are based on secondary analyses nested within the main trial. All data collection forms were monitored prior to double data entry. Cleaned data were exported to Stata version 9.0 (Stata Corporation, College Station, Texas) for analysis. In the MOPA, the lowest dilution of serum tested in the assay is 4. The OIs of samples that did not kill 50% of bacteria were reported as “2” for analysis purposes. The threshold of the assay was set at 8 [13]. Pair-wise comparisons of proportions with OIs ≥ 8 were performed using exact McNemar’s test. Comparisons of the proportion of infants with OIs ≥ 8 between groups were performed using Fisher’s exact test. Serotype-specific OIs were natural log transformed to calculate geometric mean titers (GMT). Simple and multi-variable regression analyses were undertaken to adjust for both the pre-mPPS OPA titer for all 23 serotypes, and the number of PCV-7 doses administered for all 7 PCV-7 serotypes. We defined hyporesponsiveness to a particular serotype as a significantly lower OPA titer observed post-mPPS, in the 12 month PPV-23 group compared to the no 12 month PPV-23 group, controlling for pre-mPPS OPA titer levels, using multivariable regression analysis. Serotype-specific antibody concentrations by ELISA were log (base e) transformed to calculate geometric mean concentrations (GMC). Serotype-specific IgG GMC are presented but analyses have been reported in detail elsewhere [14–16]. PCV-7 serotypes were classified as serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. All other serotypes were classified as non-PCV-7 serotypes. Due to the multiple comparisons, a p-value of <0.01 was considered statistically significant.

3. Results

There were 552 children enrolled in the study. Characteristics and the number of children randomized to the 8 groups have been reported elsewhere [16,19]. There were 90 (16.3%) withdrawals. No participants were withdrawn due to an adverse event attributable to any of the vaccines.

3.1 PCV-7 serotypes

One month following the PCV-7 primary series there were no significant differences between the 3 and 2 PCV-7 dose groups in the proportion of infants with OI ≥ 8 for 4 of 6

PCV-7 serotypes (Table 1). OPA GMT following 2 PCV doses were 3 to 10-fold lower for serotypes 23F and 6B respectively compared with a 3 dose schedule, with serotype 14 showing a similar trend. A single PCV-7 dose was inferior to 3 doses but significantly increased the proportion of infants with $\text{OI} \geq 8$ compared with the unvaccinated group for all serotypes except 6B.

At 9 months of age there were no significant differences between the 3 and 2 PCV-7 dose groups in the proportion of infants with $\text{OI} \geq 8$ for any PCV-7 serotype except serotype 23F (Table 1). However there was a trend for higher OPA GMT in the 3 dose group compared to the 2 dose group. The proportion of infants with $\text{OI} \geq 8$ was significantly higher in the 3 dose group compared with the single dose group for all serotypes except serotype 4.

At 12 months of age the proportion of infants with $\text{OI} \geq 8$ were significantly higher in the 3 dose group compared to the 2 dose group for 3 of 6 PCV-7 serotypes (6B, 18C, 23F) with approximately 24% fewer children in the 2 dose group having an $\text{OI} \geq 8$ for serotype 6B compared to the 3 dose group (Table 1). There was a trend for higher OPA GMT for serotype 14 in the 3 PCV dose group compared to the 2 PCV dose group. The proportion of infants with $\text{OI} \geq 8$ was significantly higher for all serotypes in the 3 PCV-7 dose group compared with the single dose (except serotype 4) and the unvaccinated groups. A single PCV-7 dose significantly increased the proportion of infants with $\text{OI} \geq 8$ compared to the unvaccinated group for all serotypes except 6B.

Two weeks following the PPV-23 at 12 months of age there were significant increases in the proportion of children with $\text{OI} \geq 8$ for all PCV-7 serotypes for all PCV-7 dosage groups. There were no significant differences between the 3 and 2 PCV-7 dose groups, and the 3 versus single PCV-7 dose groups in the proportion of children with $\text{OI} \geq 8$ for any PCV-7 serotype (Table 2). However there was a trend for higher OPA GMT in the 3 dose groups compared with the 2 dose group for serotypes 6B, 14, and 23F. The proportion of children with $\text{OI} \geq 8$ was significantly higher for all PCV-7 serotypes in the 3 dose group compared with those children who had not received PCV-7. A single PCV-7 dose significantly increased the proportion of children with $\text{OI} \geq 8$ compared to the unvaccinated group for all PCV-7 serotypes.

At 17 months of age, the proportion of children with $\text{OI} \geq 8$ for the 3 and 2 PCV-7 dose groups who had received the PPV-23 at 12 months continued to be significantly higher for all PCV-7 serotypes compared with the corresponding PCV-7 group who had not received the 12 month PPV-23, except for serotype 14 and 23F (Table 3). For the single dose group who had received the 12 month PPV-23, there were significantly higher proportions of children with $\text{OI} \geq 8$ for 5 of 7 PCV-7 serotypes compared with the single dose group who had not received the 12 month PPV-23. For the groups that had received no PCV-7 there were no significant differences in the proportion of children with $\text{OI} \geq 8$ for any PCV-7 serotype except serotype 4 which was significantly higher in the group that had received the 12 month PPV-23.

At 18 months of age, one month post-mPPS, there were no significant differences in the proportion of children with $\text{OI} \geq 8$ for any PCV-7 serotype comparing the 3, 2, or single PCV-7 dose groups who had also received the 12 month PPV-23 compared with the corresponding PCV-7 group who had not received the 12 month PPV-23. For example, in the 3 PCV-7 groups, 100% of children who did not receive PPV-23 at 12 months had an $\text{OI} \geq 8$ for serotype 4, compared with 98% who did receive PPV-23. The proportion of children with $\text{OI} \geq 8$ was significantly higher for 3 of the PCV-7 serotypes in the group that had received no PCV-7 in infancy or PPV-23 at 12 months compared with the group that had received no PCV-7 and the 12 month PPV-23.

To examine the effect of PPV-23 at 12 months and the number of PCV-7 doses in early infancy, multiple variable regression was undertaken. Prior to adjusting for the pre-existing OPA titer, the post-mPPS OPA titers were significantly lower for all serotypes (except 7F) in the PPV-23 group compared with the group that had not received the 12 month PPV-23. To assess whether the poor response to mPPS in the 12 month PPV-23 recipients was due to higher pre-mPPS OPA titers, adjustment was made for the pre-existing OPA titer. At any given pre-OPA titer, PPV-23 negatively affected the response to mPPS for all serotypes (examples using serotypes 4 and 6B are shown in Figure 1) except serotypes 1 and 5. At any given pre-mPPS OPA titer against serotype 4, most children who had received PPV-23 did not change titer level after re-exposure, as indicated by falling along the 45 degree line, whilst most children who did not receive PPV-23 at 12 months had an increase in OPA titer post-re-exposure. For serotypes 1 and 5, exposure to PPV-23 was not associated with a lower response to mPPS after adjusting for pre-existing OPA titer. For the PCV-7 serotypes, the impact of PPV-23 on the mPPS response was not affected by the number of PCV-7 doses received in the primary series (details not shown).

3.2 Non-PCV-7 and PCV-7 related serotypes

Following the 12 month PPV-23, there were significant increases in the proportion of children with OI ≥ 8 for the 2 serotypes (1 and 5) tested (Table 4). At 17 months of age (prior to mPPS) there were significantly higher proportions of children with OI ≥ 8 for 3 of 7 non-PCV-7 serotypes (3, 5, 7F) in those that had received the 12 month PPV-23 compared with those that had not (Table 5). One month following mPPS, there were significantly higher proportions of children with OI ≥ 8 for 4 of 7 non-PCV-7 serotypes (1, 5, 6A, 6C) in those that had not received the PPV-23 compared with those that had.

4. Discussion

This is the first randomized controlled trial of reduced pneumococcal dose schedules evaluating the OPA of all PCV-7 and many non-PCV-7 serotypes post PPV-23 booster. This study has shown that despite no significant difference found between many of the PCV-7 serotypes comparing the 3 PCV-7 dose group to the 2 dose group, a trend for higher OPA GMT were found for many of the serotypes. A significant difference may not have been found due to the small sample size. These findings are similar to the results of serotype-specific antibody concentrations measured by ELISA [14,20]. Despite the OPA being significantly lower for all PCV-7 serotypes following a single PCV-7 dose compared with the 3 doses, a single dose clearly produces substantial functional antibodies compared with the unvaccinated control group. The response to a single PCV-7 response may have been enhanced by the co-administration of Diphtheria-Tetanus-whole cell Pertussis (DTwP) due to its potential adjuvant effect [14,21] and the groups that received one or 2 doses of PCV-7 may have had augmented responses to their 14 week dose of PCV-7 as a result of extra doses of DTwP [14]. Whilst there are no other single PCV-7 dose studies for comparison our OPA findings largely replicate to the results of ELISA antibody concentrations post primary series [14].

The PPV-23 given at 12 months significantly improves the OPA for all PCV-7 serotypes tested even for the group who had not previously been primed with PCV-7. Moreover, 2 weeks following the 12 month PPV-23 there were no significant differences in OPA for all serotypes between the single PCV-7 dose group compared with the 3 dose group. In contrast, OPA was significantly higher for all serotypes comparing the single dose group and the group that had received no prior PCV-7 indicating that priming even with a single PCV-7 dose can produce better OPA responses than PPV-23 alone. These findings are similar to the results of serotype-specific antibody concentrations measured by ELISA

whereby memory responses following PPV-23 were most profound for the single PCV-7 dose group compared with the 2 or 3 dose groups [15].

The OPA continued to be significantly higher at 17 months of age for all PCV-7 serotypes in those groups that had received the PPV-23 at 12 months compared to the groups that had not. Other combined pneumococcal conjugate vaccine/PPV-23 studies have found inconsistent OPA results post booster. A 3 dose infant 11-valent pneumococcal conjugate vaccine followed by PPV-23 booster in Finnish children resulted in antibodies of greater OPA for vaccine serotypes than those children given the homologous conjugate booster [22]. However no difference was observed between groups of Israeli children vaccinated with the pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine (PncOMPC) and either the homologous or polysaccharide booster [23]. In a randomized controlled trial of 4 doses of the 11-valent pneumococcal protein D conjugate vaccine (PD conjugate vaccine) followed by a PPV-23 dose in the fourth year of life in Czech children, vigorous OPA responses were found for all of the 11 vaccine serotypes tested post PPV-23 for both primed and unprimed children [13]. However, similar to our findings, the magnitude of the response was significantly higher in primed children for most of the serotypes [13]. These differences may be due to differences in vaccines (GSK versus Merck Inc.) and/or populations.

For the non-PCV-7 serotypes, the PPV-23 at 12 months significantly improved the OPA for the 2 serotypes tested (serotypes 1 and 5). In the aforementioned study in Czech children, the strongest response post PPV-23 was found for serotype 1 (which is contained in both the PD conjugate vaccine and PPV-23) and the weakest responses were for those serotypes that maintained the higher antibody levels prior to PPV-23 [13].

Serotype 6A is a vaccine related serotype as it differs only slightly in capsular structure and can cross react with antibodies to 6B. Despite a significant reduction in 6A IPD in vaccinated children in the US, herd immunity has not been evident amongst adults [24,25]. However this lack of effect despite cross reactivity with 6B may be explained by the discovery of 6C which was originally typed as “6A” [26]. Vaccination with 6B provides some level of cross immunogenicity against 6C although the level of cross immunogenicity is much less than that of 6A. In the pre-mPPS samples, both groups showed some cross immunogenicity against both 6A and 6C. However, this data is difficult to interpret as the absolute responses are low, presumably due to the extended interval between the last vaccination and sample collection. In the group that did not receive the 12 month PPV-23, there is a significant difference in the percentage of samples with OIs ≥ 8 , again suggesting hyporesponsiveness.

For serotype 19A, the OPA titers were similar in both groups at 17 months of age, suggesting cross- opsonization from PCV-7 serotype 19F. There are conflicting data regarding whether 19F provides cross-opsonization with 19A. In the Czech study, a fourth dose of PD conjugate vaccine (which does not contain serotype 19A) increased the OPA response to 19A (but 19A is in PPV-23), again suggesting cross-opsonization with serotype 19F [13]. In contrast, another study found PCV-7 to elicit antibodies binding to 19A although the majority of these were non-opsonic and thereby suggested that PCV-7 would provide little immunoprotection against 19A if it were estimated by OPA and not ELISA [12,27]. However a recent epidemiological study pooling data from a number of studies found modest protection against 19A IPD and AOM [28]. There is data suggesting another possible explanation for the apparent cross-opsonization of 19A, being the “Dob1” epitope, which is common to both 6B and 19A but not 19F [29]. This cross- opsonization may be population, vaccination schedule, and/or vaccine-dependent.

The lack of response to the re-challenge dose shown by the groups that had received prior PPV-23 at 12 months indicates hyporesponsiveness. We have shown previously that hyporesponsiveness, as demonstrated by a poor response to a small PPV-23 re-challenge dose (mPPS) by ELISA, occurs irrespective of pre-existing antibody level for all 23 serotypes [16]. This finding has been confirmed in this study by the attenuated OPA responses to re-challenge in the PPV-23 group which were not related to the pre-existing OPA level (except for serotypes 1 and 5). This attenuated response may be due to the depletion of the memory B cell pool [25,26], with subsequent challenge with PPV-23 resulting in immune hyporesponsiveness. In this study we did not find any adverse clinical consequences from this immunological finding, although the study was not designed to evaluate this effect. For Australian Indigenous children, until recently the routine immunisation schedule consisted of 3 infant doses of PCV-7 followed by a PPV-23 booster at 18 months of age. In a recent retrospective cohort study of Indigenous children in the Northern Territory, Australia, the findings suggested an increased risk of hospitalized acute lower respiratory infections after pneumococcal vaccination particularly after receipt of the PPV-23 [30]. In contrast, results from Western Australia using a population based data linkage system, found significant reductions in acute lower respiratory tract infections in Indigenous children [31].

The clinical relevance of immunological findings from vaccine trials is obviously best demonstrated in efficacy studies. In our study there are no pneumococcal disease endpoints to evaluate the clinical relevance of our immunological findings. However, separately we have evaluated the effect on nasopharyngeal (NP) carriage. We found a trend for a PCV-7 dose effect on VT NP carriage with less VT carriage in the 3 PCV-7 group (Russell FM et al. submitted for publication). A single PCV-7 dose resulted in some initial reduction in PCV-7 serotype carriage which provides supportive evidence that one dose may offer some early protection from IPD. The addition of PPV-23 at 12 months of age had no significant impact on carriage (but a trend for higher VT NP carriage), despite the substantial boosts in antibody levels observed [15] and despite better OPA (as demonstrated in this study) and better avidity (Russell FM et al. submitted for publication).

Vaccine responses are known to be serotype-specific. A recent study in the United Kingdom evaluating the immunogenicity of reduced dose PCV-7 schedules found a 2 dose schedule given at 2 and 4 months of age had poor antibody responses to 6B [37]. The OPA for serotype 6B suggested that a lower antibody concentration of ~0.2 g/mL rather than 0.35 g/mL, correlated better with 6B OPA [37]. In our study, 68% of children following 2 PCV-7 doses had functional antibodies post primary series which is similar to that found in a study following 3 doses of 11-valent mixed carrier pneumococcal conjugate vaccine given at 2, 4, and 6 months to Israeli and Finnish children [38] and is higher than that found in Filipino infants using the same mixed carrier vaccine (43%) [39]. In contrast to the UK study, the Czech study, using PD conjugate vaccine, found 6B to have one of the highest OPA responses post primary series [13].

Another difficulty with the assay is the conflicting results found between the correlation of ELISA and OPA. The addition of pre-absorption not only with cell wall polysaccharide but also with pneumococcal 22F capsular polysaccharide, has not only improved ELISA specificity [40,41] but has increased the correlation between anti-capsular polysaccharide antibody concentration and OPA [11,41]. There is evidence of a low correlation between antibody concentration measured by ELISA and the measurement of functional antibodies especially in the sera of non-vaccinated individuals. Several studies have shown the presence of naturally acquired cross-reactive antibodies which are prevalent in sera of infants and adults suggesting that pneumococcal polysaccharides contain some non-protective cross-reactive epitopes or impurities, other than cell wall polysaccharide, that are

common to many types of pneumococcal polysaccharide [42]. In general, the correlation between antibody concentration and OPA seems to be good in infants who have been immunized with conjugate vaccines [36,43]. Correlates have been high for vaccine serotypes, but lower for cross-reacting serotypes like 6A and 19A [27,44]. Our findings seem to indicate that there appears to be a reasonable correlation between the OPA and ELISA findings, but this will be further explored in a separate analysis.

In summary, this study has shown that 3 PCV-7 doses in infancy have similar OPA compared with the 2 PCV-7 dose group up to 9 months for most of the PCV-7 serotypes except serotypes 6B and 23F. A single dose elicits OPA for all serotypes compared to the unvaccinated group. The PPV-23 significantly increases OPA for all serotypes tested. However, the poor response to re-challenge in those that had received prior PPV-23 suggests hyporesponsiveness.

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References

1. The WHO Young Infants Study Group. Conclusions from the WHO multicenter study of serious infections in young infants. *Pediatr Infect Dis J*. 1999 Oct; 18(10 Suppl):S32–4. [PubMed: 10530571]
2. O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. *Lancet*. 2009 Sep 12; 374(9693):893–902. [PubMed: 19748398]
3. Bruyn GA, Zegers BJ, van Furth R. Mechanisms of host defense against infection with *Streptococcus pneumoniae*. *Clin Infect Dis*. 1992 Jan; 14(1):251–62. [PubMed: 1571441]
4. Austrian R. Some observations on the pneumococcus and on the current status of pneumococcal disease and its prevention. *Rev Infect Dis*. 1981 Mar-Apr; 3(Suppl):S1–17. [PubMed: 7025155]
5. Tuomanen EI, Austrian R, Masure HR. Pathogenesis of pneumococcal infection. *N Engl J Med*. 1995 May 11; 332(19):1280–4. [PubMed: 7708073]
6. Usinger WR, Lucas AH. Avidity as a determinant of the protective efficacy of human antibodies to pneumococcal capsular polysaccharides. *Infect Immun*. 1999 May; 67(5):2366–70. [PubMed: 10225896]
7. Sun Y, Hwang Y, Nahm MH. Avidity, potency, and cross-reactivity of monoclonal antibodies to pneumococcal capsular polysaccharide serotype 6B. *Infect Immun*. 2001 Jan; 69(1):336–44. [PubMed: 11119522]
8. Romero-Steiner S, Musher DM, Cetron MS, Pais LB, Groover JE, Fiore AE, et al. Reduction in functional antibody activity against *Streptococcus pneumoniae* in vaccinated elderly individuals highly correlates with decreased IgG antibody avidity. *Clin Infect Dis*. 1999 Aug; 29(2):281–8. [PubMed: 10476727]
9. Jodar L, Butler J, Carlone G, Dagan R, Goldblatt D, Kayhty H, et al. Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. *Vaccine*. 2003 Jul 4; 21(23):3265–72. [PubMed: 12804857]
10. Anttila M, Voutilainen M, Jantti V, Eskola J, Kayhty H. Contribution of serotype-specific IgG concentration, IgG subclasses and relative antibody avidity to opsonophagocytic activity against *Streptococcus pneumoniae*. *Clin Exp Immunol*. 1999 Dec; 118(3):402–7. [PubMed: 10594558]

11. Romero-Steiner S, Frasch CE, Carlone G, Fleck RA, Goldblatt D, Nahm MH. Use of opsonophagocytosis for serological evaluation of pneumococcal vaccines. *Clin Vaccine Immunol.* 2006 Feb; 13(2):165–9. [PubMed: 16467321]
12. Lee H, Nahm MH, Burton R, Kim KH. Immune response in infants to the heptavalent pneumococcal conjugate vaccine against vaccine-related serotypes 6A and 19A. *Clin Vaccine Immunol.* 2009 Mar; 16(3):376–81. [PubMed: 19144787]
13. Schuerman L, Prymula R, Henckaerts I, Poolman J. ELISA IgG concentrations and opsonophagocytic activity following pneumococcal protein D conjugate vaccination and relationship to efficacy against acute otitis media. *Vaccine.* 2007 Mar 1; 25(11):1962–8. [PubMed: 17258357]
14. Russell FM, Balloch A, Tang ML, Carapetis JR, Licciardi P, Nelson J, et al. Immunogenicity following one, two, or three doses of the 7-valent pneumococcal conjugate vaccine. *Vaccine.* 2009 Sep 18; 27(41):5685–91. [PubMed: 19616498]
15. Russell FM, Licciardi PV, Balloch A, Biaukula V, Tikoduadua L, Carapetis JR, et al. Safety and immunogenicity of the 23-valent pneumococcal polysaccharide vaccine at 12 months of age, following one, two, or three doses of the 7-valent pneumococcal conjugate vaccine in infancy. *Vaccine.* 2010 Apr 19; 28(18):3086–94. [PubMed: 20199764]
16. Russell FM, Carapetis JR, Balloch A, Licciardi PV, Jenney AW, Tikoduadua L, et al. Hyporesponsiveness to re-challenge dose following pneumococcal polysaccharide vaccine at 12 months of age, a randomized controlled trial. *Vaccine.* 2010 Apr 26; 28(19):3341–9. [PubMed: 20206670]
17. Burton RL, Nahm MH. Development and validation of a fourfold multiplexed opsonization assay (MOPA4) for pneumococcal antibodies. *Clin Vaccine Immunol.* 2006 Sep; 13(9):1004–9. [PubMed: 16960111]
18. Kim KH, Yu J, Nahm MH. Efficiency of a pneumococcal opsonophagocytic killing assay improved by multiplexing and by coloring colonies. *Clin Diagn Lab Immunol.* 2003 Jul; 10(4): 616–21. [PubMed: 12853394]
19. Russell FM, Balloch A, Tang ML, Carapetis JR, Licciardi P, Nelson J, et al. Immunogenicity following one, two, or three doses of the 7-valent pneumococcal conjugate vaccine. *Vaccine.* 2009 Jul 17.
20. Givon-Lavi N, Greenberg D, Dagan R. Immunogenicity of alternative regimens of the conjugated 7-valent pneumococcal vaccine: a randomized controlled trial. *Pediatr Infect Dis J.* 2010 Aug; 29(8):756–62. [PubMed: 20661103]
21. Dagan R, Goldblatt D, Maleckar JR, Yaich M, Eskola J. Reduction of antibody response to an 11-valent pneumococcal vaccine coadministered with a vaccine containing acellular pertussis components. *Infect Immun.* 2004 Sep; 72(9):5383–91. [PubMed: 15322036]
22. Nurkka, A.; Poolman, J.; Henckaerts, I.; Kilpi, TM.; Käyhty, H. Opsonophagocytic activity of antibodies induced by 11-valent pneumococcal conjugate vaccine. 4th International Symposium on Pneumococci and Pneumococcal Disease Helsinki; Finland. May 9–13, 2004; Abstract PSV-41
23. Blum MD, Dagan R, Mendelman PM, Pinski V, Giordani M, Li S, et al. A comparison of multiple regimens of pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine and pneumococcal polysaccharide vaccine in toddlers. *Vaccine.* 2000 May 8; 18(22):2359–67. [PubMed: 10738092]
24. Haber M, Barskey A, Baughman W, Barker L, Whitney CG, Shaw KM, et al. Herd immunity and pneumococcal conjugate vaccine: a quantitative model. *Vaccine.* 2007 Jul 20; 25(29):5390–8. [PubMed: 17583392]
25. Hicks LA, Harrison LH, Flannery B, Hadler JL, Schaffner W, Craig AS, et al. Incidence of pneumococcal disease due to non-pneumococcal conjugate vaccine (PCV7) serotypes in the United States during the era of widespread PCV7 vaccination, 1998–2004. *J Infect Dis.* 2007 Nov 1; 196(9):1346–54. [PubMed: 17922399]
26. Park IH, Pritchard DG, Cartee R, Brandao A, Brandileone MC, Nahm MH. Discovery of a new capsular serotype (6C) within serogroup 6 of *Streptococcus pneumoniae*. *J Clin Microbiol.* 2007 Apr; 45(4):1225–33. [PubMed: 17267625]

27. Fernsten, P.; Hu, B.; Yu, X. Specificity of the opsonic response to serotype 19A and 19F conjugate vaccines. 6th International Symposium on Pneumococci and Pneumococcal Diseases; Reykjavik, Iceland. 2008.
28. Hausdorff W, Hoet B, Schuerman L. Do pneumococcal conjugate vaccines provide any cross-protection against serotype 19A? *BMC Pediatr.* 2010 Feb 2;10(4)
29. Park S, Parameswar A, Demchenko A, Nahm M. Identification of a simple chemical structure associated with protective human antibodies against multiple pneumococcal serogroups. *Infect Immun.* 2009; 77(8):3374–9. [PubMed: 19451241]
30. O'Grady K, Lee K, Carlin J, Torzillo P, Chang A, Mulholland E, et al. Increased risk of hospitalization for acute lower respiratory tract infection among Australian indigenous infants 5–23 months of age following pneumococcal vaccination: a cohort study. *Clin Infect Dis.* 2010 Apr 1; 50(7):970–8. [PubMed: 20192729]
31. Moore, H.; Lehmann, D.; de Klerk, N.; Jacoby, P.; Richmond, P. Reduction in disparity in pneumonia hospitalisations between Aboriginal and Non-Aboriginal children in Western Australia. 7th International Symposium on Pneumococci and Pneumococcal Diseases; Israel: Tel Aviv; 2010. Poster 16
32. Eskola J, Kilpi T, Palmu A, Jokinen J, Haapakoski J, Herva E, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *N Engl J Med.* 2001 Feb 8; 344(6):403–9. [PubMed: 11172176]
33. Kilpi T, Ahman H, Jokinen J, Lankinen KS, Palmu A, Savolainen H, et al. Protective efficacy of a second pneumococcal conjugate vaccine against pneumococcal acute otitis media in infants and children: randomized, controlled trial of a 7-valent pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine in 1666 children. *Clin Infect Dis.* 2003 Nov 1; 37(9):1155–64. [PubMed: 14557958]
34. Ekstrom N, Ahman H, Verho J, Jokinen J, Vakevainen M, Kilpi T, et al. Kinetics and avidity of antibodies evoked by heptavalent pneumococcal conjugate vaccines PncCRM and PncOMPC in the Finnish Otitis Media Vaccine Trial. *Infect Immun.* 2005 Jan; 73(1):369–77. [PubMed: 15618174]
35. Ekstrom N, Vakevainen M, Verho J, Kilpi T, Kayhty H. Functional antibodies elicited by two heptavalent pneumococcal conjugate vaccines in the Finnish Otitis Media Vaccine Trial. *Infect Immun.* 2007 Apr; 75(4):1794–800. [PubMed: 17261612]
36. Jokinen JT, Ahman H, Kilpi TM, Makela PH, Kayhty MH. Concentration of antipneumococcal antibodies as a serological correlate of protection: an application to acute otitis media. *J Infect Dis.* 2004 Aug 1; 190(3):545–50. [PubMed: 15243930]
37. Goldblatt D, Southern J, Ashton L, Andrews N, Woodgate S, Burbidge P, et al. Immunogenicity of a Reduced Schedule of Pneumococcal Conjugate Vaccine in Healthy Infants and Correlates of Protection for Serotype 6B in the United Kingdom. *Pediatr Infect Dis J.* 2009 Dec 11.
38. Wuorimaa TK, Dagan R, Bailleux F, Haikala R, Ekstrom N, Eskola J, et al. Functional activity of antibodies after immunization of Finnish and Israeli infants with an 11-valent pneumococcal conjugate vaccine. *Vaccine.* 2005 Nov 16; 23(46–47):5328–32. [PubMed: 16118028]
39. Puumalainen T, Ekstrom N, Zeta-Capeding R, Ollgren J, Jousimies K, Lucero M, et al. Functional antibodies elicited by an 11-valent diphtheria-tetanus toxoid-conjugated pneumococcal vaccine. *J Infect Dis.* 2003 Jun 1; 187(11):1704–8. [PubMed: 12751027]
40. Concepcion N, Frasch CE. Evaluation of previously assigned antibody concentrations in pneumococcal polysaccharide reference serum 89SF by the method of cross-standardization. *Clin Diagn Lab Immunol.* 1998 Mar; 5(2):199–204. [PubMed: 9521143]
41. Concepcion NF, Frasch CE. Pneumococcal type 22f polysaccharide absorption improves the specificity of a pneumococcal-polysaccharide enzyme-linked immunosorbent assay. *Clin Diagn Lab Immunol.* 2001 Mar; 8(2):266–72. [PubMed: 11238206]
42. Soininen A, van den Dobbelsteen G, Oomen L, Kayhty H. Are the enzyme immunoassays for antibodies to pneumococcal capsular polysaccharides serotype specific? *Clin Diagn Lab Immunol.* 2000 May; 7(3):468–76. [PubMed: 10799463]
43. Vidarsson G, Sigurdardottir ST, Gudnason T, Kjartansson S, Kristinsson KG, Ingolfsson G, et al. Isotypes and opsonophagocytosis of pneumococcus type 6B antibodies elicited in infants and

- adults by an experimental pneumococcus type 6B-tetanus toxoid vaccine. *Infect Immun*. 1998 Jun; 66(6):2866–70. [PubMed: 9596761]
44. Yu X, Gray B, Chang S, Ward JI, Edwards KM, Nahm MH. Immunity to cross-reactive serotypes induced by pneumococcal conjugate vaccines in infants. *J Infect Dis*. 1999 Nov; 180(5):1569–76. [PubMed: 10515817]

Figure 1. Pre- and one month post-mPPS OPA titer for serotypes 4 and 6B, in those that did (+) and did not (o) receive PPV-23 at 12 months of age. Data from infants receiving 0, 1, 2, or 3 PCV-7 doses have been combined in these graphs

Footnote: A 45-degree line, which indicates no change before and after the re-challenge (mPPS), is superimposed. The pre- and post-mPPS OPA titer of those who received PPV-23 at 12 months mostly fell along the 45-degree line, indicating no response to mPPS, except for serotypes 1 and 5. In contrast, most children who did not receive PPV-23 had an increase in OPA titer, as indicated by the data points falling above the 45-degree line

Table 1

Serotype-specific IgG geometric mean concentrations (GMCs and 95% CI), OPA geometric mean titers^a (GMT and 95% CI) and proportions of infants with OI ≥ 8 to 6 PCV-7 serotypes 4 weeks post primary series, and at 9 and 12 months of age, by number of PCV-7 administered in the primary series.

Serotype	3 PCV-7			2 PCV-7			1 PCV-7			0 PCV-7		
	GMC (95% CI)	OPA GMT (95% CI)	% OI ≥ 8	GMC (95% CI)	OPA GMT (95% CI)	% OI ≥ 8	GMC (95% CI)	OPA GMT (95% CI)	% OI ≥ 8	GMC (95% CI)	OPA GMT (95% CI)	% OI ≥ 8
4 weeks post primary series												
n = 125												
n = 119												
n = 146												
n = 141												
n = 121												
n = 118												
n = 62												
n = 61												
4	5.47 (4.84–6.19)	872.3 (709.6–1072.2)	100	5.23 (4.46–6.13)	935.6 (745.0–1175.0)	99	2.20 (1.80–2.70)	219.0 (153.0–313.5)	94 ^b	0.04 (0.03–0.04)	2.3 (1.9–2.7)	2 ^{b,c}
6B	1.66 (1.33–2.07)	660.6 (423.6–1030.2)	95	0.86 (0.70–1.07)	63.6 (40.0–101.2)	68 ^b	0.19 (0.16–0.22)	3.7 (2.8–4.9)	14 ^b	0.11 (0.10–0.13)	2.2 (1.9–2.4)	3 ^b
9V	4.76 (4.19–5.40)	801.7 (609.9–1053.8)	100	4.71 (3.88–5.71)	794.8 (581.8–1085.9)	97	0.90 (0.74–1.09)	84.3 (53.6–132.6)	76 ^b	0.07 (0.06–0.08)	2.1 (1.9–2.4)	2 ^{b,c}
14	5.51 (4.50–6.76)	984.4 (633.4–1529.9)	94	3.12 (2.42–4.03)	430.4 (267.4–692.6)	87	1.07 (0.89–1.27)	137.6 (78.8–240.5)	75 ^b	0.34 (0.27–0.43)	3.3 (2.6–4.2)	23 ^{b,c}
18C	3.20 (2.66–3.86)	696.2 (508.0–954.1)	98	2.67 (2.16–3.31)	576.1 (435.5–762.0)	98	0.58 (0.45–0.74)	35.6 (23.1–54.8)	63 ^b	0.06 (0.05–0.07)	2.2 (2.0–2.3)	2 ^{b,c}
23F	2.93 (2.39–3.59)	750.4 (515.2–1093)	97	1.65 (1.29–2.11)	278.8 (182.3–426.5)	87 ^b	0.23 (0.20–0.27)	18.2 (11.1–29.5)	46 ^b	0.11 (0.10–0.14)	2.2 (2.0–2.4)	2 ^{b,c}
9 months n = 37												
n = 36												
n = 48												
n = 43												
4	0.79 (0.55–1.14)	101.0 (60.0–170.1)	97	0.86 (0.67–1.12)	70.3 (43.1–114.6)	90	0.60 (0.42–0.85)	49.9 (26.0–95.8)	81	NA	NA	NA
6B	0.82 (0.58–1.17)	65.8 (29.6–146.5)	78	0.81 (0.59–1.12)	48.2 (25.5–91.2)	75	0.39 (0.29–0.52)	5.8 (3.0–11.2)	26 ^b	NA	NA	NA
9V	0.91 (0.71–1.16)	104.1 (56.2–192.9)	97	1.00 (0.72–1.38)	62.0 (35.1–109.7)	85	0.56 (0.40–0.77)	25.1 (12.4–50.8)	56 ^b	NA	NA	NA
14	3.99 (2.86–5.57)	401.4 (207–778.5)	94	1.93 (1.20–3.09)	124.2 (62.3–247.4)	81	1.11 (0.79–1.57)	55.4 (24.0–127.6)	67 ^b	NA	NA	NA
18C	0.49 (0.37–0.65)	134.4 (80.4–224.2)	94	0.41 (0.33–0.53)	51.8 (33.2–80.7)	88	0.18 (0.14–0.24)	13.7 (8.7–21.7)	65 ^b	NA	NA	NA
23F	0.65 (0.46–0.94)	117.2 (59.6–230.3)	94	0.44 (0.33–0.60)	29.2 (14.4–59.2)	63 ^b	0.24 (0.18–0.32)	10.5 (5.6–19.7)	47 ^b	NA	NA	NA
12 months n = 113												
n = 112												
n = 142												
n = 114												
n = 111												
n = 59												
4	0.48 (0.41–0.57)	32.5 (21.6–48.9)	71	0.47 (0.40–0.54)	27.4 (20.1–37.5)	74	0.63 (0.50–0.81)	63.2 (41.6–96.1)	81 ^b	0.07 (0.06–0.09)	6.1 (3.5–10.6)	23 ^{b,c}
6B	0.86 (0.72–1.03)	71.0 (45.6–110.6)	77	0.76 (0.63–0.92)	27.0 (17.5–41.6)	59 ^b	0.57 (0.46–0.71)	4.2 (3.1–5.8)	20 ^b	0.14 (0.12–0.17)	3.0 (2.0–4.5)	7 ^b
9V	0.59 (0.51–0.67)	48.4 (31.7–73.7)	78	0.62 (0.54–0.71)	56.9 (38.2–84.7)	79	0.50 (0.41–0.62)	37.7 (23.9–59.3)	66 ^b	0.09 (0.07–0.11)	4.9 (2.9–8.5)	19 ^{b,c}
14	2.38 (1.98–2.86)	204.6 (134.5–311.2)	87	1.52 (1.26–1.84)	79.4 (52.5–120.0)	79	1.16 (0.94–1.44)	48.6 (30.3–77.9)	73 ^b	0.19 (0.16–0.24)	3.8 (2.3–6.1)	13 ^{b,c}
18C	0.32 (0.27–0.38)	46.8 (33.0–66.4)	84	0.24 (0.21–0.28)	22.7 (16.9–30.3)	71 ^b	0.17 (0.15–0.20)	12.4 (9.0–17.2)	59 ^b	0.06 (0.05–0.08)	2.6 (1.9–3.5)	5 ^{b,c}
23F	0.54 (0.44–0.66)	64.2 (42.0–98.3)	79	0.42 (0.35–0.50)	35.4 (23.8–52.5)	65 ^b	0.26 (0.21–0.31)	12.7 (8.2–19.7)	43 ^b	0.07 (0.06–0.09)	4.5 (2.7–7.7)	16 ^{b,c}

- ^a Dilution able to kill 50% of bacteria. All 3 vs. 2, 3 vs. 1, 3 vs. 0, 1 vs. 0 PCV-7 dose comparisons of proportions with $OI \geq 8$ were not significant except those marked.
- ^b Significant difference ($p < 0.01$) comparing proportions with $OI \geq 8$ following 0, 1, or 2 doses PCV-7 to the proportions following 3 PCV-7 doses.
- ^c Significant difference ($p < 0.01$) comparing proportions with $OI \geq 8$ following a single PCV-7 dose to the proportions following no PCV-7 dose.

Table 2

OPA geometric mean titers^a (GMT and 95% CI) and proportions of children with OI $\geq$ 8 to 6 PCV-7 serotypes pre- and 14 days post-PPV-23 at 12 months of age and by number of PCV-7 administered in the primary series.

Pre-PPV-23 at 12 months of age									
3 PCV-7 (n = 52)			2 PCV-7 (n = 67)			1 PCV-7 (n = 58)			0 PCV-7 (n = 55)
OPA GMT (95% CI)	% OI $\geq$ 8		OPA GMT (95% CI)	% OI $\geq$ 8		OPA GMT (95% CI)	% OI $\geq$ 8		OPA GMT (95% CI)
4 28.7 (15.9–51.8)	69		18.2 (11.8–28.0)	70		65.0 (36.0–117.4)	81		6.2 (3.5–10.9)
6B 95.5 (48.7–187.2)	83		19.8 (10.6–36.8)	51		4.6 (2.9–7.1)	22		3.0 (2.0–4.6)
9V 48.9 (26.7–89.6)	81		48.2 (28.0–83.0)	82		61.0 (32.5–114.7)	76		5.0 (2.9–8.7)
14 156.8 (79.0–311.2)	81		86.4 (48.1–155.0)	82		60.6 (32.2–114.3)	78		3.8 (2.3–6.2)
18C 59.8 (35.3–101.2)	88		18.5 (12.0–28.5)	64		18.5 (11.4–30.2)	66		2.6 (1.9–3.6)
23F 52.4 (27.5–99.7)	75		26.9 (15.3–47.5)	61		17.0 (8.8–33.0)	48		4.6 (2.7–7.9)
14 days post-12 month PPV-23									
3 PCV-7 (n = 52)			2 PCV-7 (n = 67)			1 PCV-7 (n = 58)			0 PCV-7 (n = 55)
OPA GMT (95% CI)	% OI $\geq$ 8		OPA GMT (95% CI)	% OI $\geq$ 8		OPA GMT (95% CI)	% OI $\geq$ 8		OPA GMT (95% CI)
4 4571.8 (3104–6733.6)	100		4808.0 (3729.5–6198.4)	100		23392.4 (16960.1–32264.1)	100		249.9 (126.8–492.5)
6B 6446.7 (3419.9–12152.5)	94		3560.0 (2149.1–5897.3)	96		1361.4 (718.6–2579.2)	91		4.8 (2.6–8.6)
9V 5093.3 (3730.5–6954.0)	100		4391.1 (3417.2–5642.6)	100		13747.9 (9792.4–19301.3)	100		81.4 (37.0–179.0)
14 3023.5 (1531.6–5968.8)	92		2261.3 (1352.7–3780.0)	97		2618.5 (1221.4–5613.6)	90		9.0 (4.3–19.0)
18C 3975.9 (3018.5–5237.1)	100		3262.8 (2513.6–4235.4)	100		5302.7 (3433.1–8190.4)	100		66.7 (33.3–133.8)
23F 4109.5 (2422.8–6970.6)	98		2295.0 (1420.2–3708.5)	97		2119.6 (1161–3869.8)	93		18.1 (8.5–38.5)

^aDilution able to kill 50% of bacteria. All post PPV-23 comparisons comparing 3 vs. 2, 3 vs. 1, 3 vs. 0, and 1 vs. 0 PCV-7 doses were not significant except those marked.

^bSignificant difference ($p < 0.01$) comparing proportions with OI $\geq$ 8 following no PCV-7 dose to the proportions following 3 doses of PCV-7.

^cSignificant difference ($p < 0.01$) comparing proportions with OI $\geq$ 8 following a single PCV-7 dose to the proportions following no PCV-7 dose.

Table 3

Serotype-specific IgG geometric mean concentrations (GMCs and 95% CI)^a, OPA geometric mean titers^b (GMT and 95% CI) and proportions of infants with OI ≥ 8 pre-mPPS at 17 months of age and one month post-mPPS in those that have or have not received the 12 month PPV-23 and by number of PCV-7 doses in the primary series.

	3 PCV-7 no PPV-23			3 PCV-7 + PPV-23			2 PCV-7 no PPV-23			2 PCV-7 + PPV-23		
	Pre-mPPS	Post-mPPS		Pre-mPPS	Post-mPPS		Pre-mPPS	Post-mPPS		Pre-mPPS	Post-mPPS	
4												
GMC	0.35 (0.29–0.43)	4.69 (3.62–6.07)		2.19 (1.79–2.69)	2.01 (1.66–2.43)		0.43 (0.33–0.56)	3.99 (3.10–5.12)		2.03 (1.70–2.42)	2.00 (1.67–2.40)	
GMT	18.6 (11.3–30.7)	1627.8 (1209.1–2191.6)		403.3 (282.2–576.3)	444.1 (307.2–642.1)		37.1 (20.1–68.7)	1286.3 (922.1–1794.5)		297.5 (217.0–407.7)	340.5 (254.7–455.1)	
OI ≥ 8	64	100		98	98 ^{c,e}		72	100		97	100 ^{c,e}	
6B												
GMC	0.91 (0.69–1.21)	12.77 (9.52–17.12)		4.35 (3.22–5.89)	4.08 (3.17–5.26)		0.78 (0.58–1.04)	10.57 (7.73–14.44)		3.85 (3.10–4.79)	4.08 (3.18–5.25)	
GMT	50.6 (27.5–92.9)	2774.9 (1941.0–3967.1)		539.8 (325.8–894.3)	639.7 (390.7–1047.4)		39.9 (20.9–76.1)	1898.8 (1134.4–3178.1)		223.9 (134.1–373.7)	239.4 (144.6–396.3)	
OI ≥ 8	74	100		96	96 ^{c,e}		65	97		92	92 ^{c,e}	
9V												
GMC	0.41 (0.34–0.49)	3.92 (3.08–4.99)		2.25 (1.74–2.91)	2.10 (1.67–2.64)		0.49 (0.39–0.62)	4.34 (3.33–5.66)		2.50 (2.02–3.08)	2.46 (1.98–3.05)	
GMT	29.8 (16.7–53.1)	1317.7 (982.5–1767.3)		383.7 (245.2–600.5)	431.9 (300.7–620.4)		48.7 (27.4–86.4)	1284.8 (894.4–1845.8)		300.9 (212.0–427.1)	306.6 (210.2–447.0)	
OI ≥ 8	67	100		100	100 ^{c,e}		75	98		97	97 ^{c,e}	
14												
GMC	1.78 (1.42–2.24)	10.25 (7.79–13.49)		4.31 (3.00–6.19)	4.26 (3.08–5.90)		1.12 (0.86–1.46)	6.84 (4.80–9.77)		3.58 (2.72–4.71)	3.83 (2.91–5.03)	
GMT	117.7 (62.4–222.1)	2123.9 (1547.4–2915.2)		405.6 (222.6–739.1)	507.0 (290.7–884.1)		50.0 (24.2–103.6)	930.0 (497.8–1737.3)		276.0 (157.2–484.4)	316.7 (182.1–550.6)	
OI ≥ 8	79	100		91 ^d	94 ^{c,e}		63	90		87	89 ^{c,e}	
18C												
GMC	0.21 (0.18–0.26)	1.94 (1.56–2.42)		1.28 (1.07–1.53)	1.25 (1.04–1.49)		0.20 (0.16–0.25)	2.55 (1.97–3.29)		1.13 (0.92–1.38)	1.23 (1.00–1.51)	
GMT	18.7 (12.1–28.9)	937.6 (705.6–1246.0)		260.8 (193.9–350.9)	249.9 (184.6–338.3)		15.6 (9.9–24.8)	1025.8 (768.9–1368.5)		151.0 (100.4–227.3)	156.0 (105.0–231.8)	
OI ≥ 8	69	100		100	100 ^{c,e}		67	100		95	95 ^{c,e}	
19F												
GMC	1.19 (0.84–1.67)	15.33 (11.27–20.86)		5.55 (4.24–7.26)	5.42 (4.17–7.06)		1.06 (0.81–1.38)	15.95 (11.59–21.94)		4.53 (3.54–5.79)	5.09 (3.93–6.61)	
GMT	10.4 (5.7–19.0)	1917.5 (1222.9–3006.6)		391.2 (243.5–628.5)	479.2 (318.9–719.9)		11.5 (6.4–20.5)	1621.3 (952.3–2760.3)		257.2 (160.2–412.9)	285.0 (177.0–458.9)	
OI ≥ 8	40	97		98	98 ^{c,e}		45	97		95	95 ^{c,e}	
23F												

	3 PCV-7 no PPV-23			3 PCV-7 + PPV-23			2 PCV-7 no PPV-23			2 PCV-7 + PPV-23		
	Pre-mPPS	Post-mPPS		Pre- mPPS	Post- mPPS		Pre- mPPS	Post- mPPS		Pre-mPPS	Post-mPPS	
GMC	0.57 (0.43–0.75)	4.10 (3.26–5.17)		1.68 (1.29–2.20)	1.54 (1.21–1.96)		0.43 (0.32–0.58)	3.74 (2.74–5.12)		1.41 (1.06–1.86)	1.52 (1.17–1.97)	
GMT	71.7 (37.9–135.6)	1716.2 (1357.8–2169.3)		312.5 (189.8–514.6)	268.2 (151.7–474.2)		43.7 (22.0–86.8)	971.9 (579.4–1630.2)		141.7 (81.5–246.6)	156.0 (90.7–268.3)	
OI ≥ 8	78	100		96	94 ^{c,e}		63	95		84 ^d	87 ^{c,e}	
4												
GMC	0.56 (0.39–0.80)	10.39 (7.38–14.63)		6.36 (4.94–8.19)	6.37 (4.93–8.23)		0.11 (0.09–0.13)	0.79 (0.59–1.07)		0.70 (0.53–0.91)	0.81 (0.63–1.04)	
GMT	56.3 (30.3–104.7)	3478.8 (1957.6–6182.1)		1598.7 (1089.9–2344.9)	1829.7 (1273.5–2628.7)		4.9 (3.1–7.6)	204.9 (116.0–362.0)		18.9 (11.0–32.6)	19.1 (10.7–34.2)	
OI ≥ 8	84	95 ^c		98 ^d	98 ^{c,e}		24	88		65	61	
6B												
GMC	0.62 (0.47–0.83)	5.82 (3.90–8.68)		3.88 (2.87–5.25)	3.83 (2.80–5.26)		0.20 (0.16–0.24)	0.30 (0.24–0.37)		0.21 (0.17–0.26)	0.22 (0.17–0.29)	
GMT	6.2 (3.4–11.2)	302.9 (131.2–698.9)		81.4 (40.3–164.1)	89.9 (45.2–178.9)		3.3 (2.2–5.0)	5.4 (3.1–9.4)		4.3 (2.6–7.1)	3.7 (2.3–5.9)	
OI ≥ 8	30	81		76	80 ^{c,e}		10	21 ^c		17 ^d	13 ^e	
9V												
GMC	0.51 (0.36–0.71)	9.26 (6.45–13.28)		5.49 (4.23–7.12)	5.21 (4.05–6.70)		0.14 (0.11–0.17)	0.51 (0.39–0.68)		0.38 (0.31–0.47)	0.50 (0.40–0.62)	
GMT	40.0 (19.6–81.8)	1786.5 (1074.3–2971.1)		1127.6 (852.7–1491.1)	902.3 (619.4–1314.3)		8.8 (4.9–15.7)	60.8 (28.7–128.9)		9.6 (5.4–17.0)	9.6 (5.3–17.4)	
OI ≥ 8	74	100		100	98 ^{c,e}		34	62		39 ^d	37	
14												
GMC	0.93 (0.66–1.32)	11.09 (6.95–17.69)		4.85 (3.27–7.19)	4.94 (3.39–7.19)		0.31 (0.25–0.38)	0.51 (0.37–0.71)		0.67 (0.47–0.96)	0.97 (0.68–1.38)	
GMT	34.8 (15.7–77.3)	1005.6 (432.7–2336.8)		248.1 (114.0–540.1)	328.1 (156.0–689.8)		8.7 (4.4–17.2)	18.4 (8.0–42.2)		7.6 (3.8–15.2)	24.3 (10.6–55.4)	
OI ≥ 8	65	88		81 ^d	83 ^{c,e}		26	34 ^c		28 ^d	46 ^e	
18C												
GMC	0.15 (0.12–0.19)	2.80 (2.02–3.87)		1.79 (1.37–2.34)	1.87 (1.46–2.39)		0.10 (0.08–0.12)	0.48 (0.35–0.65)		0.50 (0.39–0.66)	0.62 (0.46–0.83)	
GMT	8.4 (5.1–13.8)	1033.0 (647.9–1647.1)		322.4 (201.7–515.2)	309.7 (192.1–499.1)		4.2 (2.8–6.4)	32.4 (17.1–61.5)		6.7 (4.3–10.7)	7.1 (4.5–11.3)	
OI ≥ 8	53	98		94	94 ^{c,e}		22	64		39 ^d	38 ^e	
19F												
GMC	0.92 (0.66–1.26)	34.74 (23.17–52.11)		13.47 (9.90–18.33)	14.34 (10.72–19.18)		0.59 (0.47–0.75)	0.99 (0.77–1.26)		0.79 (0.61–1.04)	0.88 (0.67–1.15)	
GMT	9.3 (5.0–17.2)	3657.4 (1793.1–7459.8)		1086.4 (626.7–1883.3)	1470.4 (929.2–2326.7)		3.2 (2.2–4.6)	13.0 (7.4–22.7)		5.9 (3.5–9.8)	9.7 (5.6–16.8)	
OI ≥ 8	44	93		93	96 ^{c,e}		12	50		31 ^d	44 ^e	
23F												
GMC	0.32 (0.22–0.48)	3.34 (2.22–5.03)		1.78 (1.28–2.49)	1.54 (1.10–2.16)		0.19 (0.16–0.23)	0.37 (0.29–0.47)		0.27 (0.21–0.34)	0.27 (0.21–0.35)	

	3 PCV-7 no PPV-23		3 PCV-7 + PPV-23		2 PCV-7 no PPV-23		2 PCV-7 + PPV-23	
	Pre-mPPS	Post-mPPS	Pre- mPPS	Post-mPPS	Pre- mPPS	Post-mPPS	Pre-mPPS	Post-mPPS
GMT	10.4 (4.8–22.6)	507.2 (245.3–1048.5)	175.0 (97.5–314.2)	139.0 (74.6–258.9)	6.7 (3.7–12.2)	27.0 (12.3–59.4)	5.4 (3.1–9.4)	4.3 (2.6–7.5)
OI ≥ 8	35	88	87	83 ^{c,e}	24	47	22 ^d	17

^aGMC sample size by group: 3 PCV-7 no PPV-23 *n* = 59, 3 PCV-7 + PPV-23 *n* = 48, 2 PCV-7 no PPV-23 *n* = 68, 2 PCV-7 + PPV-23 *n* = 67, 1 PCV-7 no PPV-23 *n* = 49, 1 PCV-7 + PPV-23 *n* = 59, 0 PCV-7 no PPV-23 *n* = 62, and 0 PCV-7 + PPV-23 *n* = 57.

^bDilution able to kill 50% of bacteria. GMT and OI sample size by group: 3 PCV-7 no PPV-23 *n* = 58, 3 PCV-7 + PPV-23 *n* = 47, 2 PCV-7 no PPV-23 *n* = 60, 2 PCV-7 + PPV-23 *n* = 62, 1 PCV-7 no PPV-23 *n* = 43, 1 PCV-7 + PPV-23 *n* = 54, 0 PCV-7 no PPV-23 *n* = 58, and 0 PCV-7 + PPV-23 *n* = 54.

^cAll paired group comparisons of the proportion of children with OI ≥ 8 pre/post mPPS were statistically significant (*p* < 0.01) except those marked.

^dAll comparisons of the proportion of children with OI ≥ 8 at 17 months of age (pre-mPPS) between the 3 PCV-7/12 month PPV-23 group and the 3 PCV-7 group, the 2 PCV-7/12 month PPV-23 group and the 2 PCV-7 group, the one PCV-7/12 month PPV-23 group and the single PCV-7 group, and the 12 month PPV-23 group and the unvaccinated group, were statistically significant (*p* < 0.01) except those marked.

^eAll comparisons of the proportion of children with OI ≥ 8 at 18 months of age (post-mPPS) between the 3 PCV-7/12 month PPV-23 group and the 3 PCV-7 group, the 2 PCV-7/12 month PPV-23 group and the 2 PCV-7 group, the one PCV-7/12 month PPV-23 group and the single PCV-7 group, and the 12 month PPV-23 group and the unvaccinated group, were statistically significant (*p* < 0.01) except those marked.

Serotype-specific IgG geometric mean concentrations (GMCs and 95% CI), OPA geometric mean titers^a (GMT and 95% CI) and proportions of children with OI ≥ 8 to the non-PCV-7 serotypes 1 and 5 pre- and 14 days post-PPV-23 at 12 months of age.

	Pre-PPV-23 at 12 months			14 days post-12 month PPV-23		
	GMC (95% CI) <i>n</i> = 235	OPA GMT (95% CI)	% OI ≥ 8	GMC (95% CI) <i>n</i> = 235	OPA GMT (95% CI)	% OI $\geq 8^b$
	<i>n</i> = 228			<i>n</i> = 228		
1	0.17 (0.15–0.19)	2.3 (2.1–2.5)	4	1.59 (1.38–1.82)	4.7 (3.9–5.6)	27
5	0.26 (0.23–0.29)	2.1 (2.0–2.3)	2	2.26 (2.01–2.55)	22.2 (18.2–27.1)	77

^aDilution able to kill 50% of bacteria.

^b*p*-value < 0.001 for proportions with OI ≥ 8 pre/post PPV-23 for both serotypes.

Table 5

Serotype-specific IgG geometric mean concentrations (GMCs and 95% CI), OPA geometric mean titers^a (GMT and 95% CI) and proportions of children with OI ≥ 8 to 7 non-PCV-7 serotypes at 17 months and one month post-mPPS.

	No 12m PPV-23			12m PPV-23			<i>p</i> -Value ^b
	GMC (95% CI) <i>n</i> = 238	OPA GMT (95% CI)	% OI ≥ 8	GMC (95% CI) <i>n</i> = 231	OPA GMT (95% CI)	% OI ≥ 8	
<i>n</i> = 224							
<i>n</i> = 220							
Pre-mPPS at 17 months of age							
1	0.23 (0.21–0.25)	2.2 (2.1–2.3)	4	0.63 (0.56–0.71)	2.4 (2.2–2.6)	6	0.167
3	0.30 (0.26–0.34)	7.4 (6.0–9.1)	45	1.46 (1.30–1.63)	41.2 (35.1–48.2)	92	<0.001
5	0.31 (0.28–0.34)	2.3 (2.1–2.5)	5	0.77 (0.69–0.86)	5.0 (4.3–5.8)	39	<0.001
6A	NA	5.1 (3.9–6.6)	20	NA	8.1 (6.1–10.7)	35	0.028
6C	NA	5.7 (4.2–7.9)	17	NA	5.2 (3.8–7.0)	16	0.317
7F	0.12 (0.10–0.13)	471.0 (321.2–690.8)	80	0.51 (0.45–0.57)	1593.1 (1240.7–2045.6)	95	<0.001
19A	0.65 (0.57–0.74)	7.0 (5.4–9.1)	32	1.14 (1.00–1.31)	10.1 (7.8–13.1)	45	0.017
One month post-mPPS (18 months of age)							
1	0.82 (0.73–0.93)	4.1 (3.4–4.9)	22	0.73 (0.65–0.81)	2.4 (2.2–2.5)	7	<0.001
3	2.11 (1.86–2.39)	111.2 (94.1–131.4)	96	2.32 (2.06–2.63)	77.2 (66.0–90.3)	95	0.512
5	0.86 (0.77–0.96)	12.3 (10.0–15.3)	60	0.85 (0.76–0.96)	5.7 (4.9–6.6)	41	<0.001
6A	NA	33.3 (22.6–49.2)	54	NA	8.5 (6.3–11.4)	32	<0.001
6C	NA	19.9 (13.0–30.3)	37	NA	6.3 (4.6–8.8)	20	<0.001
7F	0.83 (0.72–0.96)	3320.5 (2783.6–3960.9)	98	0.65 (0.57–0.74)	1949.2 (1538.7–2469.3)	96	0.172
19A	2.19 (1.83–2.62)	194.1 (138.4–272.2)	82	1.46 (1.28–1.68)	45.3 (33.8–60.7)	74	0.049

^a Dilution able to kill 50% of bacteria.

^b p-value for proportions with OI ≥ 8 for each serotype mPPS comparing those that did not did not receive the PPV-23.