Online Repository

Methods

Study samples

Blood samples were sent to the National Centre for Communicable Disease Reference Laboratory (Mataika House, Suva) where they were centrifuged at 1,000 x g for 10 min and the plasma was collected and stored at -80°C until analysis. Freshly-isolated peripheral blood mononuclear cells (PBMCs) were collected using Lymphoprep (Axis-Shield, Norway) density gradient centrifugation for the memory B cell assays. NP swabs were placed into 1 ml skim milk, tryptone, glucose, and glycerol (STGG) 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 h and then stored at 4°C overnight (O/N). Serum and control samples were diluted 1:100 in a pre-absorption buffer of phosphate-buffered saline with 10% (w/v) Foetal Calf Serum (PBS/FCS) containing cell-wall polysaccharide (CPS; 10μg/mL) and serotype 22F (30μg/mL) to remove non-specific antibodies and incubated O/N at 4°C. For the serotype 22F ELISA, serum samples are pre-absorbed with CPS only. The next day, plates were washed with PBS containing 0.05% (v/v) Tween20 (PBS-T) and blocked with PBS/FCS and incubated at 37°C for 1 hour. The reference standard serum 89-SF (Food and Drug Administration, Bethesda, USA) was pre-absorbed with CPS only as the assigned serotype-specific IgG values are based on ELISA measurement that used pre-absorption with CPS but not serotype 22F. Following this incubation, serial dilutions of the pre-absorbed 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. Following another wash step with PBS-T, the reaction was developed by incubation with a 3.3', 5.5'-tetramethylbenzidine (TMB) substrate solution for nine minutes and stopped with 1M phosphoric acid. Optical density at 450nm (630nm reference filter) was measured using a microplate reader. Serotype-specific IgG concentrations for each patient sample were derived from the 89-SF standard values and expressed in μg/mL.

Multiplex Opsonophagocytosis Assay

The MOPA 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 min before making serial dilutions and 20μL/well of each dilution were tested in duplicate in round-bottom 96-well plates (Corning Inc., Corning, NY). Eleven 2·3-fold serial dilutions were used for inter-assay variability experiments, and eight three-fold dilutions were used for all other experiments. Frozen aliquots of target pneumococci were thawed, washed twice with opsonisation buffer B (Hanks' balanced salt solution [HBSS] with magnesium and calcium, 0.1% gelatin, and 10% FBS) and diluted to ~$2x10^5$ CFU/ml of each serotype for multiplexed assays. For MOPA, equal volumes of four bacterial suspensions in one assay group are pooled and 10μL of bacterial suspension added to each well. After 30 min of incubation at room temperature (RT), 10μL of complement and 40μL of HL60 cells (~$4x10^5$ cells) were added to each well (HL60 cells washed twice with HBSS before use). Plates were incubated

in a tissue culture incubator (37°C, 5% CO_2) with shaking (mini orbital shaker; Bellco Biotechnology, Vineland, NJ) at $100_{x\,g}$. After a 45 min, plates were placed on ice for ~12 min and 10 µL of the final reaction mixture was spotted onto four different plates of Todd-Hewitt agar supplemented with 0.5% yeast extract. An overlay agar containing one of the four antibiotics (selective markers for target bacteria) was added to one of each of the Todd-Hewitt agar plates and incubated O/N at 37°C. The number of bacterial colonies was then enumerated. Results were expressed as opsonisation indices (OIs), defined as the interpolated dilution of serum that kills 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 analysis purposes. A cut off of 8 was used as the cut-off for a positive response.

Enumeration of pneumococcal-specific memory B cells

Enumeration of pneumococcal-specific memory B cells was done to 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 $2x10^6$ cells/mL and 100µL added to each well along with 100µL of an antigen cocktail (Staphylococcus aureus Cowan strain – Pansorbin cells [SAC; 1:5000)], 2·5µg/mL CpG and 83ng/mL pokeweed mitogen). Plates were incubated at 37°C with 5% CO_2 and 95% humidity for 5 days. At day 5, cells were harvested by gentle re-suspension, added to a 30mL tube filled with RPMI-EDTA + 0.5% FCS (RPMI-FCS). Cells were washed in RPMI-FCS following centrifugation at 800x g for 20 min and twice following centrifugation at 650x g for 15 min. After counting with trypan blue, cells were resuspended in RPMI-FCS at a final concentration of $2x10^6$ cells/mL for seeding onto antigen-coated ELISPOT plates.

Multiscreen hydrophobic polyvinyldenedifluoride (PVDF) membrane ELISPOT plates were coated with anti-IgG (10µg/mL), tetanus toxoid (5µg/mL), diphtheria toxoid (10µg/mL) or pneumococcal polysaccharides conjugated to methylated human serum albumin at concentrations in the range 10-20µg/mL and were sealed and incubated O/N at 4°C. The following day, ELISPOT plates were washed three times with PBS and blocked with RPMI-FCS for 30 mins at 37°C with 5% CO_2 and 95% humidity.

Cultured cells were seeded at $2x10^5$ cells/well into the antigen-coated ELISPOT plates and incubated O/N at 37°C with 5% CO_2 and 95% humidity. Cells were then washed with PBS-T and bound IgG was detected following incubation with an alkaline phosphatase-conjugated IgG for four hours at RT. ELISPOT plates were washed with PBS-T four times before addition of an alkaline phosphatase substrate solution (nitroblue tetrazolium plus 5-bromo-4-chloro-3-indoylphosphate in dimethyl formamide) to all wells to allow spots to develop. The reaction was stopped with two washes in distilled water. Cells were visualized and counted using an automated ELISPOT reader and software. The total frequency of IgG-secreting antibody-forming cells (AFCs) was used as the positive control and samples with <1,000 IgG AFCs/10^6 cultured PBMCs were removed from analysis.

Nasopharyngeal carriage measurement

Swab samples were thawed, diluted 1:2 in STGG and 50µl inoculated onto Columbia horse blood agar plates (Oxoid, Thermo Fisher Scientific, Australia) containing 5µg/mL gentamicin. Plates were incubated at 37°C with 5% CO_2 for 36-44 h. Two randomly selected α-haemolytic colonies, plus any additional morphologically distinct α-haemolytic colonies were subcultured and pneumococcal isolates identified by optochin sensitivity, bile solubility testing and Phadebact® Pneumococcus test (Boule Diagnostics AB, Huddinge, Sweden).

Serotyping was performed by latex agglutination as previously described (Porter et al. J Vis Exp. 2014; 91:51747). Ten percent were also serotyped by a Quellung reaction using specific antisera (Statens Serum Institute, Copenhagen, Denmark). Laboratory staff members were blinded to the group allocation for each isolate.

Figures

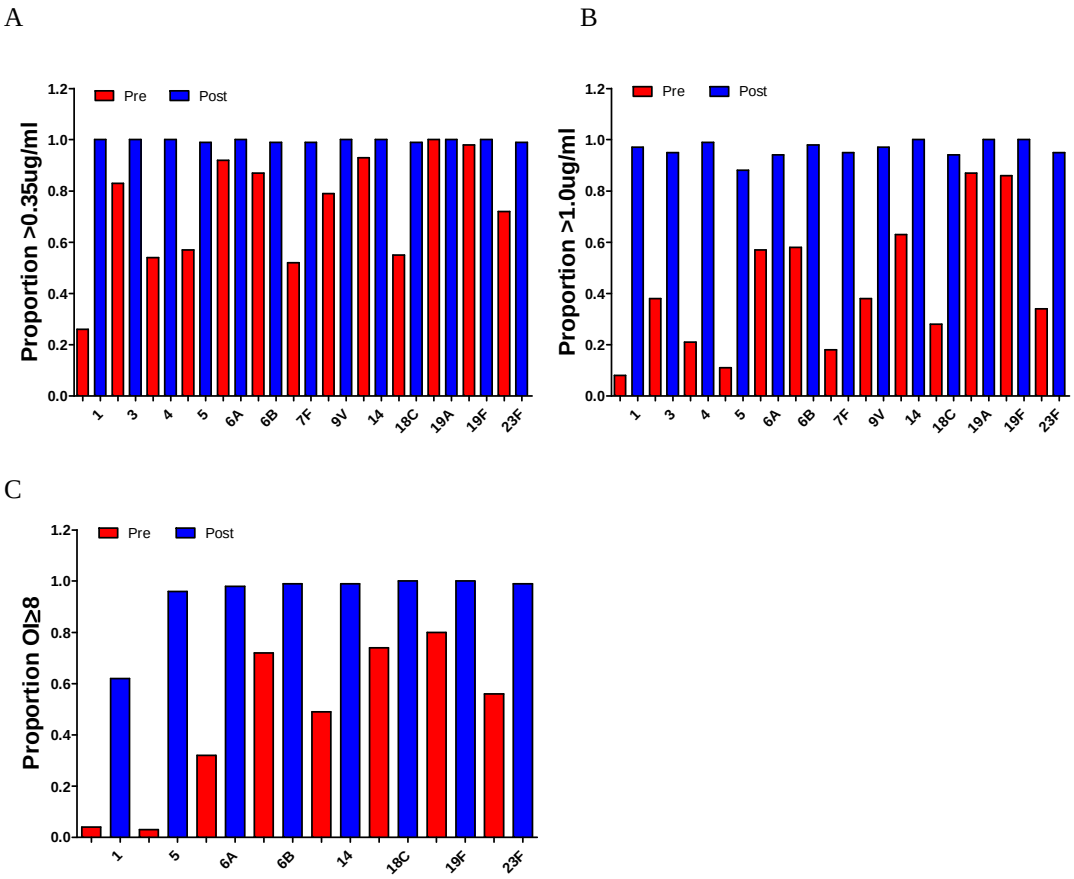

Figure E1: Response to PCV13 immunisation in children aged 5-7 years old. Proportion of children with A) serotype-specific IgG levels ≥0.35µg/ml, B) serotype-specific IgG levels ≥1.0µg/ml and C) opsonophagocytic responses, OI≥8 are shown before (red bars) and after (blue bars) immunisation with PCV13 (N=185 paired individuals). Data are taken from 185 paired samples. For Panel A, $p<0.0001$ for all serotypes except 19A, 19F (both not significant), 14 ($p=0.0002$); Panel B, $p<0.0001$ for all serotypes except 19A, 19F; Panel C, $p<0.0001$ for all serotypes.

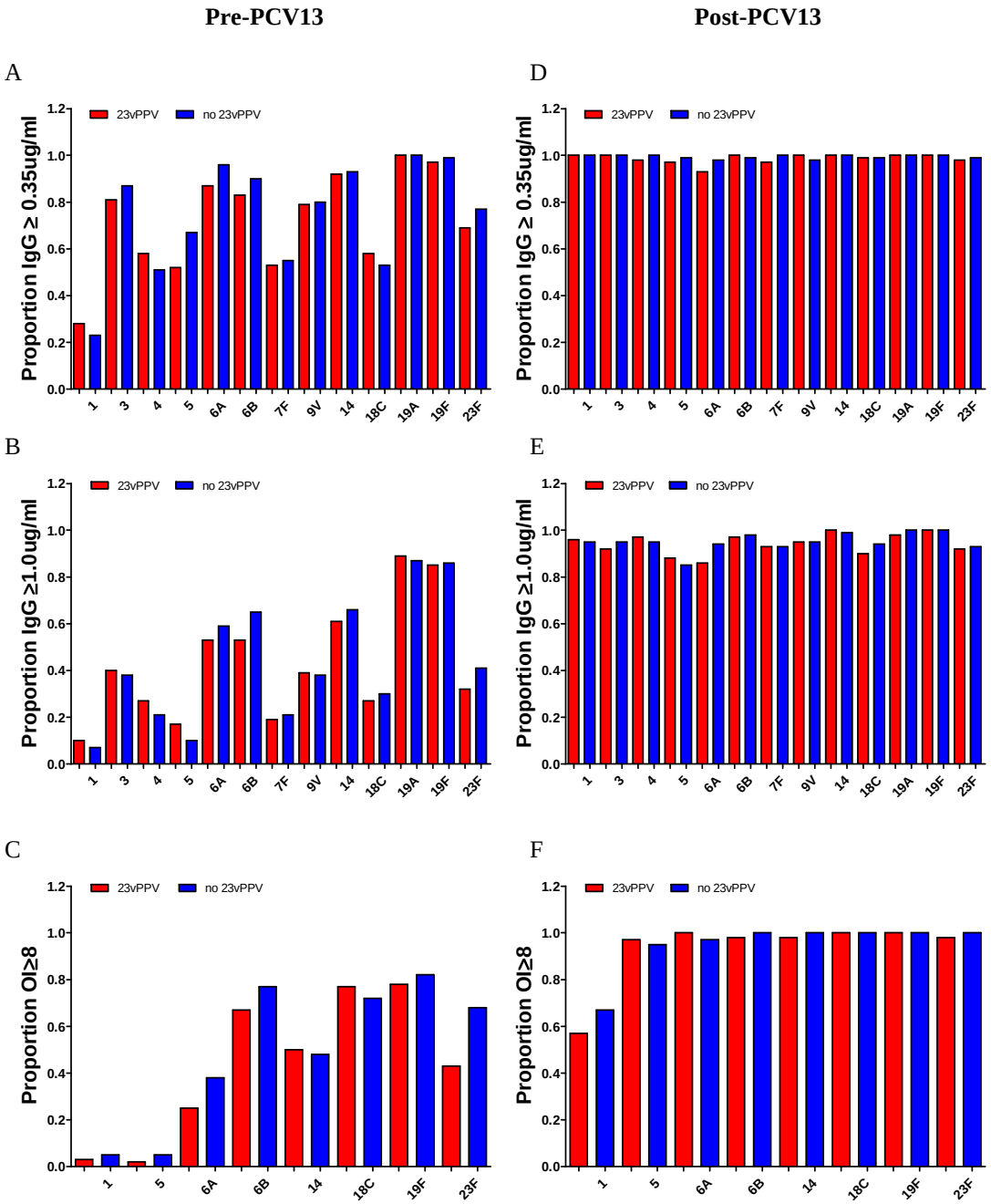

Figure E2: Response to PCV13 immunisation in children who did (red bars) or did not (blue bars) receive 23vPPV at 12-months of age. The proportion of children with serotype-specific IgG levels ≥0.35µg/ml, serotype-specific IgG levels ≥1.0µg/ml and opsonophagocytic responses are shown pre-PCV13 (Panels A-C) and post-PCV13 (Panels D-F). 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 Panel C, serotype 23F (p=0.0098).

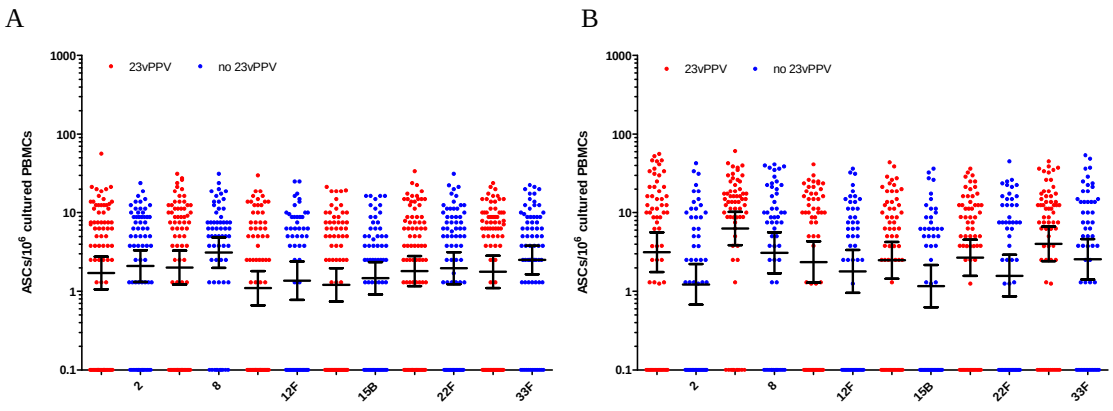

Figure E3: Response to PCV13 immunisation in children who did (red circles) or did not (blue circles) receive 23vPPV at 12-months of age. Enumeration of pneumococcal-specific memory B cells for non-PCV13 serotypes at A) pre-PCV13 and B) 28-days PCV13 post-immunisation. Data are presented as median ± IQR for children that did (N=98) or did not (N=87) receive 23vPPV at 12 months of age. No significant differences were found.

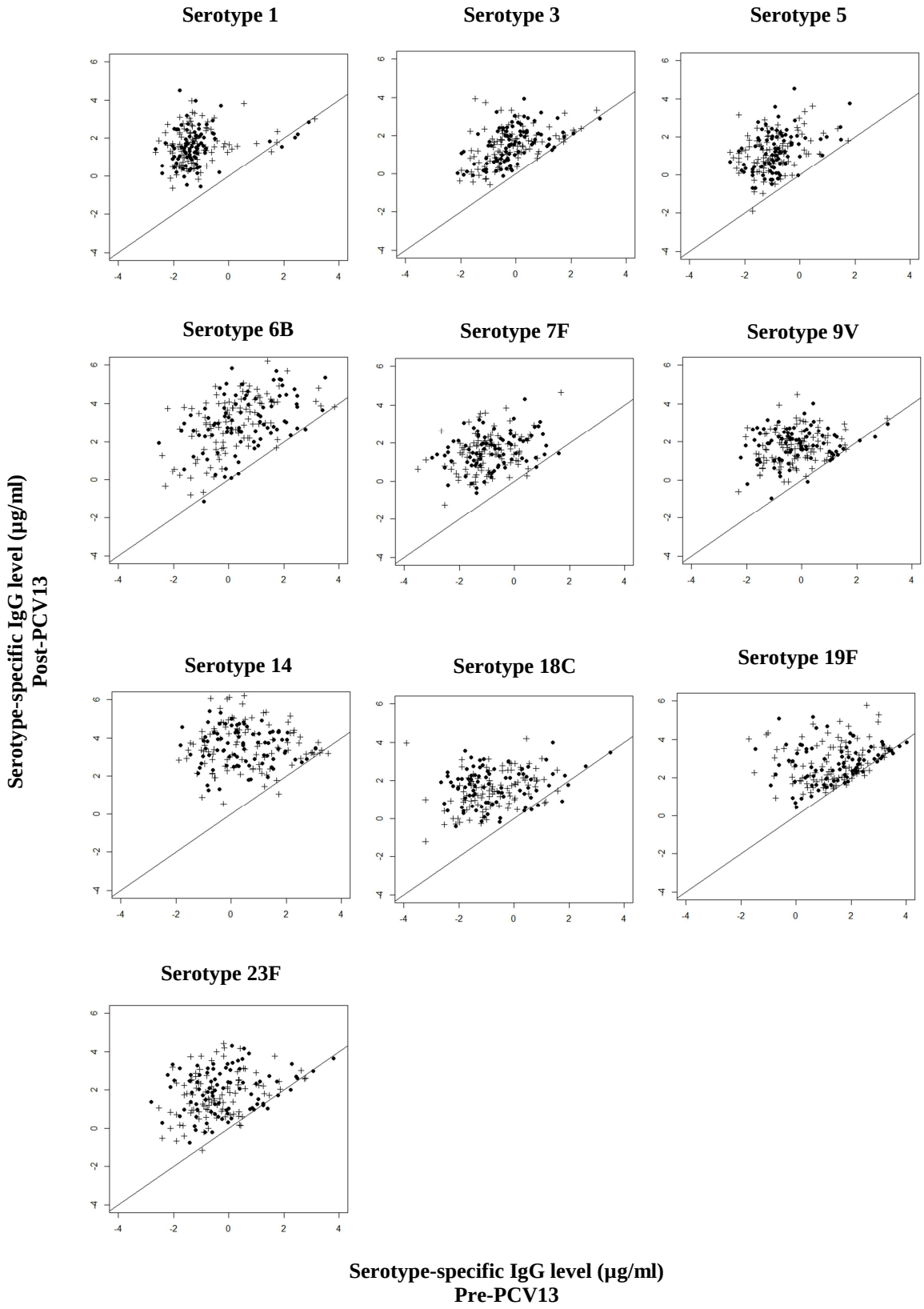

Figure E4: Response to PCV13 immunisation in children, now aged 5-7 years, who did (cross) or did not (dark circles) receive 23vPPV at 12 months of age. Children responded similarly to PCV13 serotypes regardless of 23vPPV receipt. The diagonal line represents no change from pre-PCV13 (baseline) levels.

Table E1: Serotype-specific IgG GMC ± 95% CI (µg/mL) 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)

*Serotype 6A is not included in 23vPPV; N=98 children who received 23vPPV at 12 months of age; N=87 children who did not receive 23vPPV at 12 months of age
No significant differences were found for any comparison

Table E2: Serotype-specific GMOI ± 95% CI in children who did or did not receive prior 23vPPV at 12 months of age, pre- and post-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)

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

Table E3: Serotype-specific AFCs (Median ± IQR) in children who did or did not receive prior 23vPPV at 12 months of age, pre- and post-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)

AFCs = antibody-forming cells; IQR = Interquartile range; No significant differences were found for all other comparisons

Table E4A: Proportion of children with serotype-specific IgG ≥ 0.35µg/ml 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 N (%)	**No prior 23vPPV N (%)**	**Prior 23vPPV N (%)**	**No prior 23vPPV N (%)**
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)

*Serotype 6A is not included in 23vPPV; N=98 children that received 23vPPV; N=87 children that did not receive 23vPPV

No significant differences were found for any comparison

Table E4B: Proportion of children with serotype-specific IgG ≥ 1.0µg/ml 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 N (%)	No prior 23vPPV N (%)	Prior 23vPPV N (%)	No prior 23vPPV N (%)
PCV13 serotypes	9 (9)	5 (6)	95 (97)	84 (97)
1	38 (39)	32 (37)	91 (93)	84 (97)
3	24 (24)	15 (19)	98 (100)	85 (98)
4	13 (13)	8 (9)	88 (90)	75 (86)
5	52 (53)	56 (64)	95 (97)	86 (99)
6B	17 (17)	17 (20)	93 (95)	82 94)
7F	37 (38)	25 (29)	95 (97)	84 (97)
9V	60 (61)	56 (64)	98 (100)	87 (100)
14	26 (27)	25 (29)	89 (91)	84 (97)
18C	85 (87)	76 (87)	98 (100)	87 (100)
19A	84 (86)	75 (86)	98 (100)	87 (100)
19F	30 (31)	33 (38)	92 (94)	83 (95)
23F				
Non-PCV13 serotypes	39 (40)	31 (36)	49 (50)	45 (52)
2	27 (28)	22 (25)	27 (28)	23 (26)
8	14 (14)	33 (38)	67 (68)	63 (72)
9N	30 (31)	36 (41)	37 (38)	36 (41)
10A	32 (33)	26 (30)	29 (30)	28 (32)
11A	3 (3)	3 (3)	4 (4)	3 (3)
12F	33 (34)	29 (33)	53 (54)	45 (52)
15B	17 (17)	15 (19)	18 (18)	16 (18)
17F	30 (31)	22 (25)	29 (30)	27 (31)
20	38 (39)	37 (43)	39 (40)	38 (44)
22F	19 (19)	12 (14)	22 (22)	19 (22)
33F				

*Serotype 6A is not included in 23vPPV; N=98 children that received 23vPPV; N=87 children that did not receive 23vPPV

No significant differences were found for any comparison

Table E5: Proportion of children with OI ≥ 8 in children who did or did not receive prior 23vPPV at 12 months of age, pre- and post-PCV13

Serotype	Pre-PCV13		Post-PCV13	
	Prior 23vPPV N (%)	No prior 23vPPV N (%)	Prior 23vPPV N (%)	No prior 23vPPV N (%)
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)

N=60 children per group who did or did not receive 23vPPV at 12 months of age; *p=0.0058 between children that did or did not receive 23vPPV pre-PCV13; No significant differences were found for all other comparisons

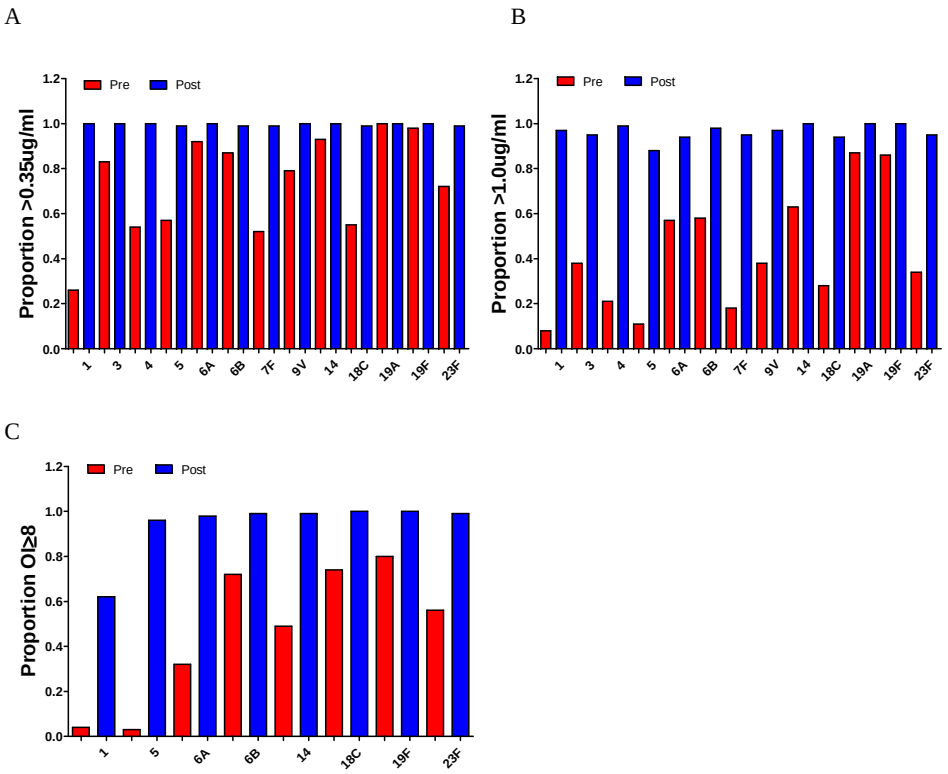

Figure E1: Response to PCV13 immunisation in children aged 5-7 years old. Proportion of children with A) serotype-specific IgG levels ≥0.35µg/ml, B) serotype-specific IgG levels ≥1.0µg/ml and C) opsonophagocytic responses, OI≥8 are shown before (red bars) and after (blue bars) immunisation with PCV13 (N=185 paired individuals). Data are taken from 185 paired samples. For Panel A, $p<0.0001$ for all serotypes except 19A, 19F (both not significant), 14 ($p=0.0002$); Panel B, $p<0.0001$ for all serotypes except 19A, 19F; Panel C, $p<0.0001$ for all serotypes.

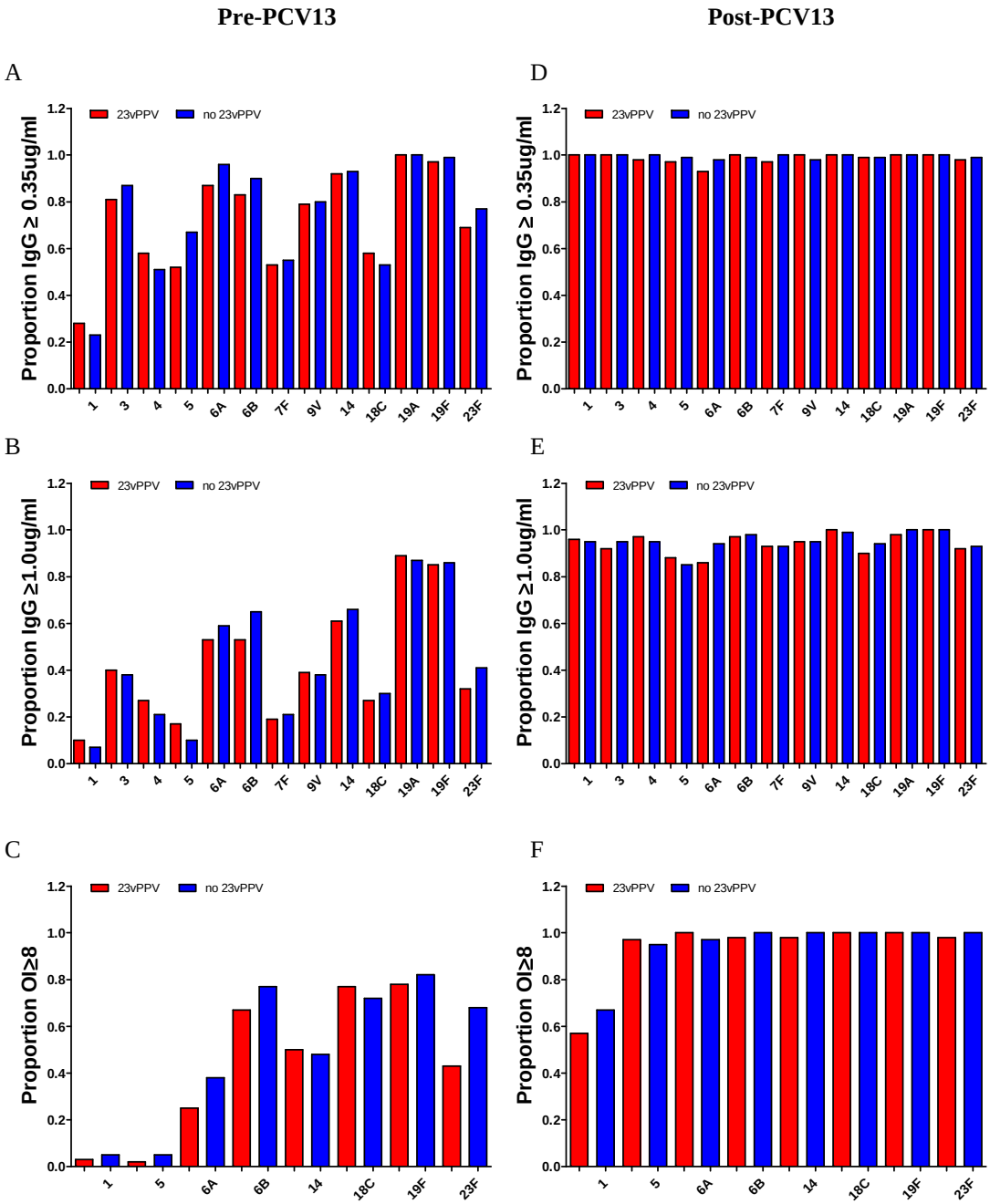

Figure E2: Response to PCV13 immunisation in children who did (red bars) or did not (blue bars) receive 23vPPV at 12-months of age. The proportion of children with serotype-specific IgG levels ≥0.35µg/ml, serotype-specific IgG levels ≥1.0µg/ml and opsonophagocytic responses are shown pre-PCV13 (Panels A-C) and post-PCV13 (Panels D-F). 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 Panel C, serotype 23F (p=0.0098).

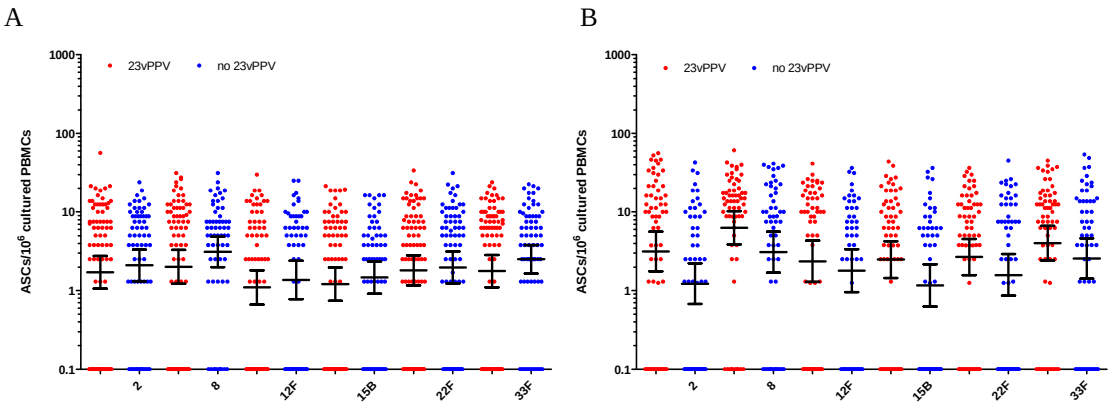

Figure E3: Response to PCV13 immunisation in children who did (red circles) or did not (blue circles) receive 23vPPV at 12-months of age. Enumeration of pneumococcal-specific memory B cells for non-PCV13 serotypes at A) pre-PCV13 and B) 28-days PCV13 post-immunisation. Data are presented as median ± IQR for children that did (N=98) or did not (N=87) receive 23vPPV at 12 months of age. No significant differences were found.

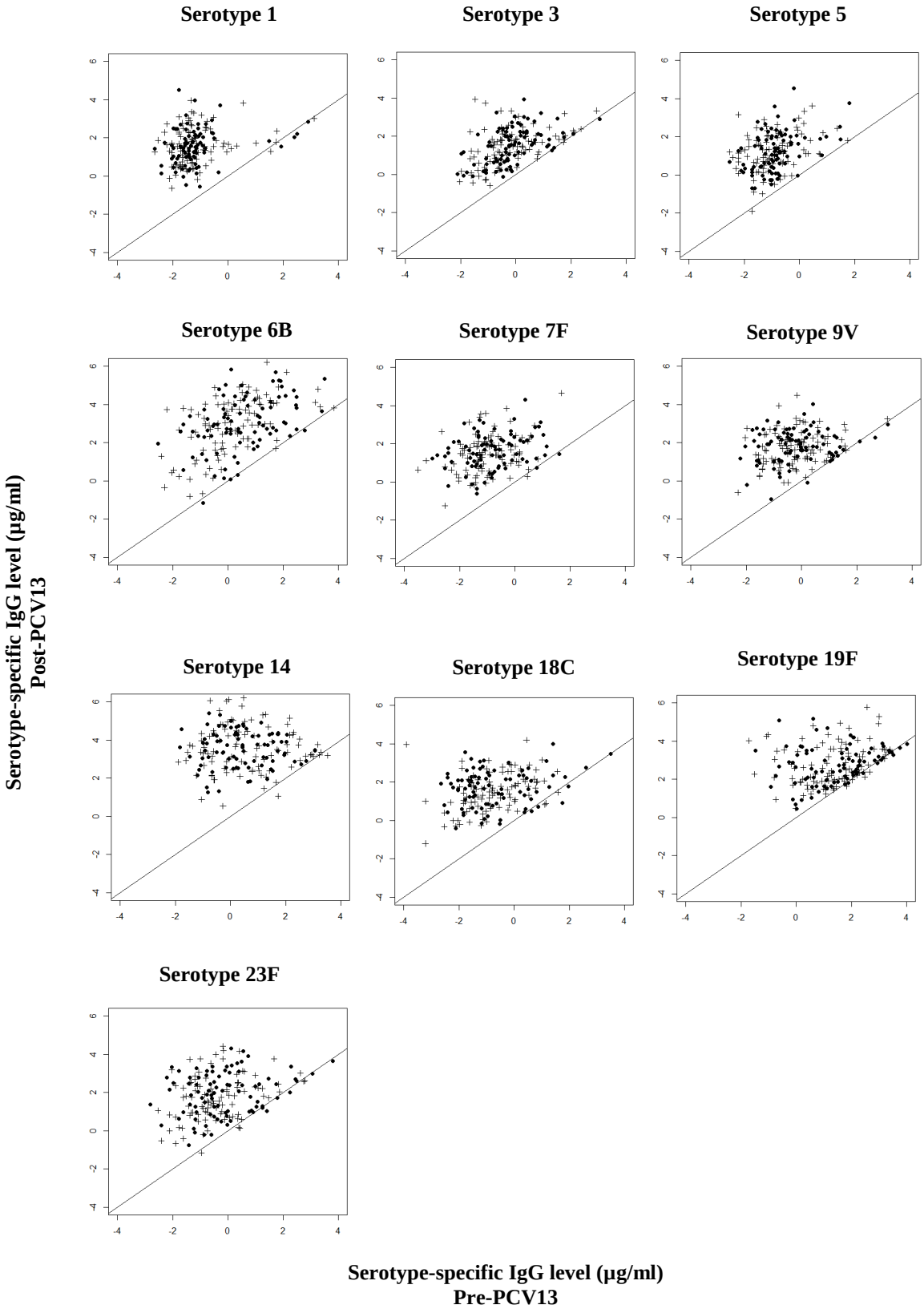

Figure E4: Response to PCV13 immunisation in children, now aged 5-7 years, who did (cross) or did not (dark circles) receive 23vPPV at 12 months of age. Children responded similarly to PCV13 serotypes regardless of 23vPPV receipt. The diagonal line represents no change from pre-PCV13 (baseline) levels.