



Bacteria and Bacterial Diseases

Immune impacts of infant whole-cell and acellular pertussis vaccination on co-administered vaccines

Gladymar Pérez Chacón ^{a,b,1,2}, Sonia McAlister ^{a,c,2}, James Totterdell ^d, Marie J. Estcourt ^d, Julie A. Marsh ^{a,e}, Mark Jones ^d, Kirsten P. Perrett ^{f,g,h}, Dianne E. Campbell ^{i,j}, Nicholas Wood ^j, Michael Gold ^k, Claire S. Waddington ^l, Michael O'Sullivan ^{c,m,n}, Nigel Curtis ^{h,o,p}, Ushma Wadia ^{a,e}, Peter B. McIntyre ^q, Patrick G. Holt ^r, Tom Snelling ^{a,b,d}, Peter C. Richmond ^{a,c,*}

^a Wesfarmers Centre of Vaccines and Infectious Diseases, The Kids Research Institute Australia, Nedlands, Western Australia, Australia

^b School of Population Health, Faculty of Health Science, Curtin University, Perth, Western Australia, Australia

^c Division of Paediatrics, School of Medicine, The University of Western Australia, Perth, Western Australia, Australia

^d Sydney School of Public Health, Faculty of Medicine and Health, University of Sydney, Sydney, New South Wales, Australia

^e Centre for Child Health Research, School of Medicine, The University of Western Australia, Perth, Western Australia, Australia

^f Population Allergy, Murdoch Children's Research Institute, Parkville, Victoria, Australia

^g Department of Allergy and Immunology, Royal Children's Hospital, Melbourne, Victoria, Australia

^h Department of Paediatrics, The University of Melbourne, Melbourne, Victoria, Australia

ⁱ Department of Allergy and Immunology, The Children's Hospital at Westmead, Sydney, New South Wales, Australia

^j Discipline of Child and Adolescent Health, The University of Sydney, Sydney, New South Wales, Australia

^k Discipline of Paediatrics, School of Medicine, University of Adelaide, Adelaide, South Australia, Australia

^l Liverpool School Tropical Medicine, Liverpool, United Kingdom

^m Department of Immunology, Perth Children's Hospital, Nedlands, Western Australia, Australia

ⁿ The Kids Research Institute Australia, Nedlands, Western Australia, Australia

^o Infectious Diseases Unit, Royal Children's Hospital Melbourne, Parkville, Victoria, Australia

^p Infectious Diseases Group, Murdoch Children's Research Institute, Parkville, Victoria, Australia

^q Dunedin School of Medicine, University of Otago, Dunedin, New Zealand

^r Wal-yan Respiratory Research Centre, The Kids Research Institute Australia, Nedlands, Western Australia, Australia

ARTICLE INFO

Article history:

Accepted 17 May 2025

Available online 23 May 2025

Keywords:

Diphtheria-Tetanus-Pertussis Vaccine/
immunology

Diphtheria-Tetanus-acellular Pertussis
Vaccines

Vaccines, Combined

Vaccines, Conjugate/immunology

Pneumococcal Conjugate Vaccine

Haemophilus Vaccines

Hepatitis B Vaccines

SUMMARY

Objectives: We compared the effect of a heterologous wP/aP/aP primary series (hereafter mixed wP/aP) versus a homologous aP/aP/aP primary schedule (hereafter aP-only) on antibody responses to co-administered vaccine antigens in infants and toddlers.

Methods: We randomised Australian infants in a 1:1 ratio to receive either a mixed wP/aP schedule (pentavalent diphtheria-tetanus-wP-hepatitis B-Haemophilus influenzae type b; DTwP-HepB-Hib vaccine at 6 weeks old, followed by hexavalent DTaP-inactivated poliovirus vaccine (IPV)-HepB-Hib vaccine at 4 and 6 months old) or aP-only priming doses of hexavalent DTaP-IPV-HepB-Hib vaccine at the same ages. All infants received 13-valent pneumococcal conjugate vaccine (13vPCV) at 6 weeks, 4 and 12 months of age and DTaP-IPV and Hib vaccine boosters at 18 months. We assessed whether the wP/aP schedule is non-inferior to the aP-only schedule for co-administered vaccine antigens (geometric mean ratio [GMR] > 2/3). Registration: ACTRN12617000065392p.

Results: Between March 2018 and January 2020, 150 infants were randomised (75 per arm). Responses to all 13vPCV serotypes and Hib-PRP at 6, 7, 18, and 19 months old, as well as HBsAg at 6 and 7 months old, were non-inferior (> 90% probability).

Conclusion: A mixed wP/aP schedule resulted in non-inferior IgG responses to co-administered vaccine antigens compared to the standard aP-only schedule for pertussis primary immunisation.

© 2025 Published by Elsevier Ltd on behalf of The British Infection Association. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Correspondence to: General Paediatrics and Immunology Departments, Child and Adolescent Health Service, Perth Children's Hospital, 15 Hospital Avenue, Nedlands, Perth 6009, Western Australia, Australia.

E-mail address: peter.richmond@uwa.edu.au (P. Richmond).

¹ Present address: The Kirby Institute, University of New South Wales, Sydney, New South Wales, Australia.

² Contributed equally as first authors.

Introduction

Vaccinating infants with whole-cell pertussis (wP) rather than acellular pertussis (aP) vaccine formulations elicits different, long-lived immune responses and provides enhanced protection against pertussis infection.^{1,2} In recipients of homologous pertussis vaccine priming, the type of pertussis vaccine used as the first dose of the primary series – formulations of diphtheria and tetanus toxoids with either wP (DTwP) or aP (DTaP) – appears to influence T-cell responses to tetanus toxoid (TT) and to diphtheria toxoid (DT).^{3,4}

Bacterial capsular polysaccharides, like those in *Streptococcus pneumoniae* or *Haemophilus influenzae* type b (Hib), trigger T-cell-independent B-cell responses. In conjugated pneumococcal or Hib vaccines, these polysaccharides are bound to an immunogenic protein carrier (such as TT or CRM₁₉₇, the non-toxic mutant of diphtheria toxin). This binding recruits carrier-specific CD4⁺ T follicular helper cells, leading to a T-dependent antibody response, which is crucial for preventing invasive pneumococcal or Hib disease in infants.⁵ However, immunisation with pertussis vaccines and conjugated polysaccharide vaccines may interact unpredictably.⁶ Interactions are influenced by the type and concentration of protein carriers,⁷ timing and order of vaccination,⁸ coadministration with inactivated poliovirus vaccine (IPV),⁹ off-target responses elicited by *Bordetella pertussis* lipopolysaccharide (present in wP but not in aP vaccines),¹⁰ and interference from the presence of antibodies prior to vaccination.^{11,12} In contrast, while historical data provide no evidence of a difference in seroprotective responses to simultaneously administered hepatitis B vaccine among recipients of wP versus aP-based primary series,¹³ IgG geometric mean concentrations (GMCs) of the hepatitis B (HepB) surface antigen (HBsAg) appear to be lower among recipients of aP versus wP combination vaccines.¹⁴

In response to reactogenicity concerns, Australia transitioned from a wP-only to an aP-only primary series in the late 1990s. Observational studies suggest that, among the birth cohorts born during the switchover period from wP-only to aP-only regimens, a first dose of wP, compared to aP, appeared to be protective against the development of childhood IgE-mediated food allergy.^{15,16} The latter remains a major public health problem in this country, affecting up to 10% of infants by age 12 months. Before a novel heterologous pertussis primary vaccination schedule can be recommended, it is important to ensure no unintended adverse impact on the immunogenicity of other routine vaccines, such as pneumococcal conjugate and Hib, and hepatitis B vaccines. We previously reported the non-inferiority of a mixed wP/aP primary schedule compared to the standard aP-only primary schedule with respect to anti-diphtheria, tetanus, and pertussis IgG responses at 7 months old, approximately one month after completion of the primary series.¹⁷ Here we report and compare antibody responses to concurrently administered 13-valent pneumococcal conjugated vaccine (13vPCV), Hib capsular polysaccharide (Hib-PRP) and HBsAg among infants randomly assigned to either a heterologous wP/aP/aP (hereafter mixed wP/aP) or homologous aP/aP/aP (hereafter aP-only) priming schedule.

Methods

Study design and participants

OPTIMUM (Optimising Immunisation Using Mixed Schedules) is a two-stage, Bayesian adaptive, group sequential, randomised, parallel-group, observer-blinded controlled trial.^{18,19} Stage one was conducted in Perth (Western Australia, WA) and planned to compare anti-TT-IgE responses at 7 months old in a cohort of infants randomly assigned to either a mixed wP/aP primary schedule or a standard aP-only primary schedule. In addition, the first stage of this trial compared the reactogenicity and immunogenicity of both

schedules.¹⁷ Stage two will evaluate atopic outcomes (i.e., IgE-mediated food allergy, atopic dermatitis, and atopic sensitisation) by 12 months old in up to 3000 infants.

In brief, eligible infants were born after 32 weeks' gestation and aged between 6 and < 12 weeks; full inclusion and exclusion criteria are detailed in the study protocol and statistical analysis plan.^{18,19} Parental consent was obtained immediately before enrolment. Approval was granted by the Child and Adolescent Health Service Ethics Committee, WA, Australia (RGS00019).

Randomisation and blinding

Infants were randomly assigned in a 1:1 ratio to receive either an intramuscular (IM) dose (0.5 mL) of a World Health Organization (WHO)-prequalified pentavalent wP combination vaccine with no IPV components (DTwP-Hib-HepB, Pentabio, PT Bio Farma, Indonesia, hereafter referred to as wP) or the standard hexavalent aP combination vaccine (DTaP-Hib-HepB-IPV, Infanrix Hexa, GlaxoSmithKline, Australia) in the anterolateral aspect of the right thigh.²⁰ In both vaccine formulations, Hib-PRP is conjugated to the TT protein.

Randomisation was performed using a computer-generated allocation sequence prepared by the trial statistician, based on randomly permuted blocks of varying size. Details of the allocation concealment were previously reported in the published protocol.¹⁸

Procedures

At 4 and 6 months old, all study infants received a dose of the hexavalent aP vaccine per the Australian routine immunisation schedule.²¹ A booster dose of aP was given at 18 months old (0.5 mL; IM) using a DTaP-IPV formulation (Infanrix IPV, GlaxoSmithKline, Australia), thereby preserving blinding while ensuring every participant received a minimum of three (and up to four) doses of IPV by 18 months old. All infants were administered two IM priming doses (each 0.5 mL) of the 13vPCV CRM₁₉₇-conjugated vaccine (Pfizer) at 6 weeks and 4 months old, and a booster dose at 12 months old (hereafter referred to as a '2 + 1' schedule). The 13vPCV was injected in the anterolateral aspect of the opposite thigh to co-administer wP or aP vaccines. Additional routine vaccines were administered per national guidelines: oral monovalent rotavirus vaccine at 6 weeks and 4 months old; measles-mumps-rubella combination vaccine and quadrivalent meningococcal (serogroups A, C, W, Y)-TT conjugate vaccine at 12 months old; Hib-PRP conjugated to TT and measles-mumps-rubella-varicella at 18 months old. As a study-specific procedure and in alignment with Australian immunisation guidelines from the wP vaccine era, oral paracetamol (15 mg/Kg) was given just before the 6-week vaccine doses to attenuate wP reactogenicity; caregivers were advised to administer two further unobserved doses of paracetamol 6 h apart. The sampling schedule was aligned with the schedule for pertussis vaccine doses; bloods were collected immediately before the 6-month dose of aP and 4 weeks afterwards (i.e. 8 and 12 weeks after the 4-month dose of the 13vPCV; up to 5 mL), and immediately before the booster dose of DTaP-IPV and Hib vaccines at age 18 months and 4 weeks afterwards (i.e. 6 and 7 months after the 12-month booster dose of the 13vPCV; up to 10 mL).

Serum serotype-specific IgG to pneumococcal capsular polysaccharides included in the 13vPCV (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) were measured at 6, 7, 18, and 19 months old using an inhouse multiplex fluorescent bead-based immunoassay, as previously described, with modifications.^{22–25} In brief, Bio-Plex® COOH-microspheres (6.25 × 10⁶, 500 µl, Bio-Rad) were conjugated overnight with optimised doses of DMTMM-modified pneumococcal polysaccharides (ATCC, Manassas, USA) in 1X PBS pH 7.2 (Life Technologies, Carlsbad, USA) as per Schlottmann et al.²⁶ The optimal

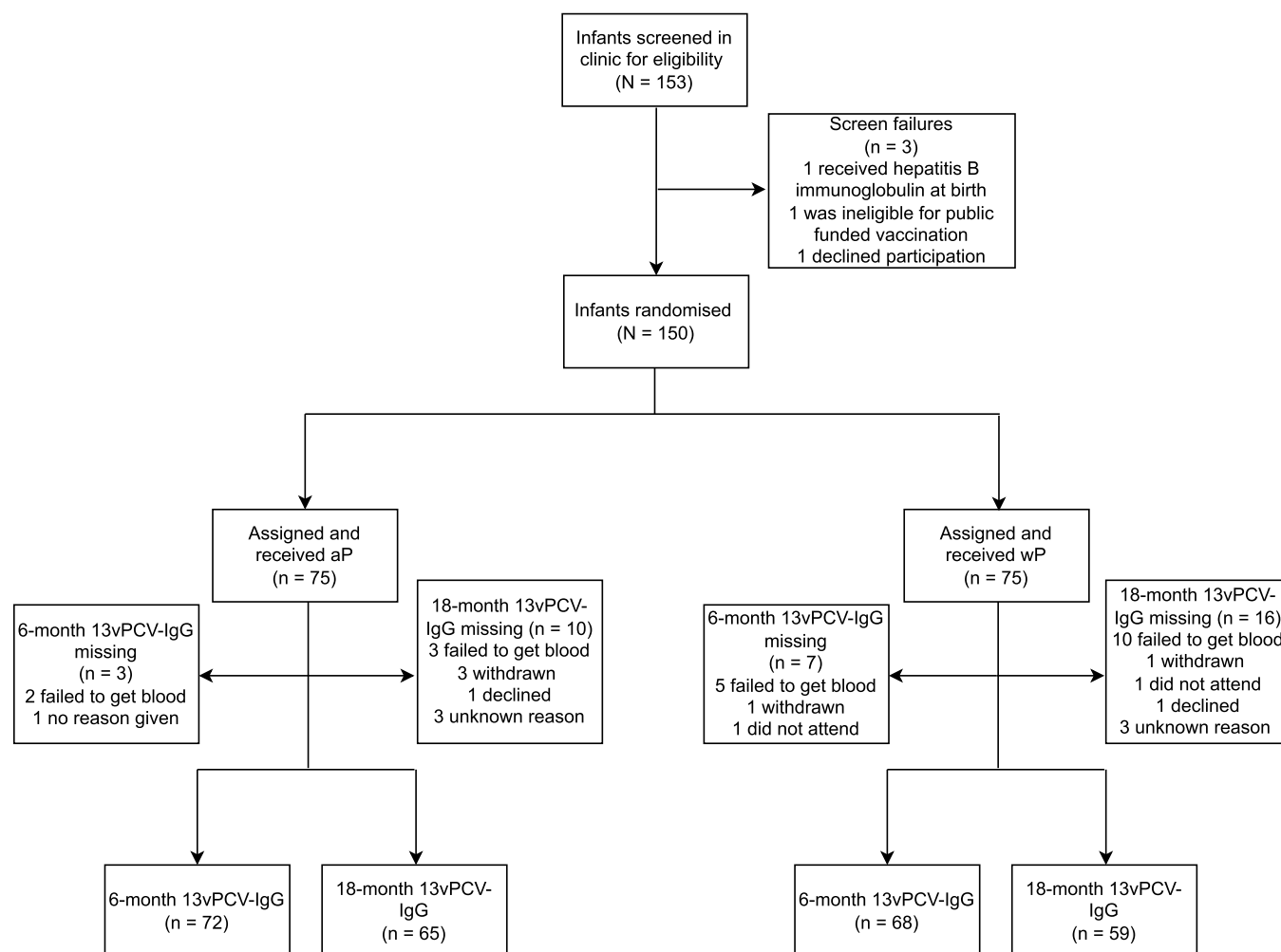


Fig. 1. Trial profile. aP, acellular pertussis vaccine; wP, whole-cell pertussis vaccine; 13vPCV, 13-valent pneumococcal conjugate vaccine.

coating doses were: a 1:1 dose; 500 µl microspheres with 500 µl DMTMM-modified polysaccharides for serotypes 1, 3, 5, 7F and 9V; and a 1:0.5 dose; 500 µl microspheres with 250 µl DMTMM-modified polysaccharides for serotypes 4, 6A, 6B, 14, 18C, 19A, 19F, and 23F. Serum IgG responses to HBsAg and the Hib-PRP were quantified in a duplex fluorescent bead-based immunoassay at the same timepoints.

In May 2023, the group assignment of participants was disclosed to parents by the site study coordinator after the last child enrolled in the stage one cohort completed the scheduled visits. Except for the study statistician, the study investigators remain blinded to the group assignments of individual participants.

Outcomes

Immunogenicity outcomes included vaccine antigen-specific IgG responses, summarised as GMCs and the proportion of participants with vaccine antigen-specific IgG concentrations meeting or exceeding established correlates of protection (i.e., 13vPCV serotype-specific IgG ≥ 0.35 µg/mL, HBsAg-IgG ≥ 10 mIU/mL, Hib-PRP-IgG ≥ 0.15 µg/mL at 6 and 7 months old and ≥ 1 µg/mL at 18 and 19 months old). Reactogenicity and parental acceptability outcomes following the 6-week, 4-month, and 6-month pertussis vaccine doses have been reported previously.¹⁷ Serious adverse events (SAEs) were defined according to the Australian National Health and Medical Research Council guidelines.²⁷ Vaccine failure (i.e., invasive Hib disease, vaccine-serotype invasive pneumococcal disease, and

hepatitis B infection) was considered an adverse event of special interest.

Statistical analysis

The statistical analysis plan and study protocol have previously been published.^{18,19} IgG concentrations for each vaccine antigen at 6, 7, 18, and 19 months old were independently modelled using Bayesian multivariate-normal linear regression on the available log₁₀ concentrations with an unstructured covariance matrix shared across both treatment groups to account for the serial correlation in the longitudinal data. The geometric mean ratio (GMR) of the mixed schedule compared to the aP-only schedule was estimated at each time point. The probability of non-inferiority was calculated using a non-inferiority margin of 2/3 on the GMR per WHO guidelines.²⁸ Additionally, IgG seropositivity for each vaccine antigen was analysed via Bayesian logistic regression models with random intercepts for individuals to account for repeat measures. All models were adjusted for sex, birth order (i.e., the order a child is born in their family), breastfeeding status, delivery method, family history of atopic disease, and parental income as baseline covariates. Missing data were assumed to be missing at random (ignorable) under the proposed models. Analyses were conducted separately for the intent-to-treat (ITT) and per-protocol (PP) analyses sets, as pre-specified in the statistical analysis plan,¹⁹ using R version 4.4.1 and Stan version 2.35.0 (via the CmdStanR library version 0.8.1). This trial

is registered at the Australian and New Zealand Clinical Trial Registry (ACTRN12617000065392p).

Role of the funding source

This study is funded by the Telethon New Children's Hospital Research Fund and Australia's National Health and Medicine Research Council (NHMRC GNT1158722). The funders have had no role in the study design, data collection, data analysis, data interpretation, or writing of this report.

Results

Between March 7, 2018 and January 13, 2020, 153 infants were assessed in clinic for eligibility (Fig. 1); two were excluded (one ineligible for public funded immunisation; one received hepatitis B immunoglobulin at birth), one declined participation due to blood sampling, and 150 underwent randomisation to either the wP/aP schedule or the aP-only schedule (75 in each group). The baseline characteristics of the randomised infants were balanced across the study groups (Table 1). The trial profile focuses on post-13vPCV priming and post-13vPCV boosting measurements at 6 and 18 months (earliest timepoints; Fig. 1); missingness patterns by assigned treatment group sets across all the scheduled phlebotomies are provided in the Appendix (p 4).

At 6 months old, the lowest pneumococcal serotype-specific IgG GMCs in both study groups were for serotypes 3, 6B, 19A, and 23F (ITT analysis set, Table 2 and Appendix p. 5; PP analysis set, Appendix pp. 11–12). The probability of the GMR exceeding 2/3 (evaluating non-inferiority) in the wP/aP group compared to the aP-only group was > 0.99 for all 13 vaccine serotypes in both ITT (Fig. 2; Appendix pp. 14–15) and PP analysis sets (Appendix pp. 16–18). The probability of the GMR exceeding 1 (evaluating superiority of wP/aP) ranged from 0.88 to > 0.99 in the ITT (Fig. 2; Appendix pp. 14–15) and in the PP analysis set (Appendix pp. 16–18) for all serotypes. For each of the 13 vaccine serotypes, the probability of a seroprotection difference > 0 (evaluating superiority of wP/aP) in the wP/aP group compared to the aP-only group ranged from 0.80 to > 0.99 in the ITT analysis set (Fig. 3 and Appendix pp. 19–20) and from 0.71 to > 0.99 in the PP analysis set (Appendix pp. 21–23). Similar findings were observed at 7 months old (3 months after 13vPCV priming); these data are presented in Table 2, Figs. 2 and 3, and in the Appendix (p. 5, pp. 11–12, and 19–23).

At 18 months old (6 months post-13vPCV booster), serotypes 1, 3, 18C, and 19A had the lowest pneumococcal serotype-specific IgG GMCs across both groups (ITT analysis set, Table 2 and Appendix p. 5; PP analysis set, Appendix pp. 11–12). The probability of the GMR exceeding 2/3 in the wP/aP group compared to the aP-only group ranged from 0.96 to > 0.99 for all 13 vaccine serotypes in the ITT analysis set (Fig. 2; Appendix pp. 14–15) and from 0.94 to > 0.99 in the PP analysis set (Appendix pp. 16–17). Additionally, the probability of the GMR exceeding 1 ranged from 0.50 to > 0.99 in the ITT (Fig. 2; Appendix pp. 14–15), with the lowest for serotype 19A, and from 0.14 to > 0.99 in the PP analysis set (Appendix pp. 16–18), with the lowest for serotype 4. For each of the 13 vaccine serotypes, the probability of a seroprotection difference > 0 in the wP/aP group compared to the aP-only group ranged from 0.51 to > 0.99 in the ITT analysis set (Fig. 3; Appendix pp. 19–20) and from 0.42 to > 0.99 in the PP analysis set (Appendix pp. 21–23), with the lowest for serotypes 3, 4, and 5. Similar findings were observed at 19 months old (7 months post-13vPCV booster); these data are presented in Table 2, Figs. 2 and 3, and in the Appendix (p. 5, pp. 11–12, 19–23).

In the ITT analysis set, both study groups exhibited low GMCs of anti-Hib-PRP immediately before the third dose of the Hib-PRP vaccine at age 6 months (wP/aP group = 0.11 µg/mL versus aP-only group = 0.04 µg/mL). A minority had anti-PRP IgG ≥ 0.15 µg/mL (wP/

Table 1
Baseline and demographic characteristics.

	aP-only schedule (n = 75)	Mixed wP/aP schedule (n = 75)	Overall (n = 150)
Sex of child - n (%)			
Female	36 (48)	38 (51)	74 (49)
Male	39 (52)	37 (49)	76 (51)
Infant's ethnicity - n (%) ^a			
European Caucasian	72 (48)	73 (49)	145 (48)
Indian subcontinent	10 (7)	12 (8)	22 (7)
Asian	6 (4)	11 (7)	17 (6)
South American	1 (1)	2 (1)	3 (1)
Black African	0 (0)	1 (1)	1 (0)
Indigenous Australian	1 (1)	0 (0)	1 (0)
Maternal parity - n (%)			
1	53 (71)	46 (61)	99 (66)
2	14 (19)	22 (29)	36 (24)
3	7 (9)	4 (5)	11 (7)
4	1 (1)	3 (4)	4 (3)
Maternal dTpa during this infant's pregnancy - n (%)			
5c-dTpa	21 (28)	20 (27)	41 (27)
3c-dTpa	43 (57)	39 (52)	82 (55)
Unknown	11 (15)	13 (17)	24 (16)
None	0 (0)	3 (4)	3 (2)
Maternal dTpa last 5 years, prior to this infant's pregnancy - n (%)			
5c-dTpa	7 (9)	6 (8)	13 (9)
3c-dTpa	6 (8)	9 (12)	15 (10)
3c-dTpa-IPV	1 (1)	0 (0)	1 (1)
Unknown	22 (29)	25 (33)	47 (31)
None	39 (52)	35 (47)	74 (49)
Gestational age at delivery (weeks)	39 (38–39)	38 (38–39)	38 (38–39)
Age (days) at enrolment	48 (45–50)	47 (44–50)	48 (45–50)

aP: acellular pertussis vaccine. wP: whole-cell pertussis vaccine as a first dose. 5c-dTpa: 5-component diphtheria-tetanus-acellular pertussis combination vaccine (reduced antigen formulation; includes pertussis toxoid, filamentous haemagglutinin, pertactin, and fimbriae types 2 and 3); 3c-dTpa: 3-component diphtheria-tetanus-acellular pertussis combination vaccine (reduced antigen formulation; includes pertussis toxoid, filamentous haemagglutinin, and pertactin); IPV: inactivated poliovirus vaccine.

^a Multiple ethnicities may apply, so percentages may not sum to 100.

aP, n = 26/68 [38%]; aP-only, n = 9/72 [12%], Table 2; Appendix p 6). By 7 months old (4 weeks post Hib-PRP priming), IgG GMCs and seroprotection rates were higher in the wP/aP group (0.64 µg/mL; n = 55/67 [82%]) compared to the aP-only group (0.20 µg/mL; n = 37/69 [54%]; Table 2; Appendix p 6). GMCs for Hib-PRP IgG were both non-inferior (GMR > 2/3) and superior (GMR > 1) with a probability > 0.99 in the wP/aP group compared to the aP-only group at both ages (Fig. 2; Appendix pp 14–15). The probability of a seroprotection difference > 0 in the wP/aP group compared to the aP-only group was also > 0.99 at both 6 and 7 months (Fig. 3; Appendix pp 19–20). Similar results were observed in the PP analysis set (Appendix pp 16–17; pp 21–22).

Anti-Hib-PRP IgG GMCs declined between 7 months and immediately before the Hib-PRP booster dose at 18 months, from 0.64 to 0.22 µg/mL in the wP/aP group and from 0.20 to 0.09 µg/mL in the aP-only group (ITT analysis set, Table 2). At 18 months, few toddlers had Hib-PRP IgG levels ≥ 1.00 µg/mL (wP/aP, n = 9/59 [15%] versus aP-only, n = 2/65 [3%]). By 19 months (4 weeks post-Hib-PRP boosting), IgG GMCs and seroprotection rates were higher in the wP/aP group (23.25 µg/mL; n = 50/54 [93%]) compared to the aP-only group (10.18 µg/mL; n = 54/64 [84%]) (Table 2). GMCs for Hib-PRP were both non-inferior (GMR > 2/3) and superior (GMR > 1) with a probability > 0.99 in the wP/aP group versus the aP-only group (Fig. 2; Appendix pp.14–15). The probability of seroprotection difference > 0 in the wP/aP group compared to the aP-only group was 0.99 and 0.98 at 18 and 19 months, respectively. Similar results were

Table 2
Descriptive statistics of IgG concentrations by antigen type, age and assigned treatment group.

	aP-only (n = 75)						wP/aP (n = 75)					
	n	GMC	GSD	Q1–Q3	Min–Max	S+	n	GMC	GSD	Q1–Q3	Min–Max	S+
PnPs 1												
6-month	72	0.63	3.02	0.29–1.56	0.04–10.5	68%	68	0.86	2.83	0.35–1.93	0.16–8.85	74%
7-month	69	0.51	2.87	0.24–1.11	0.04–7.26	55%	67	0.68	2.74	0.31–1.50	0.10–4.87	72%
18-month	65	0.55	2.86	0.25–0.95	0.07–7.37	68%	59	0.69	2.66	0.36–1.25	0.09–8.23	76%
19-month	64	0.48	2.89	0.22–0.89	0.06–8.77	61%	54	0.65	2.54	0.31–1.16	0.11–5.14	72%
PnPs 3												
6-month	72	0.28	2.39	0.19–0.47	0.02–1.89	39%	68	0.43	2.22	0.29–0.70	0.05–2.06	63%
7-month	69	0.22	2.32	0.12–0.33	0.02–1.16	23%	67	0.33	2.30	0.20–0.52	0.05–2.58	45%
18-month	65	0.25	2.67	0.13–0.51	0.03–2.35	35%	59	0.32	2.42	0.18–0.48	0.06–4.52	41%
19-month	64	0.22	2.60	0.12–0.40	0.03–3.33	30%	54	0.30	2.19	0.15–0.43	0.09–2.25	41%
PnPs 4												
6-month	72	0.66	3.60	0.29–1.64	0.02–10.5	69%	68	1.03	3.13	0.54–2.09	0.04–7.64	85%
7-month	69	0.54	3.26	0.25–1.21	0.02–9.18	62%	67	0.82	2.68	0.44–1.54	0.04–4.72	81%
18-month	65	0.74	2.42	0.45–1.46	0.09–8.31	83%	59	0.84	2.46	0.50–1.47	0.09–14.0	83%
19-month	64	0.61	2.45	0.40–1.22	0.06–10.6	80%	54	0.80	2.43	0.50–1.39	0.14–13.2	85%
PnPs 5												
6-month	72	0.53	3.69	0.17–1.40	0.02–8.19	72%	68	0.82	2.96	0.44–1.77	0.03–5.37	81%
7-month	69	0.50	3.35	0.22–1.20	0.03–4.89	70%	67	0.72	2.81	0.34–1.50	0.03–4.75	73%
18-month	65	0.72	2.28	0.44–1.27	0.15–5.39	83%	59	0.83	2.25	0.55–1.31	0.09–9.46	88%
19-month	64	0.67	2.23	0.38–1.08	0.14–5.16	77%	54	0.78	2.20	0.50–1.27	0.11–6.22	89%
PnPs 6A												
6-month	72	0.87	4.96	0.31–2.83	0.01–30.5	72%	68	1.60	3.87	0.81–4.22	0.02–14.4	85%
7-month	69	0.75	4.41	0.25–2.21	0.03–18.1	70%	67	1.39	3.38	0.75–3.45	0.03–9.41	88%
18-month	65	2.42	2.51	1.24–4.57	0.43–13.0	100%	59	2.78	2.35	1.55–4.14	0.55–45.5	100%
19-month	64	2.26	2.60	1.11–4.26	0.38–16.4	100%	54	2.57	2.36	1.48–3.40	0.42–32.2	100%
PnPs 6B												
6-month	72	0.06	5.60	0.02–0.21	0.00–5.49	14%	68	0.14	5.71	0.03–0.40	0.01–14.2	29%
7-month	69	0.07	4.95	0.02–0.25	0.00–4.25	17%	67	0.14	5.27	0.04–0.46	0.01–8.96	31%
18-month	65	0.85	4.01	0.30–2.41	0.03–8.17	72%	59	1.54	3.08	0.69–3.13	0.16–100	93%
19-month	64	0.81	4.07	0.29–2.62	0.03–15.5	73%	54	1.44	2.93	0.68–2.80	0.18–62.4	94%
PnPs 7F												
6-month	72	1.31	2.60	0.84–2.60	0.06–10.6	94%	68	1.79	2.38	1.06–3.26	0.07–8.35	99%
7-month	69	1.12	2.61	0.73–2.23	0.04–6.85	91%	67	1.47	2.36	0.84–2.94	0.07–6.32	94%
18-month	65	1.36	2.09	0.86–2.24	0.25–6.53	94%	59	1.76	1.93	1.12–2.49	0.39–18.7	100%
19-month	64	1.34	2.13	0.94–2.08	0.23–8.76	94%	54	1.79	1.86	1.21–2.51	0.47–13.5	100%
PnPs 9V												
6-month	72	0.49	4.39	0.26–1.50	0.02–14.8	68%	68	0.87	3.14	0.45–1.96	0.04–6.62	79%
7-month	69	0.43	3.56	0.20–1.07	0.02–6.72	59%	67	0.73	2.74	0.35–1.52	0.04–5.18	75%
18-month	65	0.71	2.67	0.35–1.59	0.11–6.15	75%	59	0.90	2.31	0.48–1.56	0.14–8.86	85%
19-month	64	0.64	2.72	0.29–1.22	0.11–9.05	69%	54	0.83	2.38	0.40–1.41	0.13–5.66	83%
PnPs 14												
6-month	72	1.06	4.21	0.26–3.71	0.07–15.0	69%	68	2.38	3.23	1.20–4.76	0.05–17.1	96%
7-month	69	1.02	4.20	0.39–2.86	0.04–29.3	75%	67	2.11	2.94	1.23–4.68	0.06–15.8	94%
18-month	65	1.51	3.14	0.62–3.39	0.09–15.9	91%	59	1.89	2.41	1.09–3.61	0.05–14.8	97%
19-month	64	1.33	3.17	0.57–2.73	0.09–17.6	88%	54	1.65	2.49	1.14–3.12	0.04–10.4	96%
PnPs 18C												
6-month	72	0.76	2.68	0.44–1.53	0.06–7.64	78%	68	1.03	2.63	0.60–2.04	0.05–6.08	90%
7-month	69	0.58	2.43	0.31–1.14	0.08–5.22	70%	67	0.82	2.36	0.48–1.49	0.06–4.97	87%
18-month	65	0.49	2.28	0.28–0.85	0.09–4.06	65%	59	0.58	2.20	0.36–1.04	0.11–9.65	76%
19-month	64	0.45	2.32	0.24–0.80	0.09–4.52	58%	54	0.55	2.27	0.32–0.98	0.12–9.61	70%
PnPs 19A												
6-month	72	0.36	3.45	0.12–0.90	0.04–5.78	54%	68	0.47	3.71	0.21–1.15	0.01–6.13	66%
7-month	69	0.27	3.08	0.09–0.63	0.04–4.31	46%	67	0.33	3.35	0.15–0.79	0.01–4.59	51%
18-month	65	0.67	4.21	0.24–1.42	0.07–16.4	65%	59	0.75	3.43	0.34–1.33	0.06–44.0	75%
19-month	64	0.60	4.61	0.21–1.19	0.07–15.8	58%	54	0.73	3.52	0.38–1.40	0.05–37.7	76%
PnPs 19F												
6-month	72	2.20	3.65	1.15–5.64	0.02–27.2	93%	68	3.33	3.42	1.96–7.16	0.01–36.1	97%
7-month	69	1.55	3.53	0.91–3.84	0.01–13.9	91%	67	2.49	3.11	1.50–4.30	0.01–30.5	97%
18-month	65	1.45	3.72	0.58–3.41	0.07–11.2	91%	59	2.81	3.03	1.47–4.77	0.52–57.7	100%
19-month	64	1.24	3.61	0.53–3.30	0.08–10.1	86%	54	2.71	3.24	1.49–4.91	0.30–14.5	98%
PnPs 23F												
6-month	72	0.24	4.60	0.11–0.59	0.00–4.91	44%	68	0.49	4.03	0.19–1.66	0.01–6.58	56%
7-month	69	0.19	4.17	0.09–0.49	0.00–2.83	38%	67	0.39	3.64	0.17–1.04	0.01–4.97	51%
18-month	65	0.63	3.34	0.33–1.61	0.04–5.73	71%	59	1.35	3.68	0.55–3.07	0.14–72.3	86%
19-month	64	0.62	3.41	0.29–1.48	0.05–10.8	64%	54	1.37	3.57	0.56–3.32	0.17–44.1	83%
HBsAg												
6-month	72	3.81	3.78	1.91–9.00	0.04–33.7	100%	68	4.80	3.66	2.32–12.2	0.06–51.4	100%
7-month	69	7.53	2.90	3.97–14.8	0.24–42.3	100%	67	8.66	3.08	3.82–20.5	0.53–51.8	100%
18-month	65	0.52	4.14	0.21–1.49	0.02–7.59	100%	59	0.49	4.04	0.22–1.34	0.01–14.2	100%
19-month	64	0.46	4.33	0.20–1.27	0.02–7.06	100%	54	0.40	3.81	0.21–1.13	0.02–8.35	100%
Hib-PRP												
6-month	72	0.04	3.71	0.02–0.06	0.00–1.98	12%	68	0.11	4.85	0.03–0.28	0.01–9.03	38%
7-month	69	0.20	5.54	0.07–0.67	0.00–8.28	54%	67	0.64	5.14	0.27–2.43	0.02–28.3	82%

(continued on next page)

Table 2 (continued)

	aP-only (n = 75)						wP/aP (n = 75)					
	n	GMC	GSD	Q1–Q3	Min–Max	S+	n	GMC	GSD	Q1–Q3	Min–Max	S+
18-month	65	0.09	3.11	0.04–0.20	0.01–1.62	3%	59	0.22	4.69	0.07–0.48	0.01–21.4	15%
19-month	64	10.18	5.71	3.57–36.5	0.06–153	84%	54	23.25	6.26	8.98–65.7	0.13–984	93%

aP, acellular pertussis vaccine; wP, whole-cell pertussis vaccine; GMC, geometric mean concentration; GSD, geometric standard deviation; Q1 – 25th sample percentile, Q3 – 75th sample percentile; Min–Max, minimum-maximum; S+, seroprotective at specified level; PnPs, pneumococcal polysaccharide; Hib-PRP, Haemophilus influenzae type b capsular polysaccharide polyribosylribitol phosphate; HBsAg, hepatitis B surface antigen.

observed in the PP analysis set (Appendix pp.13, 16–18, 21–23). After Hib-PRP boosting, 50 of 54 toddlers (93%) in the wP/aP group and 62 of 64 (97%) in the aP-only group achieved a fourfold seroconversion in Hib-PRP IgG. The probability of a difference > 0 in the wP/aP group compared to the aP-only group was 0.21 and 0.55 in the ITT and PP analysis sets (Appendix p 24–25), respectively.

At 6 months (pre-third dose of hepatitis B vaccine) and 7 months old (4 weeks post-hepatitis B priming), all infants across the study groups had HBsAg IgG concentrations above the specified seroprotective threshold in both ITT (Table 2; Appendix p. 6) and PP analyses sets (Appendix p 13). GMCs for HBsAg IgG were non-inferior (GMR > 2/3) with at least 95% probability in the wP/aP group compared to the aP-only group at both ages (Appendix pp. 14–18).

No episodes of vaccine-serotype invasive pneumococcal disease, invasive Hib disease, or hepatitis B infection were ascertained during the follow-up period (~18 months). Additional SAEs and adverse events of special interest (breakthrough pertussis infections and hypotonic hypo-responsive episodes) occurring within the first 6 months of follow-up were described previously¹⁷; SAEs following the 18-month vaccine doses and unsolicited adverse events by treatment group will be reported at the end of the trial.

Discussion

Here we report for the first time, the long-term immunomodulatory effect of a mixed wP/aP schedule on co-administered vaccine responses up to 19 months old. In this randomised study, compared to an aP-only schedule, a mixed wP/aP schedule resulted in non-inferior IgG responses to co-administered pneumococcal vaccine serotypes and Hib-PRP at ages 6, 7, 18, and 19 months, and to HBsAg at 6 and 7 months old, all with high probability. In many instances, antibody responses (IgG GMR) were superior in the mixed wP/aP schedule group (i.e., 6B, 19F, and 23F at all the assessed time points). Consistent with earlier reports,^{10,29} here we speculate that the innate immune activation caused by the pathogen-associated molecular patterns in the whole pertussis bacteria present in the wP vaccine, along with the subsequent activation of antigen-presenting cells and pro-inflammatory cytokines, provides stronger signals to stimulate CD4⁺ T cells. Further, these stimuli support the induction and maturation of polysaccharide-specific memory B cells and plasma cell generation, as well as memory B cell and plasma cell responses with broader isotypic profiles, providing robust and more durable protective immunity. Live attenuated vaccines, such as those for measles, mumps, rubella, and BCG, have also been suggested to boost immune responses to unrelated pathogens.³⁰ The proposed mechanisms for BCG's non-specific effects include epigenetic reprogramming of monocytes and a metabolic shift in innate immune cells in both animal experiments and ex vivo studies in humans.³¹ In the future, we will assess additional stored sera and cryopreserved peripheral blood mononuclear cells from this cohort using functional opsonophagocytic assays, global T-cell and memory B-cell phenotyping to determine the functional impact and cellular drivers of the differences observed here. The strengths of our trial lie in its rich data collection, allowing us to

measure individual-level antibody trajectories across four time points while randomisation minimised potential bias from imbalance in baseline demographics between the two schedules. This approach enabled detailed insights into the immunological outcomes of the different vaccination schedules. The study also derived comprehensive reactogenicity and parental acceptability data (which have been reported previously) that demonstrate the wP/aP strategy (using a WHO-prequalified vaccine formulation) is well-tolerated and accepted, albeit more reactogenic.¹⁷ Our previous meta-analysis found that the risk difference for SAEs in receiving a first dose of wP versus aP is likely small, ranging from three fewer to two more events per 1000 children.³² As described previously, none of the seven SAEs reported within the first six months of follow-up among five infants in the OPTIMUM stage one cohort were assessed to be related to the study vaccines.¹⁷

Our study has three main limitations. First, we did not collect baseline (pre-vaccination) 6-week samples to measure passively transferred maternal antibody levels, which are known to also influence infant vaccine immunogenicity.^{33,34} Exploratory analyses on this cohort, including history of maternal dTpa as well as data from simultaneous observational studies involving infants primed with the standard aP-only schedule, may provide further insights on the role of immune interference by maternal antibodies in this setting. Second, the phlebotomy schedule was driven by parsimony and the priority to assess the immunogenicity of the pertussis priming schedule. We were therefore not able to measure peak immune responses to PCV serotypes, which are likely to have occurred approximately one month after the 4-month and 12-month 13vPCV doses. Instead, we could only examine the impact of mixed wP/aP priming at two- and three-months post-primary 13vPCV, and at six months post-boosting, respectively. Third, it was beyond the scope of this study to assess the implications of differential immune responses on risk of disease; this will require very large population-level analyses. Fourth, we note that wP- versus aP-based formulations used as the first priming dose in this trial differ not only in their pertussis antigen content but also in their TT concentrations (higher in wP than in aP) and in the inclusion of the IPV component (present in aP but not in wP). However, on biological plausibility grounds, and consistent with prior studies, we believe that enhanced immune responses to PT and co-administered antigens are a function of the type of pertussis vaccine administered as a first dose of the primary series, and not the result of differing amounts of the TT conjugate.^{1,3,17} While the adjuvanticity of IPV as part of DTaP/dTpa formulations has been reported previously in Australian and European cohorts,^{9,35,36} we were unable to further explore the proposed immunostimulant effects of IPV co-vaccination on Hib-PRP and 13vPCV responses.

An aggregate correlate of protection of 0.35 µg/mL is established for protection against invasive pneumococcal disease;³⁷ however, higher IgG concentrations appear to be required for protection against individual serotypes and specific populations.³⁸ In the 3 + 0 and 2 + 1 PCV schedule era, serotypes 3 and 19A have been the major aetiological agents of breakthrough invasive pneumococcal disease in Australia despite high vaccine coverage.^{39–41} Here we find that regardless of the pertussis vaccine primary series strategy, serotype 3 was associated with the lowest GMCs when measured during the

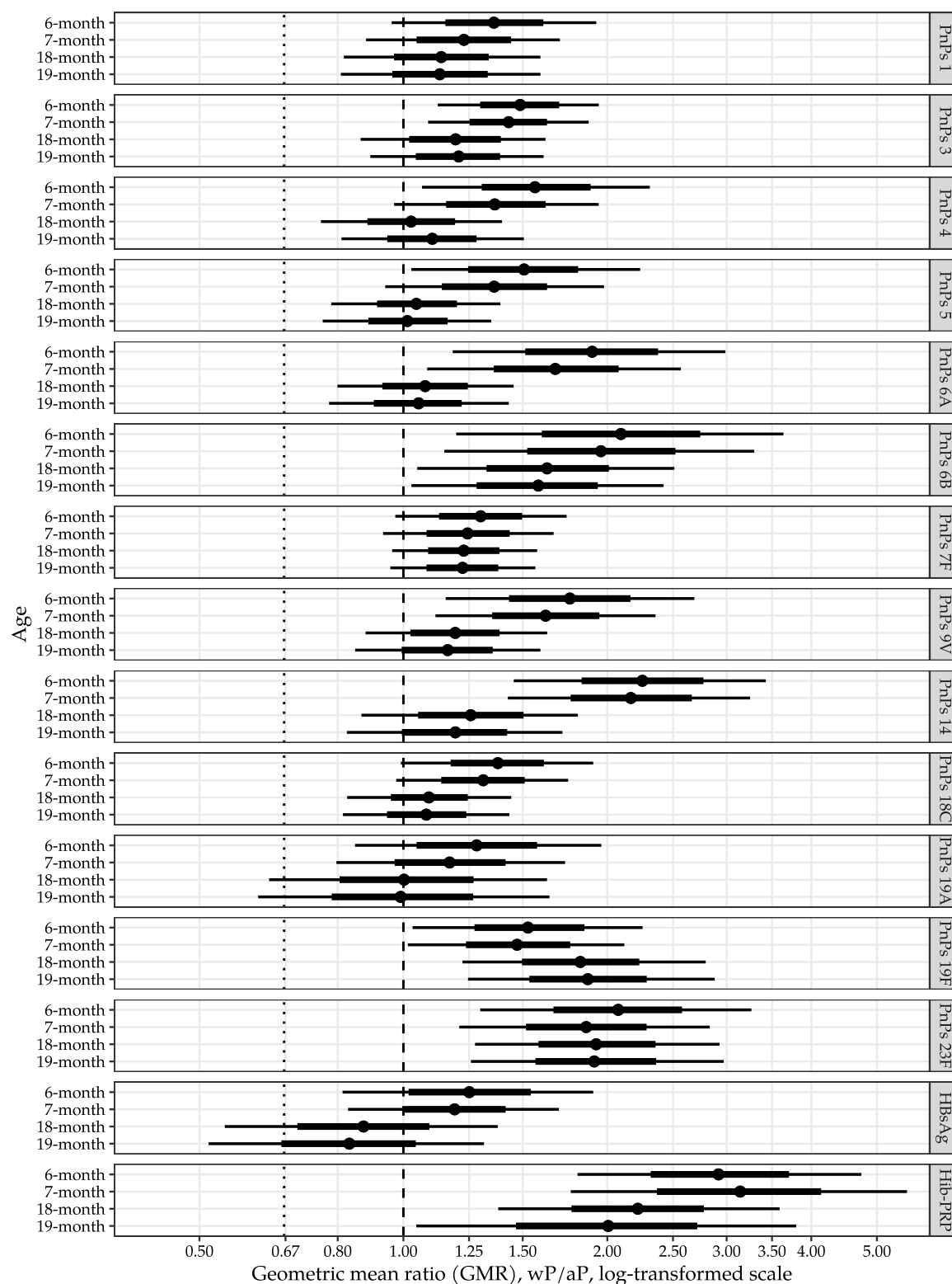


Fig. 2. IgG geometric mean ratio (GMR, wP/aP vs aP-only) by vaccination age and antigen/serotype (intention-to-treat). Points - median, rectangles - 80% credible interval (CrI), lines - 95% CrI. aP, acellular pertussis vaccine; wP, whole-cell pertussis vaccine; PnPs, pneumococcal polysaccharide; HBsAg, hepatitis B surface antigen; Hib-PRP, *Haemophilus influenzae* type b capsular polysaccharide polyribosylribitol phosphate.

second year of life. While the GMC and proportion meeting the seroprotective threshold for these serotypes were numerically higher for the mixed wP/aP group at each timepoint, the data are inconclusive for an improved immunogenic response for these specific serotypes. Finally, the multiplexed immunoassay used here has been developed for research purposes. IgG-specific

concentrations for select pneumococcal serotypes, measured with the same assay in a different cohort, correlate well with an opsonophagocytic assay and are reproducible over time.⁴² Since the established seroprotective thresholds may not perform similarly across technologies, the data therefore should be used to interpret immune kinetics only.

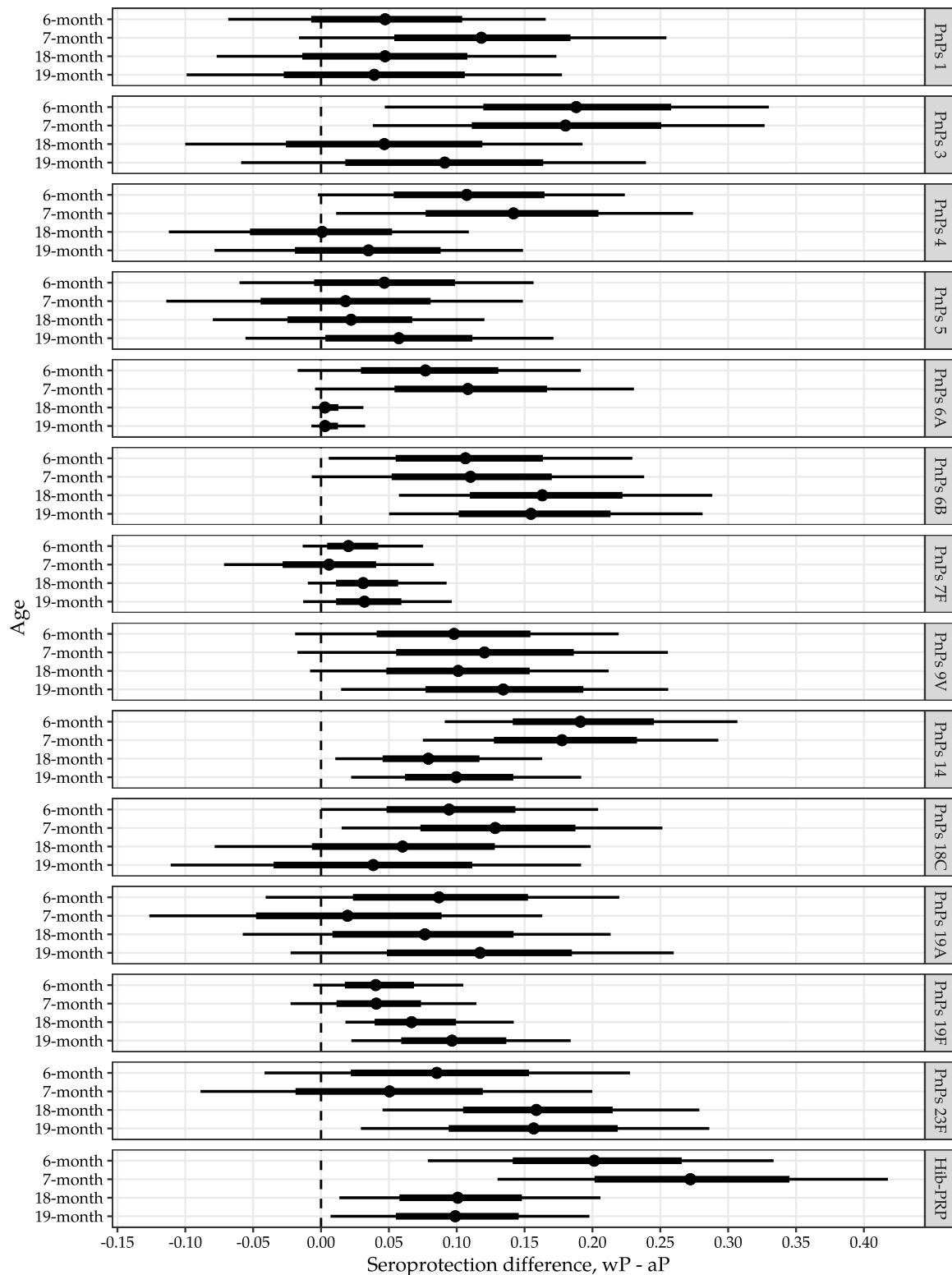


Fig. 3. Standardised probability difference (wP/aP - aP-only) for IgG seroprotection by serotype/antigen, age, and assigned treatment (intention-to-treat). Points - median, rectangles - 80% credible interval (CrI), lines - 95% CrI. aP, acellular pertussis vaccine; wP, whole-cell pertussis vaccine; PnPs, pneumococcal polysaccharide; Hib-PRP, *Haemophilus influenzae* type b capsular polysaccharide polyribosylribitol phosphate.

We conclude that the mixed wP/aP effectively boosts IgG responses to co-administered vaccine antigens and enhances the booster effect of polysaccharide vaccine antigens given in the second year of life. While the implications for vaccine-preventable diseases are uncertain, this

evidence supports countries with stable wP-based pertussis and 13vPCV programs to follow WHO recommendations for the continued use of wP schedules. These findings are also anticipated to inform 13vPCV dose-sparing policies (e.g., from 3 + 0 to 2 + 1 PCV schedules) globally.

Contributors

Conceptualisation: MJE, JAM, DEC, MG, CSW, NC, PGH, TS, PCR. Data curation: GPC, JT. Formal analysis: SM, JT. Funding acquisition: MJE, JAM, KPP, DEC, MG, CSW, NC, PBM, PGH, TS, PCR. Investigation: GPC, DEC, MoS, UW. Methodology: SM, JT, JAM, NC, TS, PCR. Project administration: MJE. Software: JT. Supervision: JT, MJE, TS, PCR. Validation: SM, JT, MJ. Visualisation: JT. Data interpretation: GPC, SM, JT, MJE, MJ, KPP, NW, CSW, UW, PBM, TS, PCR. Writing – original draft: GPC. Writing – review & editing: All authors. JT and MJ have directly accessed and verified the underlying data reported in the manuscript.

Funding

This is an investigator-initiated study supported by grants from the NHMRC (GNT1158722) awarded to TS, DEC, MG, JAM, PCR, NW, and KPP, and the Telethon New Children's Hospital Research Fund 2012 awarded to TS, PCR, and PGH. GPC was supported by PhD scholarships from the Endeavour Leadership Program (Australian Department of Education and Training), the Forrester Research Foundation, and the Wesfarmers Centre of Vaccines and Infectious Diseases during the conduct of the study. KPP is supported by a NHMRC fellowship (GNT2008911), and a Melbourne Children's Clinician-Scientist Fellowship. NC is supported by a NHMRC investigator grant (GNT1197117). Research at MCRI is supported by the Victorian Government's Operational Infrastructure Support Program. TS is supported by a Medical Research Future Fund Investigator Grant (MRF119515).

Data availability

Data from the study will be available at the completion of follow-up, analysis and reporting of OPTIMUM stage two; no end date. Deidentified, individual participant data and a data dictionary defining each field in the set, will be made available to researchers who provide a methodologically sound proposal to the University of Sydney, Australia (Ms Nicole Wong, Clinical Trial Support Lead; Department: Pro Vice-Chancellor, Research; nicole.wong@sydney.edu.au), subject to a signed data access agreement and any necessary ethics approvals (see ANZCTR trial registration). The study protocol and statistical analysis plan are published; other study-related documentation is available on request.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: DEC reports personal fees from NSW Health (salary); personal fees from DBV technologies (employment and shares); personal fees from Westmead Fertility Centre (honorarium) and grants from the Australian NHMRC and FARE (Food Allergy Research and Education) outside the submitted work during the conduct of the study. PCR has received investigator-initiated research grants to his institution from MSD and Sanofi outside the submitted work and has received institutional funding from GlaxoSmithKline and Sanofi for local and international lectures and from AstraZeneca, GlaxoSmithKline, MSD, Sanofi, and Pfizer for participation in vaccine scientific advisory boards unrelated to this work. KPP has received research grants from NIAID, Aravax, DBV Technologies, Novartis, and Siolta paid to their institution, outside the submitted work. KPP has received consultant fees from Aravax and Novartis paid to their institution, outside the submitted work. KPP is the chair of the Scientific Advisory Board for AllergyPal (MCRI founded digital app).

KPP is a Director on the Board of Omnisova Pty Ltd. All other authors declare no competing interests.

Acknowledgements

We acknowledge Aboriginal and Torres Strait Islander People as the Traditional Custodians of the land and waters of Australia. We also acknowledge the Nyoongar Wadjuk, Yawuru, Kariyarra, and Kurna Elders, their people and their land upon which The Kids Research Institute Australia is located, and where stage one of the OPTIMUM study was conducted. We seek their wisdom in our work to improve the health and development of all children.

We thank the parents and infants who took part in stage one of this trial for their commitment and continuous support. We acknowledge all members of the Vaccine Trials Group at The Kids Research Institute Australia, who were involved in the conduct of stage one of the OPTIMUM study. We would also like to express our gratitude to Kylie Rogers (Data Manager) and Prof Kathryn M. Edwards, MD (Professor of Pediatrics Emerita, Vanderbilt University School of Medicine, USA) for their valuable contributions. Additionally, we thank the independent Data Safety Monitoring Committee (Chair – Prof Keith Grimwood, Emeritus Prof Don Robertson (former Chair), Prof Allen Cheng, Prof Nigel Crawford, Prof David Isaacs, Associate Prof Stephen Lambert, Prof Helen Marshall, Prof Emma McBryde, Dr Sam Mehr, and Associate Prof John Reynolds) for their detailed oversight. Our appreciation extends to Catherine Hughes (AM), consumer representative in the OPTIMUM study Steering Committee, for her insightful input in the design and conduct of this study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jinf.2025.106515](https://doi.org/10.1016/j.jinf.2025.106515).

References

1. Bancroft T, Dillon MBC, da Silva Antunes R, Paul S, Peters B, Crotty S, et al. *Th1 versus Th2 T cell polarization by whole-cell and acellular childhood pertussis vaccines persists upon re-immunization in adolescence and adulthood*. *Cell Immunol* 2016;1:35–43.
2. Simondon F, Preziosi MP, Yam A, Kane CT, Chabirand L, Iteman I, et al. *A randomized double-blind trial comparing a two-component acellular to a whole-cell pertussis vaccine in Senegal*. *Vaccine* 1997;15(15):1606–12.
3. da Silva Antunes R, Paul S, Sidney J, Weiskopf D, Dan JM, Phillips E, et al. *Definition of human epitopes recognized in tetanus toxoid and development of an assay strategy to detect ex vivo tetanus CD4+ T cell responses*. *PLOS One* 2017;12(1):e0169086.
4. Aalberse RC, Grüber C, Ljungman M, Kakat S, Wahn U, Niggemann B, et al. *Further investigations of the IgE response to tetanus and diphtheria following covaccination with acellular rather than cellular Bordetella pertussis*. *Pedia Allergy Immunol* 2019;30(8):841–7.
5. Siegrist CA, Eberhardt CS. *Chapter 2 - Vaccine Immunology*. In: Orenstein W, Offit P, Edwards KM, Plotkin S, editors. *Plotkin's Vaccines*. 8th ed. Philadelphia: Elsevier; 2023. p. 17–36. (<https://www.sciencedirect.com/science/article/pii/B9780323790581000025>).
6. Decker MD, Howe BJ, Greenberg DP, Edwards KM. *Chapter 16 - Combination Vaccines*. In: Orenstein W, Offit P, Edwards KM, Plotkin S, editors. *Plotkin's Vaccines*. 8th ed. Philadelphia: Elsevier; 2023. p. 225–47. (<https://www.sciencedirect.com/science/article/pii/B9780323790581000165>).
7. Kitchin NRE, Southern J, Morris R, Hemme F, Thomas S, Watson MW, et al. *Evaluation of a diphtheria-tetanus-acellular pertussis-inactivated poliovirus-Haemophilus influenzae type b vaccine given concurrently with meningococcal group C conjugate vaccine at 2, 3 and 4 months of age*. *Arch Dis Child* 2007 Jan;92(1):11–6.
8. Tashani M, Heron L, Wong M, Rashid H, Booy R. *Tetanus-diphtheria-pertussis vaccine may suppress the immune response to subsequent immunization with pneumococcal CRM197-conjugate vaccine (coadministered with quadrivalent meningococcal TT-conjugate vaccine): a randomized controlled trial*. *J Travel Med* 2017 Jul 1;24(4). tax006.
9. Dagan R, Poolman JT, Zepp F. *Combination vaccines containing DTPa-Hib: impact of IPV and coadministration of CRM197 conjugates*. *Expert Rev Vaccin* 2008 Feb;7(1):97–115.
10. 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.

11. Zimmermann P, Perrett KP, Messina NL, Donath S, Ritz N, van der Klis FRM, et al. *The effect of maternal immunisation during pregnancy on infant vaccine responses*. *EClinicalMedicine* 2019 Jul 26;13:21–30.
12. McAlister S, Biggelaar A van den, Thornton R. *Suboptimal protection with 3 + 0 PCV13: a need for change?* *Lancet Infect Dis* 2022 Dec 1;22(12):1657–8.
13. Salmaso S, Piscitelli A, Rapicetta M, Chionne P, Madonna E, Argentini C. *Immunogenicity of hepatitis B vaccines among infant recipients of acellular and whole cell pertussis DTP vaccines*. *Vaccine* 1998 Apr;16(6):643–6.
14. Macías M, Lanata CF, Zambrano B, Gil AI, Amemiya I, Mispireta M, et al. *Safety and immunogenicity of an investigational fully liquid hexavalent DTaP-IPV-Hep B-PRP-T vaccine at two, four and six months of age compared with licensed vaccines in Latin America*. *Pedia Infect Dis J* 2012 Aug;31(8):e126.
15. Estcourt MJ, Campbell DE, Gold MS, Richmond P, Allen KJ, Quinn HE, et al. *Whole-cell pertussis vaccination and decreased risk of IgE-mediated food allergy: A nested case-control study*. *J Allergy* 2020;8(6):2004–14.
16. Pérez Chacón G, Fathima P, Jones M, Estcourt MJ, Gidding HF, Moore HC, et al. *Infant whole-cell versus acellular pertussis vaccination in 1997 to 1999 and risk of childhood hospitalization for food-induced anaphylaxis: Linked administrative databases cohort study*. *J Allergy Clin Immunol* 2024 Mar 1;123(3):670–80.
17. Pérez Chacón G, Estcourt MJ, Totterdell J, Marsh JA, Perrett KP, Campbell DE, et al. *Immunogenicity, reactogenicity, and IgE-mediated immune responses of a mixed whole-cell and acellular pertussis vaccine schedule in Australian infants: A randomised, double-blind, noninferiority trial*. *PLOS Med* 2024 Jun 10;21(6):e1004414.
18. Pérez Chacón G, Estcourt MJ, Totterdell J, Campbell DE, Perrett KP, Marsh JA, et al. *OPTIMUM study protocol: an adaptive randomised controlled trial of a mixed whole-cell/acellular pertussis vaccine schedule*. *BMJ Open* 2020 Dec 1;10(12):e042838.
19. Totterdell JA, Pérez Chacón G, Estcourt MJ, Jones M, Richmond P, Snelling TL, et al. *Statistical analysis plan for the OPTIMUM study: optimising immunisation using mixed schedules, an adaptive randomised controlled trial of a mixed whole-cell/acellular pertussis vaccine schedule*. *Trials* 2022 Feb 7;23(1):121.
20. Rusmil K, Gunardi H, Fadlyana E, Soedjatmiko, Dhamayanti M, Sekartini R, et al. *The immunogenicity, safety, and consistency of an Indonesia combined DTP-HB-Hib vaccine in expanded program on immunization schedule*. *BMC Pedia* 2015 Dec 19;15(1):219.
21. Australian Government Department of Health and Age Care. *National Immunisation Program schedule [Internet]*. Australian Government Department of Health and Age Care; 2023. Available from: <https://www.health.gov.au/resources/publications/national-immunisation-program-schedule?language=en>. [Accessed 29 March 2024].
22. Elberse KEM, Tcherniaeva I, Berbers GAM, Schouls LM. *Optimization and application of a multiplex bead-based assay to quantify serotype-specific IgG against Streptococcus pneumoniae polysaccharides: response to the booster vaccine after immunization with the pneumococcal 7-valent conjugate vaccine*. *Clin Vaccin Immunol* 2010 Apr;17(4):674–82.
23. McAlister SM, van den Biggelaar AHJ, Thornton RB, Richmond PC. *Optimising a 6-plex tetanus-diphtheria-pertussis fluorescent bead-based immunoassay*. *MethodsX* 2021 Jan 1;8:101360.
24. Pavliakova D, Giardina PC, Moghazeh S, Sebastian S, Koster M, Pavliak V, et al. *Development and validation of 13-plex Luminex-based assay for measuring human serum antibodies to Streptococcus pneumoniae capsular polysaccharides*. *mSphere* 2018 Aug 8;3(4):e00128–18.
25. Pickering JW, Martins TB, Greer RW, Schroder MC, Astill ME, Litwin CM, et al. *A multiplexed fluorescent microsphere immunoassay for antibodies to pneumococcal capsular polysaccharides*. *Am J Clin Pathol* 2002 Apr;117(4):589–96.
26. Schlottmann SA, Jain N, Chirmule N, Esser MT. *A novel chemistry for conjugating pneumococcal polysaccharides to Luminex microspheres*. *J Immunol Methods* 2006 Feb 20;309(1–2):75–85.
27. National Health and Medical Research Council. *Safety monitoring and reporting in clinical trials involving therapeutic goods*. NHMRC [Internet]. Canberra, Australia: National Health and Medical Research Council. 2016. Available from: (<https://www.nhmrc.gov.au/about-us/publications/safety-monitoring-and-reporting-clinical-trials-involving-therapeutic-goods>). [Accessed 29 March 2024].
28. World Health Organization Expert Committee on Biological Standardization. *Guidelines on clinical evaluation of vaccines: regulatory expectations [Internet]*. Geneva: World Health Organization. 2020. Available from: (<https://www.who.int/publications/m/item/WHO-TRS-1004-web-annex-9>). [Accessed 28 March 2024].
29. Valeri V, Sochon A, Cousu C, Chappert P, Lecoeuche D, Blanc P, et al. *The whole-cell pertussis vaccine imposes a broad effector B cell response in mouse heterologous prime-boost settings*. *JCI Insight* 2022 Nov 8;7(21):e157034.
30. Higgins JPT, Soares-Weiser K, López-López JA, Kakourou A, Chaplin K, Christensen H, et al. *Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review*. *BMJ* 2016 Oct 13;355:i5170.
31. Arts RJW, Carvalho A, La Rocca C, Palma C, Rodrigues F, Silvestre R, et al. *Immunometabolic pathways in BCG-induced trained immunity*. *Cell Rep* 2016 Dec 6;17(10):2562–71.
32. Pérez Chacón G, Ramsay J, Brennan-Jones CG, Estcourt MJ, Richmond P, Holt P, et al. *Whole-cell pertussis vaccine in early infancy for the prevention of allergy in children*. *Cochrane Database Syst Rev* 2021 Sep 6;9(9):CD013682.
33. Wanlapakorn N, Maertens K, Thongmee T, Srimuan D, Thatsanathorn T, Van Damme P, et al. *Levels of antibodies specific to diphtheria toxoid, tetanus toxoid, and Haemophilus influenzae type b in healthy children born to Tdap-vaccinated mothers*. *Vaccine* 2020 Oct 14;38(44):6914–21.
34. Knuutila A, Barkoff AM, Ivaska L, Tenhu E, Teräsjarvi J, van Gageldonk P, et al. *Effect of immunization during pregnancy and pre-existing immunity on diphtheria-tetanus-acellular pertussis vaccine responses in infants*. *Emerg Microbes Infect* 2023 Dec;12(1):2204146.
35. Rowe J, Macaubas C, Monger TM, Holt BJ, Harvey J, Poolman JT, et al. *Antigen-specific responses to diphtheria-tetanus-acellular pertussis vaccine in human infants are initially Th2 polarized*. *Infect Immun* 2000 Jul;68(7):3873–7.
36. Gillard J, Suffiotti M, Brazda P, Venkatasubramanian PB, Versteegen P, de Jonge MI, et al. *Antiviral responses induced by Tdap-IPV vaccination are associated with persistent humoral immunity to Bordetella pertussis*. *Nat Commun* 2024 Mar 8;15:2133.
37. Siber GR, Chang I, Baker S, Fernsten P, O'Brien KL, Santosham M, et al. *Estimating the protective concentration of anti-pneumococcal capsular polysaccharide antibodies*. *Vaccine* 2007 May 10;25(19):3816–26.
38. Andrews NJ, Waight PA, Burbidge P, Pearce E, Roalfe L, Zancolli M, et al. *Serotype-specific effectiveness and correlates of protection for the 13-valent pneumococcal conjugate vaccine: a postlicensure indirect cohort study*. *Lancet Infect Dis* 2014 Sep 1;14(9):839–46.
39. Jayasinghe S, Williams PCM, Macartney KK, Crawford NW, Blyth CC. *Assessing the impact of pneumococcal conjugate vaccine immunization schedule change from 3+0 to 2+1 in Australian children: A retrospective observational study*. *Clin Infect Dis* 2025 Feb 5;80(1):207–14.
40. Homaira N, Strachan R, Quinn H, Beggs S, Bhuiyan M, Bowen A, et al. *Real world impact of 13vPCV in preventing invasive pneumococcal pneumonia in Australian children: A national study*. *Vaccine* 2023 Jan 4;41(1):85–91.
41. Phuong LK, Cheung A, Templeton T, Abebe T, Ademi Z, Buttery J, et al. *Epidemiology of childhood invasive pneumococcal disease in Australia: a prospective cohort study*. *Arch Dis Child* 2024 Dec 13;110(1):52–8.
42. Ryan FJ, Clarke M, Lynn MA, Benson SC, McAlister S, Giles LC, et al. *Bifidobacteria support optimal infant vaccine responses*. *Nature* 2025 May;641(8062):456–64.