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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

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Abstract

Fijian infants aged 6 weeks were stratified by ethnicity and randomized to receive 0, 1, 2, or 3 PCV-7 doses with or without the 23-valent pneumococcal polysaccharide vaccine (PPV-23) at 12 months. Strong booster effects for all 7 PCV-7 serotypes were elicited, and for 4/7 serotypes these responses were highest in the single PCV-7 group. There were fourfold rises in GMC for all non-PCV-7 serotypes. By 17 months the PPV-23 group still had significantly higher GMC (each $p < 0.001$) for all serotypes. The PPV-23 was well tolerated and induced excellent responses for all serotypes which were greatest in the single PCV-7 group.

Keywords

Pneumococcal; polysaccharide; booster

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Introduction

Streptococcus pneumoniae is the most common cause of bacterial pneumonia in children worldwide. It 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 [2]. The case fatality rate is particularly high in infants less than 6 months old [3]. At least 48 serogroups comprising over 90 serotypes of pneumococcus have been identified [4]. Within serogroups, some serotypes cross-react immunologically, and in some cases this translates into cross-protection such as antibodies against 6B which provide cross-protection against 6A [5]. The association of particular serotypes with disease varies according to age, geography, and clinical presentation [6]. In general, the range of serotypes causing invasive pneumococcal disease (IPD) in affluent countries like the United States and in Europe is relatively narrow and largely confined to the serotypes found in the 7-valent pneumococcal conjugate vaccine (PCV-7, *Prevenar*TM, Wyeth Vaccines). In contrast, the range of serotypes causing disease in low-income countries is wider.

The 10-valent pneumococcal conjugate vaccine has recently been licensed in some countries, and a 13-valent vaccine is likely to be licensed by 2010. Some health authorities have decided or are considering a combination of an infant PCV-7 primary series with a booster of the 23-valent pneumococcal polysaccharide vaccine (PPV-23) in the second year of life to address the limited serotype coverage offered by PCV-7. There have been several studies involving children in a number of countries using different pneumococcal conjugate formulations and schedules, comparing the immunogenicity of a PPV-23 or PCV-7 booster following a pneumococcal conjugate vaccine primary series. The majority of studies have shown that serotype-specific antibody concentrations are generally higher following PPV-23 than PCV-7 booster [7-12]. The higher response may be due to the higher dose of pneumococcal polysaccharide in the PPV-23, compared to PCV-7, enhancing the stimulation of memory B cells or by stimulating a greater number of B cells overall [13]. Despite this, only pneumococcal conjugate vaccines provide mucosal immunity and have shown a reduction in nasopharyngeal (NP) carriage (NP carriage being an antecedent event for all pneumococcal disease) for conjugate serotypes, compared with unvaccinated infants [14-16] or toddlers [17-19]. In contrast, pneumococcal polysaccharide vaccines have shown no effect on pneumococcal carriage [20-24]. Most studies evaluating the impact of pneumococcal polysaccharide immunization in the absence of additional PCV-7 in infants or children have not shown any impact on pneumococcal disease or carriage [25-27].

Data from Fiji shows that the 7 serotypes included in PCV-7, plus the cross reactive serotype 6A, would potentially cover 63.3% of invasive pneumococcal disease (IPD) cases in children under 5 years [28]. This coverage would potentially increase to 83% if the PPV-23 was used, and would increase to 87% if the new 13-valent pneumococcal conjugate vaccine produced by Wyeth Vaccines (which includes serotypes 1, 3, 5, 6A, 7F and 19A) was used, largely due to the inclusion of 6A which is not included in the PPV-23 [28]. The aim of this study was to find an optimal vaccination strategy suitable for resource poor countries in terms of serotype coverage, flexibility, and affordability. To address these issues, 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 1, 2, or 3 doses of PCV-7 in infancy. In order to broaden the serotype coverage, the additional benefit of a PPV-23 booster at 12 months of age was also assessed. Presented are the geometric mean serotype-specific IgG antibody concentrations (GMC) prior to and 2 weeks following the 12 month PPV-23, and at 17 months of age.

Methods

Study participants

The study was a single blind, open-label randomized Phase II vaccine trial undertaken in Suva, the capital of Fiji. Healthy infants aged between six and eight weeks were eligible for enrolment. Details of the selection criteria and the randomization procedure have been reported elsewhere [29].

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

Study procedures and vaccines

Infants were stratified by ethnicity and randomized into one of eight groups. The seven-valent CRM₁₉₇ protein-polysaccharide conjugate vaccine containing polysaccharide antigen from pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F, 23F (*Prevenar*TM, Wyeth Vaccines) was used. The vaccine contains 2 µg of each serotype, except serotype 6B which contains 4µg. The three dose group received PCV-7 at 6, 10, 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 (*Hiberix*TM mixed with *Tritanrix*TM-*HepB*TM, GlaxoSmithKline) and oral polio were given with the primary series. *Hiberix*TM contains 10µg of purified Hib capsular polysaccharide covalently bound to approximately 30µg tetanus toxoid mixed with *Tritanrix*TM-*HepB*TM which contains not less than 30 IU of adsorbed D toxoid, not less than 60 IU of adsorbed T toxoid, not less than 4 IU of whole cell Pertussis, and 10µg of recombinant HBsAg protein. The children in all primary series groups were further randomized to receive a dose of PPV-23 (*Pneumovax*TM, Merck & Co., Inc., which consists of a purified mixture of 25µg of capsular polysaccharide from 23 pneumococcal serotypes) or no vaccine at 12 months of age (window: 12 months plus 4 weeks). In addition, all children received Measles-Rubella vaccine at 12 months of age co-administered with PPV-23. The children randomized to receive 0 or 1 PCV-7 dose in infancy had a single dose of PCV-7 administered at 2 years of age.

Children were reviewed on day 1, 2 and 7 following PPV-23 and assessed for any adverse event (AE). An AE was defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of PPV-23, whether or not related to PPV-23. A severe non-serious AE was defined as an event which prevented normal activities but did not meet the criteria of a serious AE (SAE). A SAE was defined as an AE meeting one of the following conditions: death in the 2 year follow up period; a life threatening event; hospitalization or prolongation of existing hospitalization during the 2 year period; or resulting in a persistent or significant disability/incapacity.

SAEs were sourced from parent interview at each study visit and via a search of computerized hospital discharge data. Causality of any non-serious AE were assigned by the study doctor and reviewed by a pediatrician (FR). Causality of SAEs were assigned by the study doctor and assessed by an independent external safety monitor and regularly reviewed by the study's Data Safety and Monitoring Board.

Laboratory procedures

Children who received the 12 month PPV-23 had blood drawn immediately prior to and 14 days following the PPV-23 (window: 10-21 days post PPV-23). All children had blood drawn at 17 months of age. Blood was separated by centrifugation in the health centre, kept

chilled and transported to the Colonial War Memorial Hospital laboratory, Suva, where it was divided into aliquots and stored at -20°C on the same day, until transported to the Pneumococcal Laboratory, Murdoch Childrens Research Institute, Melbourne, on dry ice for analysis.

Anticapsular pneumococcal antibody levels were assayed for all PPV-23 serotypes (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33F), using a modified 3rd generation ELISA based on current WHO recommendations [30]. In brief, microtiter wells were coated with pneumococcal polysaccharide diluted in phosphate buffered saline by incubating at room temperature overnight. To neutralise non-specific antibodies to cell wall polysaccharide (C-PS), serum samples for all serotypes (except serotype 22F) were diluted 1/100 in pre-absorption buffer containing C-PS ($10\mu\text{g}/\text{mL}$) and serotype 22F ($30\mu\text{g}/\text{mL}$) and incubated overnight at 4°C . Absorption with $30\mu\text{g}/\text{mL}$ serotype 22F overnight has been reported previously [31,32] and unpublished data from our laboratory have shown this to further improve the specificity of the pneumococcal ELISA. The reference serum standard 89-SF (Food and Drug Administration, Bethesda MD) and samples for measurement of specific IgG to serotype 22F were pre-absorbed with C-PS at $10\mu\text{g}/\text{mL}$ and incubated overnight at 4°C . Horseradish peroxidase conjugated anti-human IgG and a TMB (3,3', 5,5'-tetramethylbenzidine) substrate solution was used for detection. A high, medium, and low control serum were used on each plate to assess assay performance and inter-assay variation. Results from an inter-laboratory comparison between the Pneumococcal Laboratory, Murdoch Childrens Research Institute, (Melbourne, Australia), Wyeth Vaccine Research Laboratory (USA) and the KTL laboratory (Finland) demonstrated a good correlation of serotype-specific antibody concentrations [33]. Laboratory staff members were blinded to the group allocation of each serum sample.

Statistical analysis

This manuscript reports analytic results concerning the secondary purpose of the trial. Cleaned data were exported to Stata version 9.0 (Stata Corporation, College Station, Texas) for analysis. Serotype-specific antibody concentrations by ELISA were log (base e) transformed to calculate GMC. Comparisons of serotype-specific GMC between 0-3 dose PCV-7 groups were performed using a two sample t-test. Comparisons of serotype-specific GMC before and after the PPV-23 were performed using the paired t test. Comparisons of the proportion of infants between groups with serotype-specific antibody concentrations ≥ 0.35 and $\geq 1\mu\text{g}/\text{mL}$ were performed using Fisher's exact test. Comparisons of serotype-specific antibody concentrations ≥ 0.35 and $\geq 1\mu\text{g}/\text{mL}$ before and after the PPV-23 were performed using exact McNemar's test. A p-value of <0.01 was considered statistically significant due to the multiple comparisons.

Results

There were 552 infants enrolled in the study (Figure 1) and the characteristics of the randomized infants have been described elsewhere (15). The 552 participants represent a consent rate of 30.5%, of which 10% had withdrawn by 12 months and 15% by 17 months of age. The commonest reason for withdrawal was relocation outside the study area. No participant was withdrawn due to a reaction to any of the vaccines. The 12 month PPV-23 was administered to 245 children with all groups having blood drawn a median of 14 days (IQR 14-15 days) post booster.

Immunogenicity to PCV-7 serotypes

Two weeks following the PPV-23, GMC were significantly higher (each $p<0.001$) for all PCV-7 serotypes for children that had received either 1, 2, or 3 PCV-7 doses in the primary

series compared to levels prior to receiving PPV-23 (Table 1). For 4 of 7 serotypes (4, 9V, 18C, 19F) this response was most profound in the single PCV-7 dose group. There were no significant differences in GMC 2 weeks following the PPV-23 for any PCV-7 serotype between the 3 and 2 PCV-7 dose groups. GMC were significantly higher (each $p < 0.001$) 2 weeks following the PPV-23 compared with the pre-PPV-23 levels, for all PCV-7 serotypes in the group that had not received PCV-7 in infancy (Table 1).

Two weeks following the 12 month PPV-23, there was no significant difference between the 3 and 2 dose PCV-7 groups or between the 3 and single dose groups in the proportion of children with antibody concentrations ≥ 0.35 and $\geq 1\mu\text{g/mL}$ for the PCV-7 serotypes (Table 2).

At 17 months of age the groups that had received the 12 month PPV-23 continued to have significantly higher GMC (each $p < 0.001$) for all PCV-7 serotypes compared to those that had not received the 12 month PPV-23 but the same number of PCV-7 doses (Table 3). The single PCV-7 dose group which received the PPV-23 continued to have higher GMC compared to the 2 or 3 dose PCV-7 groups which did or did not receive the PPV-23. There were significantly higher proportions with antibody concentrations $\geq 1\mu\text{g/mL}$ for the PCV-7 serotypes in those groups that had received the 12 month PPV-23 compared with those that had not received the PPV-23 (Table 3).

Immunogenicity to non-PCV-7 serotypes

Two weeks following the 12 month PPV-23, GMC and the proportions with antibody concentrations ≥ 0.35 and $\geq 1\mu\text{g/mL}$ for all non-PCV-7 serotypes in the PPV-23 were significantly higher (each $p < 0.001$) than pre-PPV-23 levels (Table 4). To assess for non-specific effects, the proportion of children with antibody concentrations $\geq 0.35\mu\text{g/mL}$ were compared between the 3, 2, and single PCV-7 dose groups with the group that had received no prior PCV-7. There were no significant differences in responses to the non-PCV-7 serotypes following the 12 month PPV-23 between the 3 and 0 PCV dose groups (data not shown). However for serotypes 15B and 19A, the proportion of children with antibody concentrations $\geq 0.35\mu\text{g/mL}$ were significantly higher in the 2 and single dose groups compared with the 0 PCV dose group (data not shown)."

By 17 months of age, GMC and the proportion with antibody concentrations $\geq 0.35\mu\text{g/mL}$ were still significantly higher (each $p < 0.001$) for all non-PCV-7 serotypes in the groups that had received the PPV-23 vaccine at 12 months compared to the groups that had not (Table 5).

Adverse events

Following PPV-23 at 12 months of age, low grade fever was common (28.2%) while high grade fever occurred in 6.1%. The description of other general reactions are shown in Table 6. Local injection site reactions occurred in a minority of recipients. All events resolved within 48 hours. There were 101 SAEs throughout the 2 year follow up period, with none attributable to receipt of any of the study vaccines. One child who had received 2 doses of PCV-7 at 6 and 14 weeks of age died at 9 months of age from dehydration secondary to acute gastroenteritis. For children over 12 months of age, there were 14 SAEs in children who had received the 12 month PPV-23 and 22 SAEs in children who had not received PPV-23, during the follow up period up to 2 years of age. For children over 12 months of age, there were 4 cases of inpatient pneumonia in children who had received the 12 month PPV-23 compared with 7 cases in those that had not during the same follow up period. There were no cases of IPD throughout the study period.

Discussion

This study has shown that 1, 2, or 3 doses of PCV-7 in infancy primed infants sufficiently elicit an excellent booster response to the PPV-23 at 12 months of age for all PCV-7 serotypes. Furthermore, there were good antibody responses to the 16 non-PCV-7 serotypes following PPV-23 at 12 months. The antibody concentrations for all 23 serotypes remained significantly higher at 17 months of age in the PPV-23 group compared to the group that had not received PPV-23.

In addition, this study has shown that priming with a single PCV-7 dose in infancy produced the greatest booster (memory) response for most serotypes following PPV-23 at 12 months compared with 2 or 3 PCV-7 doses. Responses following the PPV-23 were similar for those children that had received either 2 or 3 PCV-7 doses in infancy and lower than that in children who received a single PCV-7 dose. The immunological explanation for the single PCV-7 dose having a better booster response is not clear. Post booster antibody concentrations are usually higher in those that have had a stronger primary response [34]. One study found that a stronger primary response was more likely following higher doses of antigen and/or a higher concentration of carrier protein, possibly through the enhanced induction of antibody producing plasma cells [35]. However this would not explain the findings in our study of a better booster response in the single dose group as our previously published data has shown that a single PCV-7 dose (lower antigen dose) administered at 14 weeks of age induced a weaker primary response [29]. In that previous study, a significant immunological response was found in the single dose group compared with an unvaccinated control group, but significantly lower GMC for all PCV-7 serotypes compared to 2 or 3 PCV-7 doses [29].

Another possible explanation for the better booster response in the single PCV-7 dose group may be that a single antigen challenge rather than multiple antigen exposures, may preferentially drive the induction of memory B cells (which are required for a booster response), rather than plasma cells [36]. Having a greater pool of memory B cells would subsequently elicit a greater booster response. A fewer dose (single PCV-7 dose) primary series may preferentially induce B cell differentiation away from plasma cells, towards memory B cells compared to repeated antigen exposure associated with 2 or 3 PCV-7 dose primary series [8,11]. In summary, our findings are consistent with the suggestion that 2 or 3 PCV-7 doses in infancy are more effective at producing plasma cells [29] and less effective at producing memory B cells than a single PCV-7 dose. Alternatively, it is speculated that our findings may be explained by some form of immunological tolerance following 2 or 3 PCV-7 doses.

Our findings indicate that PCV-7/PPV-23 compared to the PCV-7 primary series without a booster should offer superior protection from pneumococcal disease lasting at least 5 months following the 12 month PPV-23. A recent study of asthmatic children aged 2-5 years underwent sequential immunization of PCV-7 followed by PPV-23 either 2 or 10 months post PCV-7 [37]. Antibody concentrations for PCV-7 and 2 non-PCV-7 serotypes (5 and 7F) were higher following the PPV-23 booster than after PCV-7 alone [37]. Despite superior antibody concentrations being demonstrated for PCV-7/PPV-23 compared with PCV-7/PCV-7, we would not advise PCV-7/PPV-23 for 3 reasons. Firstly, superior vaccine efficacy using PCV-7/PPV-23 against clinical disease has not been demonstrated. A study of vaccine efficacy against acute otitis media found that a PCV-7/PPV-23 compared to a PCV-7/PCV-7 schedule had similar results despite higher antibodies generated post PCV-7/PPV-23 [12]. This may be due to inferior quality of antibodies being produced following PPV-23. However previous studies have found that the quality of antibody, measured by avidity or opsonophagocytic activity, can differ in those that have received PPV-23 or PCV-7 as a

booster, however results have been conflicting and therefore inconclusive [8,10,38-40]. Finnish studies have shown the concentration of antibodies required for 50% killing was higher [38] and that the avidity of such antibodies was lower after PCV-7/PPV-23 compared with PCV-7/PCV-7 [8,39,41]. In contrast, another study in Finland using the 11-valent pneumococcal conjugate vaccine showed that opsonophagocytic activity was better in the group that received a PPV-23 booster at 12-15 months than those that had the conjugate booster [40]. A study in Israeli children who received 1 dose of the 7-valent pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine followed by either a conjugate or PPV-23 booster, achieved similar opsonic antibody titers in each group for the 1 serotype tested (6B) [8]. Data from the assessment of functional antibody responses in our study documenting the avidity to 23 serotypes and opsonophagocytic activity to 8 serotypes will be forthcoming.

Secondly, conjugate vaccines are the only vaccines that provide mucosal immunity. As nasopharyngeal (NP) carriage is an antecedent event in IPD, the reduction or prevention of NP carriage reduces the transmission of pneumococci and prevents IPD in the vaccinated individual and provides herd immunity [42-44]. In contrast, pneumococcal polysaccharide vaccines have shown no effect on pneumococcal carriage [20-24]. Most studies evaluating the impact of pneumococcal polysaccharide immunization in the absence of additional PCV-7 in infants or children have not shown any impact on pneumococcal disease or carriage [25-27]. This finding may be at least partially explained by the lack of effect that pneumococcal polysaccharide vaccine has on NP carriage. In contrast, one study in Papua New Guinea, where children aged six months to five years of age were given either the 14-valent or PPV-23 in one or two doses according to age, there was a (non-significant) 19% reduction in mortality from any cause, and a 50% reduction in pneumonia mortality (95%CI, 1-75%) [45]. Natural exposure in a population with a high incidence of pneumococcal infections, resulting in regular antigenic stimulation may explain this finding [13].

Thirdly, immunological hyporesponsiveness following PPV-23 at 12 months of age has been demonstrated by reduced responses to a small re-challenge dose of PPV-23 administered at 17 months of age (Russell FM, Carapetis JR, Balloch A, Licciardi PV, Jenney AWJ, Tikoduadua L, Waqatakirewa L, Pryor J, Nelson J, Byrnes GB, Cheung YB, Tang MLK, Mulholland EK, submitted). This attenuated response to the re-challenge dose may be due to depletion of the memory B cell pool [46]. A study documenting immunologic memory 5 years after meningococcal A/C conjugate vaccination in infancy showed that challenge with the meningococcal polysaccharide or conjugate at 2 years of age demonstrated immunologic memory. However subsequent challenge with polysaccharide at 5 years of age resulted in an inability to demonstrate memory in the polysaccharide group. The authors concluded that polysaccharide immunization at 2 years of age interfered with the immune response to subsequent polysaccharide vaccination [46]. One explanation for this is that polysaccharide immunization induces memory B cells to differentiate into plasma cells and secrete antibody but does not replenish the memory B cell pool [47]. Subsequent challenge with PPV-23 may then result in immune hyporesponsiveness. No adverse clinical effects have ever been documented due to repeated exposure to the meningococcal polysaccharide vaccine. In this study we demonstrated no adverse clinical consequences, although the study was not designed to evaluate this effect.

In summary, PPV-23 at 12 months induces an excellent booster response following 1, 2, or 3 doses of PCV-7 in infancy for all PCV-7 and significant responses for non-PCV-7 serotypes up to 5 months following vaccination. Booster responses were greatest for a single PCV-7 dose compared to 2 or 3 doses of PCV-7.

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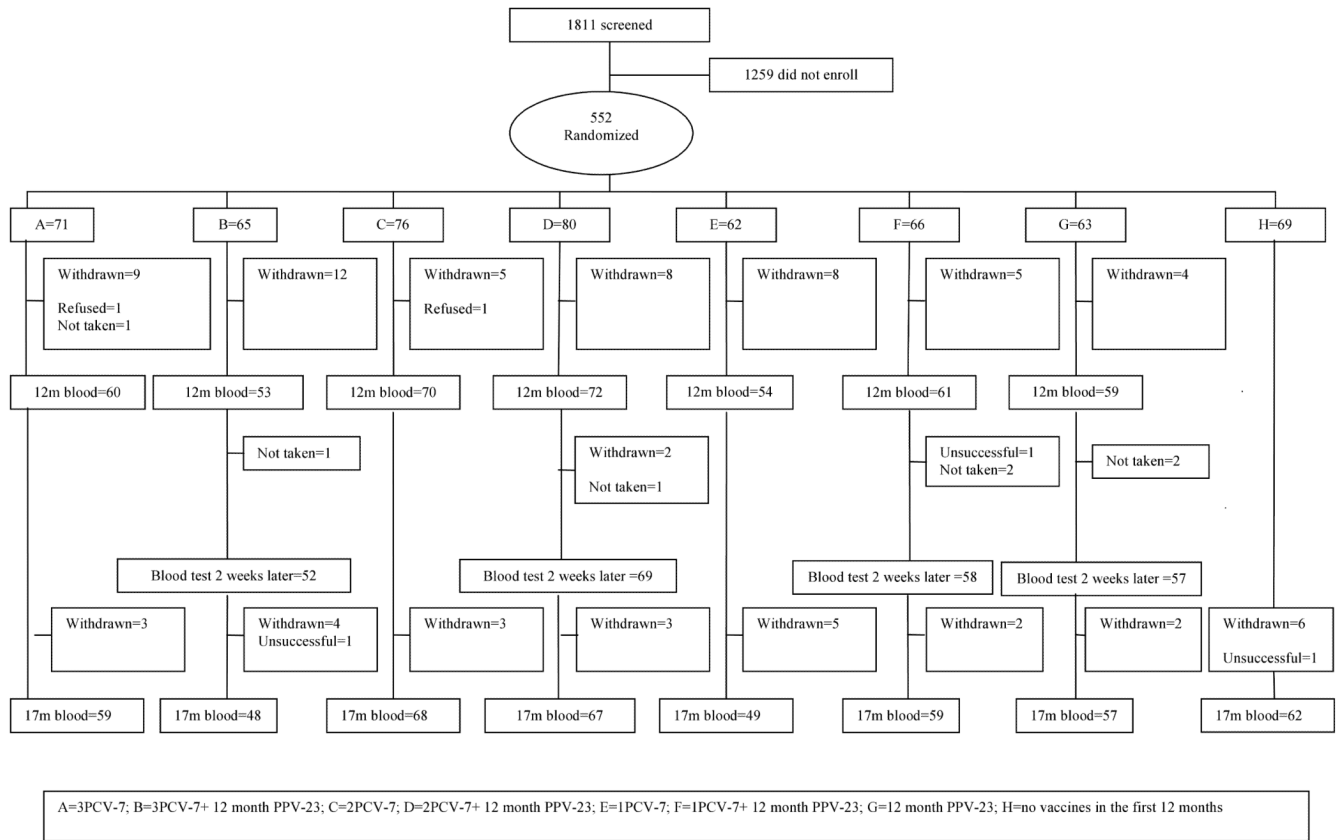


Figure 1. CONSORT chart of the screened and enrolled children to 17 months of age

Table 1
Serotype-specific IgG geometric mean concentrations (GMC and 95% confidence intervals) to PCV-7 serotypes before and 14 days following the 12 month PPV-23 and by number of PCV-7 doses administered in the primary series

	Pre-PPV-23 at 12 months of age				14 days post-12 month PPV-23			
	3 PCV-7 (n=52)	2 PCV-7 (n=69)	1 PCV-7 (n=58)	0 PCV-7 (n=57)	3 PCV-7/1.2 (n=52)	2 PCV-7/1.2 (n=69)	1 PCV-7/1.3 (n=58)	0 PCV-7/1.3 (n=57)
4	0.46 (0.37-0.57)	0.42 (0.34-0.52)	0.76 (0.53-1.08)	0.08 (0.06-0.09)	14.68 (11.10-19.42)	14.70 (11.72-18.43)	46.47 (36.65-58.92)	2.36 (1.76-3.16)
6B	0.85 (0.64-1.12)	0.79 (0.59-1.07)	0.69 (0.49-0.98)	0.14 (0.12-0.17)	29.58 (21.58-40.54)	23.83 (17.99-31.57)	18.07 (13.55-24.11)	0.31 (0.23-0.42)
9V	0.59 (0.49-0.73)	0.62 (0.53-0.71)	0.67 (0.49-0.89)	0.09 (0.07-0.11)	14.10 (10.60-18.75)	15.20 (12.03-19.21)	34.84 (27.03-44.90)	1.20 (0.90-1.61)
14	2.00 (1.48-2.72)	1.55 (1.21-1.99)	1.36 (1.01-1.83)	0.19 (0.16-0.24)	15.73 (10.48-23.60)	13.91 (9.87-19.60)	20.08 (12.19-33.05)	0.41 (0.29-0.58)
18C	0.37 (0.27-0.49)	0.23 (0.19-0.27)	0.22 (0.17-0.29)	0.06 (0.05-0.08)	8.71 (6.70-11.34)	10.51 (8.21-13.46)	16.03 (11.33-22.67)	1.22 (0.88-1.69)
19F	0.86 (0.64-1.16)	1.20 (0.93-1.55)	1.05 (0.79-1.39)	0.47 (0.36-0.61)	27.91 (19.98-38.99)	24.77 (18.21-33.68)	84.47 (55.80-127.87)	1.13 (0.80-1.59)
23F	0.52 (0.39-0.69)	0.41 (0.32-0.53)	0.31 (0.23-0.42)	0.19 (0.14-0.25)	10.87 (7.41-15.96)	10.29 (7.19-14.73)	8.33 (5.25-13.20)	0.42 (0.31-0.57)

¹ P-values were <0.001 for all serotypes comparing GMC pre/post 12 month PPV-23 for all PCV-7 dosage groups.

² For GMC comparisons between a single or 2 doses of PCV-7 with 3 PCV-7 doses, the p-values were not significant for all serotypes except for the 3 versus single dose comparison for serotypes 4, 9V, 18C, and 19F (each p<0.01).

³ P-values were <0.001 for all serotypes comparing GMC between 0 or a single dose of PCV-7 with 3 PCV-7 doses.

Table 2
Proportions of children with antibody concentrations ≥ 0.35 and $\geq 1\mu\text{g/mL}$ to PCV-7 serotypes before and 14 days post-12 month PPV-23 and by number of PCV-7 doses administered in the primary series

	Pre-PPV-23 at 12 months of age			14 days post-12 month PPV-23				
	3 PCV-7 (n=52)	2 PCV-7 (n=69)	1 PCV-7 (n=58)	0 PCV-7 (n=57)	3 PCV-7 (n=52)	2 PCV-7 (n=69)	1 PCV-7 (n=58)	0 PCV-7 (n=57)
$\geq 0.35\mu\text{g/mL}$								
4	63.5	60.9	64.9	7.0	98.1	100 ²	100 ²	94.7 ^{2,3}
6B	78.8	72.5	70.2	12.3	100	100 ²	100 ²	38.6
9V	73.1	81.2	75.4	8.8	100	100 ²	100 ²	87.7 ²
14	92.3	92.8	89.5	12.3	94.2 ¹	95.7 ^{1,2}	93.1 ^{1,2}	43.9
18C	44.2	27.5	31.6	5.3	100	100 ²	98.3 ²	82.5
19F	86.5	91.3	84.2	54.4	100 ¹	100 ^{1,2}	100 ²	84.2
23F	59.6	53.6	42.1	21.1	98.1	98.6 ²	93.1 ²	50.9
$\geq 1\mu\text{g/mL}$								
4	13.5	8.7	36.8	0	98.1	100 ²	100 ²	84.2
6B	46.2	36.2	33.3	3.5	98.1	100 ²	100 ²	14.0 ¹
9V	26.9	27.5	21.1	1.8	98.1	100 ²	100 ²	54.4
14	76.9	72.5	63.2	8.8	92.3	95.7 ²	89.7 ²	21.1 ¹
18C	13.5	2.9	3.5	0	98.1	95.7 ²	96.6 ²	57.9
19F	34.6	57.9	52.6	24.6	98.1	98.6 ²	96.6 ²	50.9
23F	30.8	15.9	15.8	5.3	94.2	88.4 ²	86.2 ²	17.5 ¹

A significant difference was observed for all comparisons except those marked:

¹ Comparison of proportions pre/post 12 month PPV-23.

² Comparison of proportions following 0, 1, or 2 doses with 3 doses of PCV-7.

³ Comparison of proportions following a single and no dose of PCV-7.

Table 3

Serotype-specific IgG GMC (and 95% confidence intervals) and proportions of children with antibody concentrations ≥ 0.35 and $\geq 1\mu\text{g/mL}$ to PCV-7 serotypes at 17 months in those who did or did not receive the 12 month PPV-23 and by number of PCV-7 doses in the primary series

	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)	0 PCV-7, PPV-23 (n=57)
GMC (95%CI)								
4	0.35 (0.29-0.43)	2.19 (1.79-2.69)	0.43 (0.33-0.56)	2.03 (1.70-2.42)	0.56 (0.39-0.80)	6.36 (4.94-8.19)	0.11 (0.09-0.13)	0.70 (0.53-0.91)
6B	0.91 (0.69-1.21)	4.35 (3.22-5.89)	0.78 (0.58-1.04)	3.85 (3.10-4.79)	0.62 (0.47-0.83)	3.88 (2.87-5.25)	0.20 (0.16-0.24)	0.21 (0.17-0.26) /
9V	0.41 (0.34-0.49)	2.25 (1.74-2.91)	0.49 (0.39-0.62)	2.50 (2.02-3.08)	0.51 (0.36-0.71)	5.49 (4.23-7.12)	0.14 (0.11-0.17)	0.38 (0.31-0.47)
14	1.78 (1.42-2.24)	4.31 (3.00-6.19)	1.12 (0.86-1.46)	3.58 (2.72-4.71)	0.93 (0.66-1.32)	4.85 (3.27-7.19)	0.31 (0.25-0.38)	0.67 (0.47-0.96)
18C	0.21 (0.18-0.26)	1.28 (1.07-1.53)	0.20 (0.16-0.25)	1.13 (0.92-1.38)	0.15 (0.12-0.19)	1.79 (1.37-2.34)	0.10 (0.08-0.12)	0.50 (0.39-0.66)
19F	1.19 (0.84-1.67)	5.55 (4.24-7.26)	1.06 (0.82-1.38)	4.53 (3.54-5.79)	0.92 (0.66-1.26)	13.47 (9.89-18.33)	0.59 (0.47-0.75)	0.79 (0.61-1.04) /
23F	0.57 (0.43-0.75)	1.68 (1.29-2.20)	0.43 (0.32-0.58)	1.41 (1.06-1.86)	0.32(0.22-0.48)	1.78 (1.28-2.49)	0.19 (0.16-0.23)	0.27 (0.21-0.34) /
Proportion $\geq 0.35\mu\text{g/mL}$								
4	52.5	100	51.5	100	63.3	98.3	6.5	73.7
6B	83.1	97.9 ²	72.1	100	73.5	98.3	21.0	24.6 ²
9V	61.0	97.9	60.3	98.5	61.2	100	12.9	56.1
14	98.3	95.8 ²	91.2	97.0 ²	83.7	94.9 ²	41.9	73.7
18C	22.0	97.9	22.1	94.0	10.2	93.2	9.7	66.7
19F	89.8	100 ²	91.2	100 ²	87.8	98.3 ²	72.6	82.5 ²
23F	64.4	93.8	54.4	88.1	34.7	89.3	17.7	43.9
Proportion $\geq 1\mu\text{g/mL}$								
4	5.1	85.4	23.5	88.1	30.6	96.6	1.6	38.6
6B	39.0	89.6	41.2	95.5	34.7	84.7	3.2	5.3 ²
9V	10.2	85.4	16.2	82.1	22.4	91.5	1.6	5.3 ²
14	69.5	89.6	47.1	85.1	42.9	81.4	9.7	28.1 ²
18C	3.4	66.7	5.9	59.7	2.0	69.5	3.2	31.6
19F	40.7	95.8	47.1	94.0	42.9	94.9	29.0	29.8 ²

	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)	0 PCV-7, PPV-23 (n=57)
GMC (95%CI)								
23F	22.0	75.0	22.1	65.7	10.2	72.9	1.6	5.3 ²

All comparisons were significant except:

¹ Comparison of GMC for 3, 2, 1 or 0 PCV-7 doses with or without the 12 month PPV-23.

² Comparison of the proportions with antibody concentrations ≥ 0.35 and $\geq 1\mu\text{g/mL}$ for 3, 2, 1 or 0 PCV-7 doses with or without the 12 month PPV-23.

Table 4
Serotype-specific IgG GMC (and 95% confidence intervals) and proportions of children with antibody concentrations ≥ 0.35 and $\geq 1\mu\text{g/mL}$ to non-PCV-7 serotypes before and 14 days post-12 month PPV-23

Serotype	Pre-PPV-23 at 12 months of age (n=235) ¹			14 days post-12 month PPV-23 (n=235) ¹		
	GMC ² (95%CI)	% $\geq 0.35\mu\text{g/mL}$ ³	% $\geq 1\mu\text{g/mL}$ ³	GMC ² (95%CI)	% $\geq 0.35\mu\text{g/mL}$ ³	% $\geq 1\mu\text{g/mL}$ ³
1	0.17 (0.15-0.19)	17.9	6.4	1.59 (1.38-1.82)	91.9	65.9
2	0.41(0.36-0.46)	54.5	13.6	10.73 (9.50-12.11)	100	98.7
3	0.27 (0.23-0.32)	34.9	10.6	8.28 (7.26-9.44)	99.1	96.6
5	0.26 (0.23-29)	35.7	11.5	2.26 (2.01-2.55)	97.9	78.7
7F	0.09 (0.08-0.10)	11.5	2.9	1.73 (1.51-1.99)	92.3	73.6
8	0.24 (0.21-0.28)	30.6	7.7	8.88 (7.82-10.09)	98.7	97.4
9N	0.23 (0.19-0.26)	27.7	7.7	8.31 (7.04-9.82)	98.3	93.6
10A	0.21 (0.19-0.24)	22.9	6.4	0.76 (0.66-0.89)	73.6	38.7
11A	0.09 (0.09-0.11)	12.8	7.2	1.51 (1.28-1.77)	87.2	68.9
12F	0.07 (0.07-0.08)	7.2	1.3	0.37 (0.31-0.43)	50.6	23.4
15B	0.29 (0.27-0.34)	37.9	9.4	2.15 (1.84-2.51)	91.1	76.2
17F	0.10 (0.09-0.11)	5.1	1.3	0.81 (0.68-0.96)	73.2	43.2
19A	0.46 (0.40-0.51)	56.6	17.9	1.93 (1.60-2.32)	86.8	63.8
20	0.09 (0.09-0.10)	4.3	1.3	0.68 (0.57-0.83)	66.1	39.1
22F	0.40 (0.36-0.46)	54.5	15.3	4.73 (3.81-5.87)	94.9	81.3
33F	0.13 (0.12-0.14)	7.7	2.9	1.66 (1.40-1.97)	84.3	69.4

¹ 235 pairs available for comparison. One pre-PPV-23 sample was not available for testing.

² P-values were <0.001 for all pre/post PPV-23 GMC comparisons for all serotypes.

³ P-values were <0.001 for comparisons of proportions with antibody concentrations $\geq 0.35\mu\text{g/mL}$ and $\geq 1\mu\text{g/mL}$ for all serotypes.

Table 5
Serotype-specific IgG GMC (and 95% confidence intervals) and proportions of children with antibody concentrations ≥ 0.35 and $\geq 1\mu\text{g/mL}$ to non-PCV-7 serotypes at 17 months of age in those that did or did not receive the 12 month PPV-23

	No PPV-23 at 12 months of age (n=238)		PPV-23 at 12 months of age (n=231)	
	GMC ¹ (95%CI)	% $\geq 0.35\mu\text{g/mL}^2$	GMC ¹ (95%CI)	% $\geq 1\mu\text{g/mL}^3$
1	0.23 (0.21-0.25)	25.6	0.63 (0.56-0.71)	27.3
2	0.51 (0.45-0.57)	65.5	2.88 (2.60-3.18)	92.2
3	0.30 (0.26-0.34)	34.9	1.46 (1.30-1.63)	69.3
5	0.31 (0.28-0.34)	41.6	0.77 (0.69-0.86)	39.4
7F	0.12 (0.10-0.13)	11.3	0.51 (0.45-0.57)	22.9
8	0.29 (0.26-0.33)	34.9	2.31 (2.08-2.56)	86.6
9N	0.23 (0.20-0.26)	24.4	1.90 (1.64-2.19)	74.0
10A	0.19 (0.17-0.21)	16.4	0.27 (0.25-0.31)	6.5
11A	0.14 (0.12-0.16)	19.7	0.35 (0.31-0.41)	15.6
12F	0.09 (0.08-0.10)	5.0	0.18 (0.16-0.21)	6.1
15B	0.30 (0.27-0.34)	37.4	0.74 (0.66-0.84)	39.8
17F	0.12 (0.11-0.13)	6.3	0.36 (0.31-0.41)	16.9
19A	0.59 (0.53-0.67)	71.4	1.14 (1.00-1.31)	57.1
20	0.13 (0.12-0.15)	12.6	0.26 (0.23-0.30)	10.0
22F	0.46 (0.41-0.51)	59.7	1.43 (1.22-1.68)	59.7
33F	0.18 (0.17-0.20)	15.1	0.62 (0.54-0.71)	36.4

¹ P-values were <0.001 for all serotypes.

² P-values were <0.001 comparing proportions $\geq 0.35\mu\text{g/mL}$ for all serotypes.

³ P-values were <0.01 comparing proportions $\geq 1\mu\text{g/mL}$ for all serotypes except serotypes 10A and 11A.

Table 6
Non-serious adverse events¹ in those children who received PPV-23 at 12 months of age (n=245)

Systemic adverse events	Number (%)
High fever ($\geq 38^{\circ}\text{C}$ per axilla)	15 (6.1)
Low grade fever ($>37^{\circ}\text{C}$ & $<38^{\circ}\text{C}$ per axilla)	69 (28.2)
Diarrhoea	2 (0.8)
Vomiting	4 (1.6)
Severe vomiting ²	1 (0.4)
Anorexia	11 (4.5)
Severe anorexia ²	2 (0.8)
Drowsiness	13 (5.3)
Severe drowsiness ²	2 (0.8)
Irritability	23 (9.4)
Severe irritability ³	2 (0.8)
Local reactions	
Erythema	15 (6.1)
$>30\text{mm}^2$	3 (1.2)
Tenderness	13 (5.3)
Induration	13 (5.3)
$>30\text{mm}^2$	3 (1.2)

¹ All cases were classified as mild or moderate unless otherwise stated

² Resolved within 2 days

³ Resolved within 1 day