Vaccine 39 (2021) 1598–1608

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

# Impact of maternal diphtheria-tetanus-acellular pertussis vaccination on pertussis booster immune responses in toddlers: Follow-up of a randomized trial

Federico Martinón-Torres [a,b], Scott A. Halperin [c], Terry Nolan [d,1], Bruce Tapiéro [e], Kirsten P. Perrett [d,2], Ignacio Salamanca de la Cueva [f], José García-Sicilia [g], Zbynek Stranak [h], Otto G. Vanderkooi [i], Pavel Kosina [j], Sarka Rumlarova [j], Miia Virta [k], Jose M. Merino Arribas [l], Mariano Miranda-Valdivieso [m], Begoña Arias Novas [n], Jan Bozensky [o], María José Cilleruelo Ortega [p], Jose Tomas Ramos Amador [q], Manuel Baca [r], Esperanza Escribano Palomino [s], Gian Vincenzo Zuccotti [t], Jan Janota [u], Paola Giovanna Marchisio [v,w], Lusine Kostanyan [x], Nadia Meyer [x,*], Maria Angeles Ceregido [x], Brigitte Cheuvart [x], Sherine O. Kuriyakose [y], Narcisa Mesaros [x]

[a] Hospital Clínico Universitario de Santiago, Pediatría Clínica, Infectológica y Traslacional, 15706 Santiago de Compostela, Spain
[b] Genetics, Vaccines and Pediatrics Research Group, University of Santiago de Compostela, Instituto de Investigación Sanitaria de Santiago de Compostela, 15706 Santiago de Compostela, Spain
[c] Dalhousie University, Canadian Center for Vaccinology, Halifax, NS B3K 6R8, Canada
[d] Murdoch Children's Research Institute, and the Melbourne School of Population and Global Health at The University of Melbourne, Melbourne, VIC 3052, Australia
[e] CHU Sainte Justine, Université de Montréal, Montréal, QC H3T 1C5, Canada
[f] Instituto Hispalense de Pediatría, Unidad de Investigación, 41014 Seville, Spain
[g] Hospital Universitario Madrid Sanchinarro, Servicio de Pediatría, 28050 Madrid, Spain
[h] Institute for the Care of Mother and Child, Neonatology Department, 147 00 Prague, Czech Republic
[i] Alberta Children's Hospital, University of Calgary, Departments of Pediatrics, Microbiology, Immunology and Infectious Diseases, Pathology and Laboratory Medicine and Community Health Sciences, Alberta Children's Hospital Research Institute, Alberta, Calgary AB T3B 6A8, Canada
[j] University Hospital, Department of Infectious Diseases, 500 05 Hradec Kralove, Czech Republic
[k] Tampere Vaccine Research Center, Tampere University, 33100 Tampere, Finland
[l] Nuevo Hospital Universitario de Burgos, Departamento de Pediatría, 09006 Burgos, Spain
[m] Hospital de Antequera, Servicio de Pediatría, 29200 Antequera, Spain
[n] Hospital Universitario Sanitas La Zarzuela, Servicio de Pediatría, 28023 Aravaca, Spain
[o] Vitkovice Hospital, Pediatrics Department, 703 00 Ostrava, Czech Republic
[p] Hospital Universitario Puerta de Hierro Majadahonda, Servicio de Pediatría, 28222 Madrid, Spain
[q] Departamento de Salud Pública y Materno-Infantil, Hospital Clínico San Carlos, 28040 Madrid, Spain
[r] Hospital Quiron Malaga, Departamento de Pediatría y Neonatología, 29004 Malaga, Spain
[s] Hospital Universitario La Paz, Servicio de Neonatologia, 28046 Madrid, Spain
[t] Ospedale dei Bambini Vittore Buzzi, and University of Milan, 20154 Milan, Italy
[u] Thomayer Hospital Prague, Department of Neonatology, 140 59 Prague, Czech Republic
[v] Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
[w] Università degli Studi di Milano, 20122 Milan, Italy
[x] GSK, Vaccines, 1300 Wavre, Belgium
[y] GSK, Vaccines, Bangalore-560001, India

*Abbreviations:* AE, adverse event; ATP, according-to-protocol; CI, confidence interval; CPS, capsular polysaccharide; DTaP-HepB-IPV/Hib, diphtheria-tetanus-three-com ponent acellular pertussis-hepatitis B virus-inactivated poliovirus/*Haemophilus influenzae* type b vaccine; DU, D antigen units; ECL, electrochemiluminescence; ELISA, enzyme-linked immunosorbent assay; FHA, filamentous hemagglutinin; GMC, geometric mean concentration; GMT, geometric mean titer; HBs, hepatitis B surface antigen; Hib, *Haemophilus influenzae* type b; IPV, inactivated poliovirus; IU, international units; PCV13, 13-valent pneumococcal conjugate vaccine; PRN, pertactin; PRP, polyribosylribitol phosphate; PT, pertussis toxoid; SAE, serious adverse event; Tdap, tetanus-reduced-antigen-content diphtheria-acellular pertussis vaccine; TT, tetanus toxoid; TVC, total vaccinated cohort; UK, United Kingdom; US, United States; WHO, World Health Organization.

* Corresponding author at: GSK, Avenue Fleming 20, 1300 Wavre, Belgium.
*E-mail address:* nadia.x.meyer@gsk.com (N. Meyer).

[1] Current affiliation: Doherty Institute, The University of Melbourne, Melbourne, VIC 3000, Australia.
[2] Current affiliation: Department of Paediatrics, The University of Melbourne, Melbourne, VIC 3052, Australia.

https://doi.org/10.1016/j.vaccine.2021.02.001
0264-410X/

ARTICLE INFO

*Article history:*
Received 9 September 2020
Received in revised form 26 November 2020
Accepted 2 February 2021
Available online 19 February 2021

*Keywords:*
Tdap vaccine
Pertussis
Maternal immunization
Blunting
Toddlers
Booster

ABSTRACT

*Background:* Transplacentally transferred antibodies induced by maternal pertussis vaccination interfere with infant immune responses to pertussis primary vaccination. We evaluated whether this interference remains in toddlers after booster vaccination.

*Methods:* In a prior phase IV, observer-blind, placebo-controlled, randomized study (NCT02377349), pregnant women in Australia, Canada and Europe received intramuscular tetanus-reduced-antigen-content diphtheria-three-component acellular pertussis vaccine (Tdap group) or placebo (control group) at $27^{0/7}$–$36^{6/7}$ weeks' gestation, with crossover immunization postpartum. Their infants were primed (study NCT02422264) and boosted (at 11–18 months; current study NCT02853929) with diphtheria-tetanus-three-component acellular pertussis-hepatitis B virus-inactivated poliovirus/*Haemophilus influenzae* type b vaccine (DTaP-HepB-IPV/Hib) and 13-valent pneumococcal conjugate vaccine. Immunogenicity before and after booster vaccination, and reactogenicity and safety of the booster were evaluated descriptively.

*Results:* 263 (Tdap group) and 277 (control group) toddlers received a DTaP-HepB-IPV/Hib booster. Pre-booster vaccination, observed geometric mean concentrations (GMCs) for the three pertussis antigens and diphtheria were 1.4–1.5-fold higher in controls than in the Tdap group. No differences were observed for the other DTaP-HepB-IPV/Hib antigens. One month post-booster vaccination, booster response rates for pertussis antigens were $\geq$ 92.1% and seroprotection rates for the other DTaP-HepB-IPV/Hib antigens were $\geq$ 99.2% in both groups (primary objective). Higher post-booster GMCs were observed in controls versus the Tdap group for anti-filamentous hemagglutinin (1.2-fold), anti-pertussis toxoid (1.5-fold) and anti-diphtheria (1.4-fold). GMCs for the other DTaP-HepB-IPV/Hib antigens were similar between groups. Serious adverse events were reported for three toddlers (controls, not vaccination-related). One death occurred pre-booster (Tdap group, not vaccination-related).

*Conclusions:* As a consequence of interference of maternal pertussis antibodies with infant immune responses to pertussis primary vaccination, pertussis antibody concentrations were still lower in toddlers from Tdap-vaccinated mothers before DTaP-HepB-IPV/Hib booster vaccination. After the booster, antibody concentrations were lower for filamentous hemagglutinin and pertussis toxoid but not for pertactin. The clinical significance of this interference requires further evaluation.

Clinical Trial Registration.
ClinicalTrials.gov: NCT02853929.



## 1. Introduction

Vaccination of women during pregnancy (maternal immunization) is increasingly being recommended as a strategy to protect young infants from infectious diseases [1]. The strategy relies on boosting pathogen-specific maternal antibodies, which are then transferred through the placenta to the fetus and enhance passive immunity during the infant's first vulnerable months of life, before protection through routine infant immunization is established [1]. One target for maternal immunization is pertussis (whooping cough), a contagious respiratory tract infection which has its highest burden in infants < 3 months [2–4]. Pertussis vaccination of pregnant women was first recommended in the United States (US) and the United Kingdom (UK) nearly a decade ago, in response to the resurgence of pertussis disease in the general population and multiple deaths in infants [5,6]. It is now implemented in several industrialized and developing countries [7] and recommended by the World Health Organization (WHO) in settings with high pertussis-related infant morbidity or mortality [8]. Several studies have shown that maternal pertussis vaccination has a favorable safety profile, results in efficient placental transfer of pertussis-specific antibodies and provides high effectiveness (69%–93%) against pertussis in infants < 2–3 months [7,9–19].

We performed a large, placebo-controlled, randomized maternal immunization trial with tetanus-reduced-antigen-content diphtheria-three-component acellular pertussis vaccine (Tdap), which confirmed that high levels of pertussis antibodies were transferred from mother to infant after vaccination during pregnancy [20]. However, a follow-up study in infants showed that maternal pertussis antibodies interfered with infant immune responses to routine primary vaccination with diphtheria-tetanus-three-component acellular pertussis-hepatitis B virus-inactivated poliovirus and *Haemophilus influenzae* type b-tetanus toxoid conjugate vaccine (DTaP-HepB-IPV/Hib), as evident from lower antibody concentrations to pertussis antigens in infants from mothers who received Tdap during pregnancy compared to those who did not [21]. This was in line with results from prior studies with Tdap vaccines showing interference of maternal antibodies with infant immune responses [22–30]. The current paper describes the results of a subsequent follow-up study of our maternal immunization trial, in which we evaluated whether antibody concentrations in toddlers from Tdap-vaccinated mothers remain lower after a DTaP-HepB-IPV/Hib booster dose.

## 2. Methods

### 2.1. Study design and participants

We previously performed a phase IV, observer-blind, randomized, placebo-controlled maternal immunization trial (ClinicalTrials.gov: NCT02377349), in which pregnant women in Australia, Canada, Czech Republic, Finland, Italy and Spain received either Tdap (*Boostrix*, GSK; Tdap group) or placebo (control group) at $27^{0/7}$–$36^{6/7}$ weeks' gestation and crossover administration within 72 h postpartum (Fig. 1) [20]. In a follow-up study ("infant study", NCT02422264), infants born to these women received 2 or 3 doses of DTaP-HepB-IPV/Hib (*Infanrix Hexa*, GSK) and 13-valent pneumococcal conjugate vaccine (PCV13, *Prevnar 13*, Pfizer Inc.) according to national/local immunization schedules: 2 doses at 2 and 4 or at

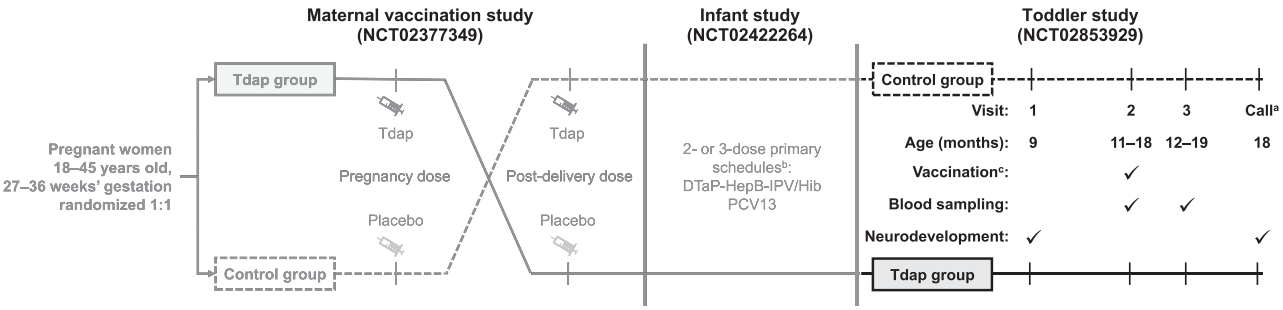


**Fig. 1. Study design.** DTaP-HepB-IPV/Hib, diphtheria-tetanus-three-component acellular pertussis-hepatitis B virus-inactivated poliovirus/*Haemophilus influenzae* type b vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; Tdap, tetanus-reduced-antigen-content diphtheria-acellular pertussis vaccine. [a]If the booster vaccination visit (visit 2) or post-booster vaccination blood sampling visit (visit 3) took place at 18 months of age, the Ages and Stages Questionnaire-3 (ASQ-3) was preferentially completed during that visit. If visit 3 was completed before 18 months of age, the study staff contacted the parents/legally acceptable representatives to complete the ASQ-3 at 18 months of age via phone. For the Czech Republic, the call at 18 months could be replaced by a clinic visit if preferred by the study team. [b]2- or 3-dose primary vaccination schedules according to national/local schedules (2 and 4 months [Spain]; 3 and 5 months [Finland, Italy]; 2, 4 and 6 months [Australia, Canada, Spain]; 2, 3 and 4 months [Czech Republic]). [c]Booster vaccination with DTaP-HepB-IPV/Hib and PCV13 according to national/local schedules.

3 and 5 months; 3 doses at 2, 4 and 6 or at 2, 3 and 4 months of age (Fig. 1) [21]. In the present follow-up study ("toddler study", NCT02853929), toddlers received a DTaP-HepB-IPV/Hib booster dose, co-administered with PCV13 at 11–18 months of age, according to national/local immunization schedules. The toddler study comprised three visits (at 9, 11–18 and 12–19 months) and, for some, a phone contact at 18 months of age (Fig. 1 and "2.5 Safety assessments" section for further details).

Healthy 9-month-old infants, who were born to mothers from the maternal immunization trial [20], had completed their primary vaccination per protocol in the infant study [21] and whose parents/legally acceptable representatives had provided written informed consent, were enrolled in the toddler study between September 2016 and May 2018. Infants were excluded if they had received prior booster vaccination against diphtheria, tetanus, pertussis, poliovirus, hepatitis B, Hib or pneumococcus or had a history of the corresponding diseases since the end of the infant study. Inclusion and exclusion criteria are detailed in the Supplementary methods.

Group allocation (Tdap or control) was based on that of the mothers in the maternal immunization trial [20]. The toddler study was open label as all toddlers received the same vaccines. Personnel evaluating study endpoints and performing laboratory testing remained blinded to the mothers' treatment allocation.

The study was conducted according to the principles of Good Clinical Practice, the Declaration of Helsinki and applicable regulations. The centers' ethics committees (Supplementary material) approved the protocol and other study-related documents. An independent data monitoring committee oversaw the participants' safety.

### 2.2. Vaccines

Each 0.5 mL dose of DTaP-HepB-IPV/Hib contained ≥ 30 international units (IU) diphtheria toxoid; ≥40 IU tetanus toxoid (TT); 25 μg filamentous hemagglutinin (FHA); 8 μg pertactin (PRN); 25 μg pertussis toxoid (PT); 10 μg hepatitis B surface antigen (HBs); 40 D antigen units (DU) IPV type 1 (Mahoney strain); 8 DU IPV type 2 (MEF-1 strain); 32 DU IPV type 3 (Saukett strain); and 700 μg $Al^{3+}$; provided as liquid component and mixed with lyophilized Hib-TT (10 μg Hib polyribosylribitol phosphate [PRP], ~25 μg TT and 0.12 mg aluminum as salts). PCV13 contained 2.2 μg capsular polysaccharide (CPS) of serotypes 1, 3, 4, 5, 6A, 7F, 9 V, 14, 18C, 19A, 19F and 23F, and 4.4 μg CPS of serotype 6B (each conjugated to diphtheria toxoid variant, $CRM_{197}$), and 125 μg $Al^{3+}$. DTaP-HepB-IPV/Hib and PCV13 were injected intramuscularly in opposite thighs or deltoids.

### 2.3. Objectives

The primary objective was to assess booster response rates for pertussis antigens and seroprotection rates for the other DTaP-HepB-IPV/Hib antigens 1 month post-booster. Booster response was defined as a post-vaccination antibody concentration ≥ 4 times the assay cut-off for toddlers with a pre-vaccination concentration below the assay cut-off, a post-vaccination concentration ≥ 4 times the pre-vaccination concentration for toddlers with a pre-vaccination concentration between the assay cut-off and < 4 times the assay cut-off, or a post-vaccination concentration ≥ 2 times the pre-vaccination concentration for toddlers with a pre-vaccination concentration ≥ 4 times the assay cut-off.

Secondary objectives were to assess the immune response to DTaP-HepB-IPV/Hib and PCV13 booster vaccination in terms of antibody concentrations/titers for all vaccine antigens and pertussis seropositivity 1 month post-booster (defined as antibody concentrations greater than or equal to the assay cut-offs); and antibody persistence after DTaP-HepB-IPV/Hib and PCV13 primary vaccination in terms of pre-booster antibody concentrations/titers for all vaccine antigens.

The safety and reactogenicity of both vaccines in terms of solicited and unsolicited adverse events (AEs) and serious adverse events (SAEs); and the toddlers' neurodevelopmental status at 9 and 18 months of age were also evaluated as secondary objectives.

### 2.4. Immunogenicity assessments

Blood samples were collected immediately before and 1 month after the booster dose (allowed interval: 21–48 days between booster and blood draw). Antibodies against pertussis antigens, diphtheria, tetanus and Hib PRP were quantified using validated enzyme-linked immunosorbent assays (ELISAs). Assay cut-offs defining seropositivity were 2.046 IU/mL (anti-FHA), 2.187 IU/mL (anti-PRN), 2.693 IU/mL (anti-PT), 0.057 IU/mL (anti-diphtheria), 0.043 IU/mL (anti-tetanus) and 0.066 μg/mL (anti-PRP). Seroprotection cut-offs were 0.1 IU/mL for diphtheria and tetanus [31,32] and 0.15 μg/mL and 1.0 μg/mL for short- and long-term protection, respectively, for Hib PRP [33,34]. There is no established correlate of protection for pertussis [35]. Anti-HBs antibodies were quantified with a commercial chemiluminescence

immunoassay (ADVIA Centaur anti-HBs2, Siemens Healthcare). The assay cut-off was 6.2 mIU/mL and seroprotection cut-off 10 mIU/mL [35–37]. Anti-poliovirus types 1, 2 and 3 antibodies were quantified with a microneutralization test [38]. Antibody titers $\geq 8$ $ED_{50}$ were considered protective. Serotype-specific anti-pneumococcal CPS antibodies (for the PCV13 serotypes) were quantified using validated multiplex electrochemiluminescence (ECL) assays [39]. A threshold of 0.35 μg/mL for the ECL assays is considered equivalent to the 0.35 μg/mL PCV licensure threshold established for the WHO pneumococcal reference ELISA [39,40]. All assays were performed at GSK, Rixensart/Wavre, Belgium.

### 2.5. Safety assessments

The participants' parents/legally acceptable representatives received diary cards at the booster vaccination visit to record solicited local and general AEs and body temperature on the day of vaccination and 3 subsequent days (days 0–3) and unsolicited AEs occurring on days 0–30. The diary cards were returned at the next visit, AEs were discussed with the investigator and transcribed onto electronic case report forms. Large injection site reactions (defined as any local swelling with a diameter > 50 mm, any noticeable diffuse injection site swelling [diameter not measurable] or any noticeable increased circumference of the injected limb) occurring on days 0–3 post-booster were also recorded. SAEs were collected from booster vaccination until each participant's last visit/phone contact. (S)AEs leading to study withdrawal were recorded from enrollment until study end.

The intensity of solicited and unsolicited AEs was graded on a scale from 1 (mild) to 3 (severe). Definitions of grade 3 AEs were crying when the limb was moved or the limb being spontaneously painful (pain); diameter > 20 mm (swelling, redness); crying inconsolably or preventing normal activity (irritability); not eating at all (loss of appetite); temperature $\geq$ 39.0 °C (fever); preventing normal activity (drowsiness, unsolicited AEs). The investigators assessed the causality between each (S)AE and vaccination based on their clinical judgement. Solicited local AEs were all considered causally related.

Together with the investigators, the participants' parents/legally acceptable representatives completed a standardized developmental screening tool (Ages & Stages Questionnaire, third edition [ASQ-3]) that assesses five neurodevelopmental domains: communication, gross motor, fine motor, problem solving and personal-social skills [41]. This was done at 9 months of age (max. 9 months + 30 days) during the first visit and at 18 months of age (±30 days), either during the second visit (booster vaccination) or third visit (blood draw), or during a separate phone call if the second and third visits occurred before 18 months. Participants with a score below the cut-off for any of the five domains were referred to a developmental specialist for a formal neurodevelopmental assessment using the Bayley Scale for Infant Development, version III (BSID-III), which assesses cognitive, language, motor, social-emotional and adaptive behaviors [42].

An overview of all congenital anomalies reported in the maternal immunization trial and the infant and toddler studies as AE or pre-existing condition in the infants' medical history is also presented.

### 2.6. Statistical analyses

All endpoints were analyzed descriptively, i.e., no tests of statistical significance were performed. Claims of interference were guided by observed differences in antibody GMCs between the Tdap and the control group and consistency with other published studies. Sample size calculations for the maternal immunization trial were described previously [20]. The sample size of the current toddler study was not based on power-based calculations. Instead, the cohort of the toddler study consisted of all available, eligible infants from the preceding infant study, which depended on the number of mothers enrolled in the maternal immunization trial. Primary immunogenicity analyses were performed on the according-to-protocol (ATP) cohort for immunogenicity (all eligible participants who complied with protocol-defined procedures and intervals and had post-booster immunogenicity results available for at least one antigen). A second analysis based on the total vaccinated cohort (TVC, all toddlers who received the DTaP-HepB-IPV/Hib booster) was also performed to complement the ATP analysis. Seroprotection, seropositivity and booster response rates were calculated with exact 95% confidence intervals (CIs). Geometric mean antibody concentrations and titers (GMCs, GMTs) were calculated with 95% CIs by taking the anti-log of the mean of the $log_{10}$ concentration or titer transformations. Antibody concentrations/titers below the assay cut-offs were given arbitrary values of half the cut-offs for GMC/GMT calculations. We performed subgroup analyses by primary vaccination dose schedule (2-dose and 3-dose), gestational age (27–32 and 33–36 weeks) and mother's age (18–24, 25–34 and 35–45 years) at maternal vaccination.

Safety analyses were performed on the TVC. Percentages of toddlers with solicited AEs, unsolicited AEs or congenital anomalies were calculated with exact 95% CIs. The analysis of neurodevelopmental status was performed on the total enrolled cohort (to account for participants who withdrew between the first and second visit). The proportion of participants with scores below the cut-off for each and any of the ASQ-3 domains, the proportion referred for BSID-III neurodevelopmental evaluation and those with at least one indicator of neurodevelopmental impairment using BSID-III were calculated.

Analyses were performed using SAS Drug Development (SAS Institute Inc., Cary, NC, USA).

## 3. Results

### 3.1. Study population

Of the 686 infants born to women enrolled in the maternal immunization study [20] and 592 infants completing the infant study [21], 551 were enrolled in the current toddler study: 270 whose mothers had received Tdap and 281 whose mothers had received placebo during pregnancy (Fig. 2). 540 toddlers received the DTaP-HepB-IPV/Hib booster, 536 completed the study and 479 were included in the ATP cohort for immunogenicity (Fig. 2). Demographic characteristics were comparable between groups (Table 1). The mean age at booster vaccination was 15 months.

### 3.2. Immunogenicity

Results are shown for the primary analyses on the ATP cohort for immunogenicity. Results for the TVC were very similar to those for the ATP cohort and the same overall conclusions could be drawn.

#### 3.2.1. Antibody persistence after the DTaP-HepB-IPV/Hib and PCV13 primary series (pre-booster)

Before the DTaP-HepB-IPV/Hib booster, observed antibody GMCs for FHA, PRN, PT and diphtheria were higher in the control than in the Tdap group (approximately 1.4- to 1.5-fold). GMCs for the other DTaP-HepB-IPV/Hib antigens were comparable between groups (Tables 2 and 3). Comparable proportions of toddlers in both groups were seroprotected against diphtheria, tetanus, hepatitis B, polio and Hib (Table 3). The proportions of toddlers with pneumococcal antibody concentrations $\geq$ 0.35 μg/mL

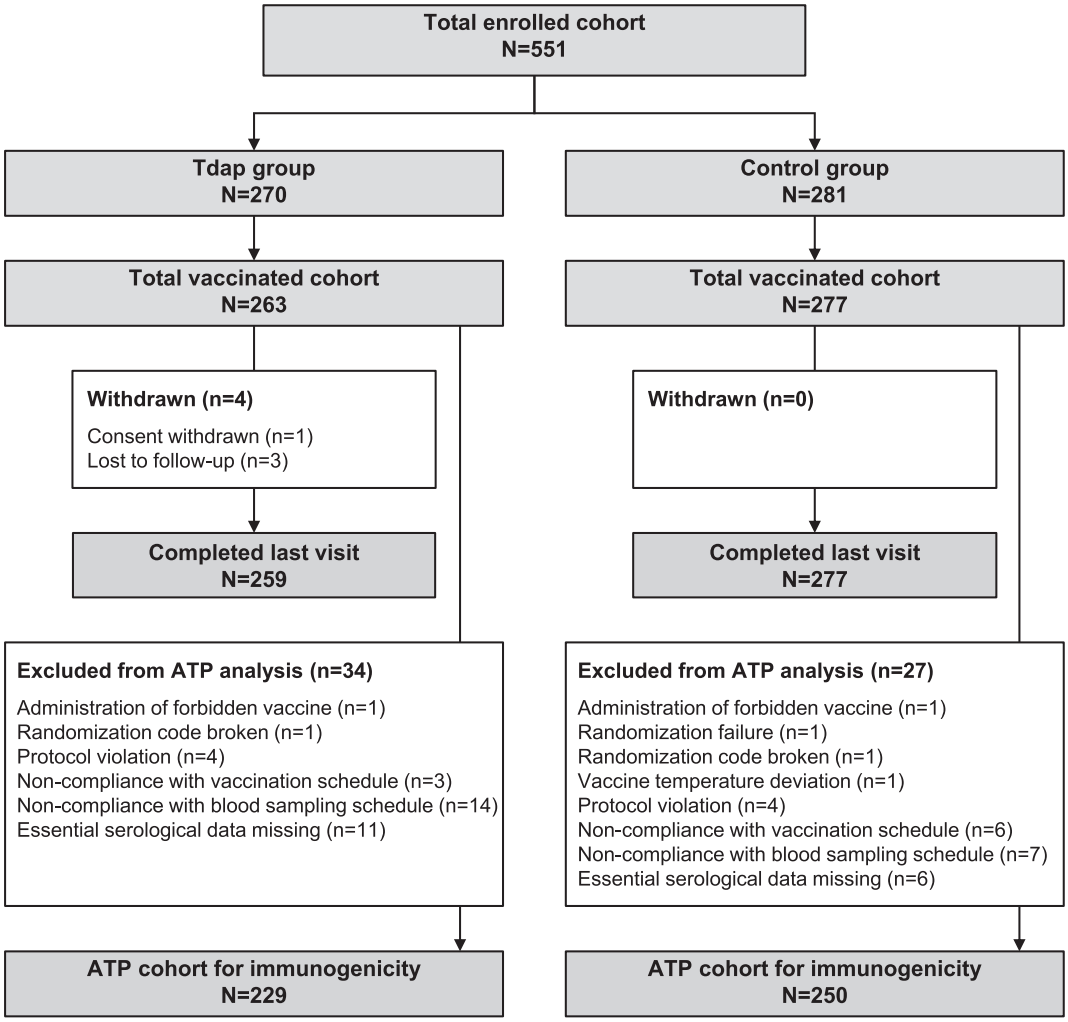


**Fig. 2. Participant flow diagram.** ATP, according-to-protocol; N, number of toddlers per cohort/group; n, number of toddlers with the specified elimination code assigned (excluding those for whom a lower elimination code number was assigned; elimination codes from the maternal immunization trial and the primary vaccination follow-up study carried forward into the booster vaccination follow-up study); Tdap, tetanus-reduced-antigen-content diphtheria-acellular pertussis vaccine.

and pneumococcal antibody GMCs were similar between groups for most PCV13 serotypes (Table 4).

*3.2.2. Response to the DTaP-HepB-IPV/Hib and PCV13 booster doses*

Substantial increases in antibody GMCs were observed from pre- to post-booster for each DTaP-HepB-IPV-Hib antigen in both groups (pertussis antigens: 13.6- versus 11.3-fold [anti-FHA], 48.4- versus 27.3-fold [anti-PRN], 11.9- versus 12.7-fold [anti-PT] in the Tdap versus the control group, respectively) (Tables 2 and 3). Accordingly, pertussis booster response rates were similarly high in both groups: 92.1%–98.1% (Tdap) and 96.7%–99.6% (control) (Table 2), as were seroprotection rates for the other DTaP-HepB-IPV/Hib antigens: ≥99.5% (Tdap) and ≥ 99.2% (control) (Table 3). Higher post-booster antibody GMCs were observed in controls than in the Tdap group for anti-FHA (1.2-fold), anti-PT (1.5-fold) and anti-diphtheria (1.4-fold). GMCs for the other DTaP-HepB-IPV/Hib antigens were similar between groups (Tables 2 and 3).

Fig. 3 shows pertussis antibody GMCs in the two groups from before the pregnancy dose until the post-booster timepoint.

At least 98.1% of toddlers in the Tdap group and ≥ 98.7% in the control group had post-booster pneumococcal antibody concentrations ≥ 0.35 μg/mL for each PCV13 serotype, except serotype 3 (Tdap: 79.3%; control: 79.6%) (Table 4). Pneumococcal antibody GMCs were similar between groups (Table 4).

*3.2.3. Subgroup analyses*

Subgroup analyses by primary series dose schedule, or by gestational or maternal age at the pregnancy dose did not indicate that lower post-booster immunogenicity after maternal Tdap immunization was specific to some subgroups (Supplementary tables 1–3 and not shown). In each subgroup, observed post-booster antibody GMCs for FHA, PT and diphtheria tended to be higher in the control versus the Tdap group.

*3.3. Reactogenicity and safety*

Solicited AEs were reported at similar rates in both groups, with redness at the DTaP-HepB-IPV/Hib injection site being the most common local (Tdap: 49.0%; control: 53.5%) and irritability the most common general solicited AE (Tdap: 63.2%; control: 68.4%) (Table 5). A minority of toddlers experienced grade 3 solicited AEs (Table 5). Large injection site reactions occurred in two toddlers in the Tdap group and three in the control at the DTaP-HepB-IPV/Hib site and in one toddler in each group at the PCV13 site. Reporting rates for unsolicited AEs were also similar

**Table 1**
Characteristics of participants in the total vaccinated cohort.

| Parameter | Tdap group (N = 263) | Control group (N = 277) |
|---|---|---|
| Country, n (%) | | |
| Australia | 12 (4.6) | 12 (4.3) |
| Canada | 66 (25.1) | 74 (26.7) |
| Czech Republic | 32 (12.2) | 35 (12.6) |
| Finland | 20 (7.6) | 29 (10.5) |
| Italy | 4 (1.5) | 4 (1.4) |
| Spain | 129 (49.0) | 123 (44.4) |
| Mean age ± SD at booster vaccination, months | | |
| Overall | 15.1 ± 2.5 | 14.9 ± 2.5 |
| Australia | 17.1 | 17.1 |
| Canada | 15.3 | 15.4 |
| Czech Republic | 12.9 | 13.2 |
| Finland | 12.3 | 12.3 |
| Italy | 11.8 | 11.5 |
| Spain | 16.7 | 16.6 |
| Female sex, n (%) | 122 (46.4) | 125 (45.1) |
| Ethnic origin, n (%) | | |
| White[a] | 240 (91.3) | 262 (94.6) |
| Asian | 3 (1.1) | 0 (0.0) |
| African/African American | 4 (1.5) | 8 (2.9) |
| Other | 16 (6.1) | 7 (2.5) |
| DTaP-HepB-IPV/Hib primary vaccination schedule, n (%) | | |
| 2-dose | 26 (9.9) | 36 (13.0) |
| 3, 5 months | 24 (9.1) | 33 (11.9) |
| 2, 4 months | 2 (0.8) | 3 (1.1) |
| 3-dose | 237 (90.1) | 241 (87.0) |
| 2, 4, 6 months | 205 (77.9) | 206 (74.4) |
| 2, 3, 4 months | 32 (12.2) | 35 (12.6) |
| Maternal age category at pregnancy dose, n (%) | | |
| 18–24 years | 4 (1.5) | 12 (4.3) |
| 25–34 years | 164 (62.4) | 172 (62.1) |
| 35–45 years | 95 (36.1) | 93 (33.6) |
| Gestational age category at pregnancy dose, n (%) | | |
| 27–32 weeks | 156 (59.3) | 165 (59.6) |
| 33–36 weeks | 107 (40.7) | 112 (40.4) |

DTaP-HepB-IPV/Hib, diphtheria-tetanus-acellular pertussis-hepatitis B virus-inactivated poliovirus and *Haemophilus influenzae* type b vaccine; N, total number of infants per group; n (%), number (percentage) of infants in the specified category; SD, standard deviation; Tdap, tetanus-reduced-antigen-content diphtheria-acellular pertussis vaccine.

[a] Includes White – Caucasian/European heritage (majority) and White – Arabic/North African heritage (1 in Tdap and 3 in control group).

between groups (Tdap: 35.7%; control: 40.1%), as were those for grade 3 AEs and AEs related to booster vaccination (Table 5). The most common related AEs were vomiting in the Tdap group (4 [1.5%] toddlers) and injection site mass in the control group (2 [0.7%] toddlers). No grade 3 unsolicited AEs were considered vaccination-related.

After the booster dose, three SAEs were reported in three toddlers in the control group (periorbital cellulitis [resolved after 13 days], pneumonia [resolved after 11 days] and sleep apnea syndrome [resolved after 133 days]). None of these were deemed vaccination-related. Before the booster dose, there was one fatality in the Tdap group: a 16-month-old toddler with congenital cytomegalovirus died as a result of several complications (enterovirus gastroenteritis, encephalopathy, astrovirus gastroenteritis, shock, increased intracranial pressure, dehydration and rhabdomyolysis). None of these complications were considered related to prior vaccination. This was the only participant who was withdrawn from the study due to an SAE.

During the maternal immunization trial, the infant and toddler studies, congenital anomalies were reported for 35 participants (10.4%, 95% CI 7.3–14.1%) in the Tdap group and 44 (12.9%, 9.5–16.9%) in the control group (Supplementary table 4). The most common congenital anomaly in both groups was atrial septal defect (Tdap: 1.5%, 0.5–3.4%; control: 2.3%, 1.0–4.6%).

At either 9 or 18 months of age, 11.5% of toddlers in the Tdap group and 11.0% in the control group had an ASQ-3 score below to cut-off for at least one neurodevelopmental domain; 40.0% and 52.9%, respectively, were referred for formal neurodevelopmental evaluation (based on BSID-III) and 4.6% and 5.8%, respectively, had at least one indicator of neurodevelopmental delay.

Supplementary Fig. 1 represents a plain language summary, summarizing the findings and highlighting their clinical relevance.

## 4. Discussion

This study showed that—as a consequence of the interference of maternally transferred Tdap-induced antibodies with the infant immune response to pertussis and diphtheria primary vaccination (as observed in the infant study [21])—antibody concentrations for

**Table 2**
Booster response rates, seropositivity rates and geometric mean concentrations for pertussis antibodies in toddlers before and 1 month after booster vaccination (ATP cohort for immunogenicity).

| Antibody (Cut-off) | Time-point | Tdap group | | | | | Control group | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N | Booster response[a], % (95% CI) | N' | Sero-positivity, % (95% CI) | GMC, IU/mL (95% CI) | N | Booster response[a], % (95% CI) | N' | Sero-positivity, % (95% CI) | GMC, IU/mL (95% CI) |
| **Anti-FHA** (2.046 IU/mL) | Pre | na | na | 223 | 96.4 (93.1–98.4) | 11.2 (9.6–13.1) | na | na | 244 | 98.8 (96.4–99.7) | 16.5 (14.4–18.8) |
| | Post | 215 | 96.7 (93.4–98.7) | 221 | 100 (98.3–100) | 152.5 (136.3–170.6) | 241 | 96.7 (93.6–98.6) | 247 | 100 (98.5–100) | 187.2 (172.7–202.9) |
| **Anti-PRN** (2.187 IU/mL) | Pre | na | na | 223 | 83.9 (78.4–88.4) | 6.9 (5.8–8.2) | na | na | 244 | 87.3 (82.5–91.2) | 9.6 (8.3–11.2) |
| | Post | 214 | 98.1 (95.3–99.5) | 220 | 100 (98.3–100) | 333.9 (285.4–390.7) | 241 | 99.6 (97.7– 100) | 247 | 100 (98.5–100) | 262.3 (230.9–298.1) |
| **Anti-PT** (2.693 IU/mL) | Pre | na | na | 223 | 68.6 (62.1–74.6) | 4.4 (3.8–5.0) | na | na | 244 | 82.4 (77.0–86.9) | 6.3 (5.5–7.1) |
| | Post | 215 | 92.1 (87.6–95.3) | 221 | 99.5 (97.5–100) | 52.4 (46.9–58.4) | 241 | 97.5 (94.7–99.1) | 247 | 100 (98.5–100) | 80.3 (73.3–88.1) |

%, percentage of toddlers who mounted a vaccine response or were seropositive (antibody concentration equal to or above the specified assay cut-offs); ATP, according-to-protocol; CI, confidence interval; FHA, filamentous hemagglutinin; GMC, geometric mean concentration; IU, international unit; N, number of toddlers with pre- and post-vaccination results available; N', number of toddlers with results available at the specified timepoint; na, not applicable; PRN, pertactin; PT, pertussis toxoid; Tdap, tetanus-reduced-antigen-content diphtheria-acellular pertussis vaccine.

[a] Booster response to FHA, PRN and PT was defined as a post-vaccination concentration ≥ 4x the assay cut-off for toddlers with a pre-vaccination concentration < assay cut-off, a post-vaccination concentration ≥ 4x the pre-vaccination concentration for toddlers with a pre-vaccination concentration between the assay cut-off and < 4x the assay cut-off, a post-vaccination concentration ≥ 2x the pre-vaccination concentration for toddlers with a pre-vaccination concentration ≥ 4x the assay cut-off.

**Table 3**
Seroprotection rates and geometric mean concentrations or titers for diphtheria, tetanus, hepatitis B, poliovirus and Hib antibodies in toddlers before and 1 month after booster vaccination (ATP cohort for immunogenicity).

| Antibody (Cut-off) | Time-points | Tdap group | | | Control group | | |
|---|---|---|---|---|---|---|---|
| | | N | Seroprotection, % (95% CI) | GMC or GMT (95% CI) | N | Seroprotection, % (95% CI) | GMC or GMT (95% CI) |
| Anti-D | Pre | 223 | 81.2 (75.4–86.1) | 0.21 (0.18–0.23) | 244 | 90.2 (85.7–93.6) | 0.32 (0.29–0.36) |
| (0.1 IU/mL) | Post | 221 | 100 (98.3–100) | 6.11 (5.58–6.70) | 247 | 100 (98.5–100) | 8.40 (7.69–9.17) |
| Anti-T | Pre | 223 | 96.4 (93.1–98.4) | 0.75 (0.65–0.88) | 244 | 95.1 (91.6–97.4) | 0.58 (0.51–0.66) |
| (0.1 IU/mL) | Post | 221 | 100 (98.3–100) | 8.20 (7.32–9.18) | 247 | 100 (98.5–100) | 6.76 (6.14–7.43) |
| Anti-HBs | Pre | 219 | 94.1 (90.1–96.8) | 158.7 (129.9–194.0) | 243 | 94.2 (90.5–96.8) | 193.4 (158.4–236.1) |
| (10 mIU/mL) | Post | 216 | 99.5 (97.4–100) | 4858.3 (3918.4–6023.7) | 241 | 99.2 (97.0–99.9) | 5031.2 (4072.7–6215.4) |
| Anti-polio 1 | Pre | 213 | 88.3 (83.2–92.3) | 64.9 (52.0–80.9) | 237 | 89.5 (84.8–93.1) | 83.3 (67.7–102.5) |
| (8 ED50) | Post | 204 | 100 (98.2–100) | 1611.7 (1381.2–1880.6) | 228 | 100 (98.4–100) | 1532.1 (1322.2–1775.3) |
| Anti-polio 2 | Pre | 210 | 89.5 (84.6–93.3) | 71.7 (57.6–89.4) | 236 | 91.1 (86.7–94.4) | 79.2 (64.4–97.5) |
| (8 ED50) | Post | 201 | 100 (98.2–100) | 2232.4 (1931.2–2580.5) | 227 | 100 (98.4–100) | 2371.2 (2097.9–2680.1) |
| Anti-polio 3 | Pre | 205 | 91.7 (87.1–95.1) | 106.0 (84.1–133.4) | 226 | 95.1 (91.5–97.5) | 118.4 (97.0–144.5) |
| (8 ED50) | Post | 188 | 100 (98.1–100) | 2944.6 (2529.4–3427.9) | 210 | 100 (98.3–100) | 2891.8 (2496.2–3350.2) |
| Anti-PRP | Pre | 222 | 72.5 (66.1–78.3) | 0.37 (0.30–0.45) | 244 | 68.0 (61.8–73.8) | 0.29 (0.24–0.35) |
| (0.15 μg/mL) | Post | 221 | 100 (98.3–100) | 26.19 (22.61–30.33) | 247 | 99.6 (97.8–100) | 19.71 (16.89–23.01) |
| Anti-PRP | Pre | 222 | 22.5 (17.2–28.6) | | 244 | 17.6 (13.1–23.0) | |
| (1.0 μg/mL) | Post | 221 | 99.5 (97.5–100) | | 247 | 97.6 (94.8–99.1) | |

%, percentage of toddlers with antibody concentrations equal to or above the specified seroprotection cut-offs; ATP, according-to-protocol; CI, confidence interval; D, diphtheria; ED50, effective dose causing 50% effect; GMC, geometric mean concentration; GMT, geometric mean titer; HBs, hepatitis B surface antigen; Hib, *Haemophilus influenzae* type b; polio 1–3, poliovirus types 1–3; (m)IU, (milli)international unit; N, number of toddlers with available results; PRP, Hib polyribosylribitol phosphate; T, tetanus; Tdap, tetanus-reduced-antigen-content diphtheria-acellular pertussis vaccine.

**Table 4**
Percentages of toddlers with pneumococcal serotype-specific antibody concentrations ≥ 0.35 μg/mL and geometric mean concentrations before and 1 month after booster vaccination (ATP cohort for immunogenicity).

| Vaccine serotype | Time-point | Tdap group | | | Control group | | |
|---|---|---|---|---|---|---|---|
| | | N | % ≥0.35 μg/mL (95% CI) | GMC, μg/mL (95% CI) | N | % ≥0.35 μg/mL (95% CI) | GMC, μg/mL (95% CI) |
| 1 | Pre | 211 | 28.9 (22.9–35.5) | 0.22 (0.19–0.24) | 232 | 39.2 (32.9–45.8) | 0.27 (0.24–0.30) |
| | Post | 208 | 100 (98.2–100) | 3.22 (2.88–3.60) | 236 | 100 (98.4–100) | 3.64 (3.28–4.04) |
| 3 | Pre | 211 | 3.8 (1.7–7.3) | 0.08 (0.07–0.09) | 232 | 5.2 (2.7–8.9) | 0.10 (0.09–0.11) |
| | Post | 208 | 79.3 (73.2–84.6) | 0.59 (0.53–0.65) | 235 | 79.6 (73.8–84.5) | 0.62 (0.57–0.69) |
| 4 | Pre | 209 | 13.4 (9.1–18.8) | 0.15 (0.13–0.16) | 232 | 27.2 (21.5–33.4) | 0.19 (0.17–0.22) |
| | Post | 208 | 99.5 (97.4–100) | 2.91 (2.54–3.33) | 234 | 99.1 (96.9–99.9) | 3.28 (2.89–3.72) |
| 5 | Pre | 211 | 47.4 (40.5–54.4) | 0.33 (0.29–0.37) | 229 | 54.1 (47.5–60.7) | 0.34 (0.31–0.38) |
| | Post | 204 | 100 (98.2–100) | 2.66 (2.39–2.97) | 229 | 99.1 (96.9–99.9) | 2.81 (2.52–3.14) |
| 6A | Pre | 211 | 53.1 (46.1–60.0) | 0.38 (0.33–0.43) | 232 | 62.9 (56.4–69.2) | 0.44 (0.39–0.50) |
| | Post | 208 | 100 (98.2–100) | 9.07 (8.05–10.22) | 236 | 100 (98.4–100) | 9.49 (8.45–10.67) |
| 6B | Pre | 211 | 45.5 (38.6–52.5) | 0.29 (0.25–0.33) | 232 | 49.6 (43.0–56.2) | 0.33 (0.29–0.38) |
| | Post | 208 | 100 (98.2–100) | 7.83 (6.82–8.98) | 236 | 99.6 (97.7–100) | 8.00 (7.06–9.06) |
| 7F | Pre | 211 | 69.7 (63.0–75.8) | 0.49 (0.44–0.54) | 232 | 75.4 (69.4–80.8) | 0.56 (0.51–0.61) |
| | Post | 208 | 100 (98.2–100) | 5.00 (4.55–5.50) | 235 | 100 (98.4–100) | 4.96 (4.50–5.48) |
| 9 V | Pre | 211 | 33.6 (27.3–40.5) | 0.26 (0.23–0.29) | 232 | 45.7 (39.2–52.3) | 0.32 (0.28–0.36) |
| | Post | 208 | 100 (98.2–100) | 3.74 (3.35–4.16) | 235 | 100 (98.4–100) | 3.91 (3.52–4.35) |
| 14 | Pre | 211 | 87.2 (81.9–91.4) | 0.97 (0.85–1.11) | 232 | 88.8 (84.0–92.5) | 1.19 (1.04–1.37) |
| | Post | 208 | 100 (98.2–100) | 10.36 (9.22–11.64) | 236 | 100 (98.4–100) | 11.62 (10.34–13.06) |
| 18C | Pre | 211 | 19.9 (14.7–25.9) | 0.19 (0.17–0.21) | 232 | 34.1 (28.0–40.5) | 0.23 (0.21–0.26) |
| | Post | 208 | 99.5 (97.4–100) | 3.23 (2.86–3.65) | 236 | 100 (98.4–100) | 3.57 (3.21–3.98) |
| 19A | Pre | 211 | 40.8 (34.1–47.7) | 0.32 (0.27–0.37) | 232 | 50.9 (44.2–57.5) | 0.37 (0.32–0.43) |
| | Post | 208 | 100 (98.2–100) | 7.90 (7.06–8.83) | 236 | 100 (98.4–100) | 8.68 (7.82–9.63) |
| 19F | Pre | 211 | 49.3 (42.4–56.2) | 0.37 (0.32–0.43) | 232 | 61.6 (55.0–67.9) | 0.47 (0.41–0.55) |
| | Post | 208 | 100 (98.2–100) | 7.66 (6.84–8.57) | 236 | 100 (98.4–100) | 8.63 (7.75–9.62) |
| 23F | Pre | 210 | 15.7 (11.1–21.4) | 0.14 (0.12–0.16) | 229 | 25.3 (19.8–31.5) | 0.19 (0.16–0.22) |
| | Post | 207 | 98.1 (95.1–99.5) | 2.07 (1.83–2.34) | 235 | 98.7 (96.3–99.7) | 2.38 (2.10–2.69) |

%, percentage of toddlers with antibody concentrations ≥ 0.35 μg/mL; ATP, according-to-protocol; CI, confidence interval; GMC, geometric mean concentration; N, number of toddlers with available results; Tdap, tetanus-reduced-antigen-content diphtheria-acellular pertussis vaccine.

these antigens were still lower in toddlers from Tdap-vaccinated mothers before DTaP-HepB-IPV/Hib booster vaccination. After the booster, differences in antibody concentrations between toddlers from the Tdap versus the control group were still apparent for FHA, PT and diphtheria but not for PRN. While observed anti-FHA, anti-PT and anti-diphtheria antibody GMCs were lower in toddlers born to mothers who had received Tdap during pregnancy compared to mothers who had not, pre- to post-booster GMC increases were similarly high in both groups, as were booster response (for pertussis antigens) and seroprotection rates (for diphtheria), indicating induction of immune memory in infants born to Tdap-vaccinated mothers.

For diphtheria, as all toddlers achieved seroprotective antibody levels after the booster dose, the clinical significance of the lower antibody GMCs is likely limited. For pertussis, the clinical relevance is difficult to ascertain since no correlate of protection has been established [35] and only humoral responses were assessed. Effectiveness studies in the UK and the US, and UK pertussis disease surveillance data do not indicate an increased risk of pertussis disease in the first and second years of life in infants and toddlers

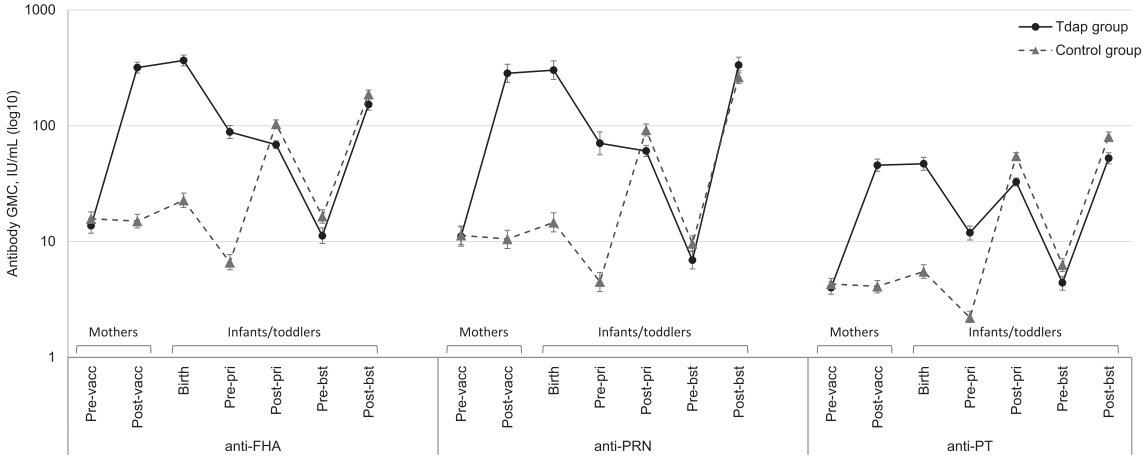


**Fig. 3. Geometric mean concentrations for pertussis antibodies in mothers before and after the pregnancy dose and in infants and toddlers before and after primary and booster vaccination (ATP cohorts for immunogenicity).** ATP, according-to-protocol; FHA, filamentous hemagglutinin; GMC, geometric mean concentration; IU, international unit; PRN, pertactin; PT, pertussis toxoid; Tdap, tetanus-reduced-antigen-content diphtheria-acellular pertussis vaccine. Error bars depict 95% confidence intervals. Timepoints: before and 30 days after the pregnancy dose (pre-vacc and post-vacc), birth (infant cord blood), before and 1 month after 2-dose or 3-dose primary vaccination (pre-pri and post-pri), before and 1 month after booster vaccination (pre-bst and post-bst).

**Table 5**
Solicited and unsolicited adverse events after booster vaccination (total vaccinated cohort).

| Adverse event | Tdap group | | Control group | |
|---|---|---|---|---|
| | n | % (95% CI) | n | % (95% CI) |
| | | **(N = 257)** | | **(N = 275)** |
| **Pain** | | | | |
| Any | 119 | 46.3 (40.1–52.6) | 137 | 49.8 (43.8–55.9) |
| Grade 3 | 8 | 3.1 (1.4–6.0) | 12 | 4.4 (2.3–7.5) |
| **Redness** | | | | |
| Any | 126 | 49.0 (42.8–55.3) | 147 | 53.5 (47.4–59.5) |
| >20 mm | 21 | 8.2 (5.1–12.2) | 24 | 8.7 (5.7–12.7) |
| **Swelling** | | | | |
| Any | 103 | 40.1 (34.0–46.3) | 106 | 38.5 (32.8–44.6) |
| >20 mm | 16 | 6.2 (3.6–9.9) | 16 | 5.8 (3.4–9.3) |
| | | **(N = 257)** | | **(N = 274)** |
| **Pain** | | | | |
| Any | 113 | 44.0 (37.8–50.3) | 124 | 45.3 (39.3–51.4) |
| Grade 3 | 8 | 3.1 (1.4–6.0) | 16 | 5.8 (3.4–9.3) |
| **Redness** | | | | |
| Any | 120 | 46.7 (40.5–53.0) | 132 | 48.2 (42.1–54.3) |
| >20 mm | 14 | 5.4 (3.0–9.0) | 14 | 5.1 (2.8–8.4) |
| **Swelling** | | | | |
| Any | 87 | 33.9 (28.1–40.0) | 86 | 31.4 (25.9–37.2) |
| >20 mm | 9 | 3.5 (1.6–6.5) | 15 | 5.5 (3.1–8.9) |
| | | **(N = 258)** | | **(N = 275)** |
| **Drowsiness** | | | | |
| Any | 128 | 49.6 (43.4–55.9) | 138 | 50.2 (44.1–56.2) |
| Grade 3 | 5 | 1.9 (0.6–4.5) | 7 | 2.5 (1.0–5.2) |
| **Irritability** | | | | |
| Any | 163 | 63.2 (57.0–69.1) | 188 | 68.4 (62.5–73.8) |
| Grade 3 | 13 | 5.0 (2.7–8.5) | 28 | 10.2 (6.9–14.4) |
| **Loss of appetite** | | | | |
| Any | 104 | 40.3 (34.3–46.6) | 116 | 42.2 (36.3–48.3) |
| Grade 3 | 10 | 3.9 (1.9–7.0) | 10 | 3.6 (1.8–6.6) |
| **Fever**[a] | | | | |
| Any | 73 | 28.3 (22.9–34.2) | 85 | 30.9 (25.5–36.7) |
| >39.0 °C | 7 | 2.7 (1.1–5.5) | 7 | 2.5 (1.0–5.2) |
| | | **(N = 263)** | | **(N = 277)** |
| Any | 94 | 35.7 (29.9–41.9) | 111 | 40.1 (34.3–46.1) |
| Grade 3 | 19 | 7.2 (4.4–11.1) | 16 | 5.8 (3.3–9.2) |
| Related | 10 | 3.8 (1.8–6.9) | 10 | 3.6 (1.7–6.5) |

AE, adverse event; CI, confidence interval; DTaP-HepB-IPV/Hib, diphtheria-tetanus-acellular pertussis-hepatitis B virus-inactivated poliovirus and *Haemophilus influenzae* type b vaccine; N, number of toddlers with the documented dose (for solicited AEs) or with the administered dose (for unsolicited AEs); n/%, number/percentage of toddlers for whom the specified AE was reported at least once during the follow-up period; PCV13, 13-valent pneumococcal conjugate vaccine; Tdap, tetanus-reduced-antigen-content diphtheria-acellular pertussis vaccine.

[a] Any fever was defined as a temperature ≥ 37.5 °C (oral, axillary, tympanic), or ≥ 38.0 °C (rectal).

born to mothers vaccinated with pertussis-containing vaccines during pregnancy [13,14,43,44]. A transmission model by Bento and Rohani showed that maternal immunization is an effective strategy to protect newborns, but—depending on the settings—if maternal antibodies interfere with infant immune responses, increases in pertussis disease can be expected in older children, with a decade's (or more) delay [45]. Therefore, continuous surveillance is needed to monitor the pertussis incidence in different age groups in countries with maternal pertussis immunization programs. This will further elucidate the clinical impact of maternal immunization on protection conferred by pediatric vaccines in infants and toddlers. In addition, further research is needed to fully understand other factors that can influence clinical protection through maternal immunization. These include the immune response of pregnant woman to different vaccine antigens, the relevance of the quantity and composition of the antigens in inducing functional vaccine-specific immune responses, the optimal timing of vaccine administration in pregnancy, the duration of vaccine-induced protection in the mothers and their infants, and the role of breastmilk antibodies in protecting infants [46].

Our results are in line with those from randomized maternal immunization trials in Canada and the Netherlands, showing significantly lower antibody GMCs for FHA and PT (and PRN, in the Dutch trial only) after pertussis booster vaccination in toddlers born to women vaccinated versus not vaccinated with 5-component or 3-component Tdap during pregnancy [22,23]. A prospective cohort study in Belgium showed significantly lower post-booster anti-PT GMCs in toddlers from 3-component Tdap-vaccinated mothers versus unvaccinated mothers [47]. In other smaller studies, non-significant trends for lower post-booster antibody GMCs for some or all pertussis antigens were apparent in toddlers born to Tdap-vaccinated versus unvaccinated mothers [24,28,29]. A recent review considered the observed interference as a class effect of pertussis-containing vaccines, regardless of the type of maternal or childhood vaccine used [48].

Based on our subgroup analyses, interference was apparent regardless of the primary series dose schedule and the gestational and maternal age at the pregnancy Tdap dose; however, the small sample size in some of these subgroups limits interpretation of these analyses.

In the infant study, we found no evidence for interference of Tdap-induced maternal antibodies with the immune response to tetanus, hepatitis B, poliovirus or Hib, nor with the response to pneumococcal conjugates [21]. Likewise, in the toddler study, no interference was observed for these antigens prior to or after DTaP-HepB-IPV/Hib and PCV13 booster vaccination. While this is largely in line with previous studies [23,49,50], some have shown higher post-primary or post-booster tetanus responses [25,26,28,29] and lower responses to a number of pneumococcal conjugates after the primary infant series [26,49–51], which mostly disappeared post-booster [49,51].

The reactogenicity and safety profiles of the DTaP-HepB-IPV/Hib and PCV13 booster doses in toddlers did not change depending on the exposure/non-exposure to Tdap during pregnancy. Solicited and unsolicited AE rates were similar between groups, no vaccination-related SAEs were reported and no differences were observed in terms of neurodevelopment and congenital anomalies.

Limitations of our study include a limited generalizability because the study was performed in high-income countries and mothers were mostly white Caucasian with low-risk pregnancies. In addition, because our study was descriptive and no adjustments for multiple comparisons were made, the observed differences between toddlers from mothers who received Tdap during compared to after pregnancy cannot be taken as evidence of statistically significant differences between these two groups.

## 5. Conclusion

Our randomized, placebo-controlled maternal immunization trial and follow-up infant and toddler studies showed that Tdap-induced pertussis antibodies were transferred efficiently from vaccinated mothers to their infants and persisted in infants during their first vulnerable months of life. However, these antibodies caused interference with the immune response to pertussis primary vaccination, the effect of which was still apparent before boosting for the three pertussis antigens and after boosting for FHA and PT. The clinical relevance of this interference requires further evaluation through continuous long-term monitoring of the pertussis incidence in older infants and children in countries with a maternal pertussis immunization program. We found no evidence for interference with the response to the other DTaP-HepB-IPV/Hib or PCV13 antigens, aside from potential minimal interference for diphtheria. The reactogenicity/safety profiles of DTaP-HepB-IPV/Hib and PCV13 in toddlers did not change depending on the exposure/non exposure to Tdap during pregnancy.

## Funding

This work was supported by GlaxoSmithKline Biologicals S.A., which was the funding source, was involved in all stages of the study conduct and analysis and paid for all costs associated with the development and publishing of this manuscript.

## Author contributions

MB, BC, SAH, PK, LK, SOK, FMT, NMes, TN, KPP, ZS and GVZ were involved in study conception and design. BAN, MB, JB, MAC, MJCO, EEP, JGS, SAH, JJ, PK, LK, PGM, FMT, JMMA, NMes, NMey, MMV, TN, KPP, JTRA, SR, ISC, ZS, BT, OGV, MV and GVZ performed the study or participated in data collection. MAC, BC, SAH, LK, SOK, PGM, FMT, JMMA, NMes, NMey, MMV, TN, KPP, ZS, BT, OV and MV were involved in data analysis and/or interpretation. All authors had access to the data and granted their final approval of the paper before submission.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: BAN reports grants from the GSK group of companies (GSK) and support from other vaccine manufacturers. BC, MAC, NMes, NMey and SOK are employees of GSK, and BC, MAC and NMes own GSK restricted shares. FMT, JGS, SAH, TN and KPP's institutions received grants from GSK during the conduct of the study. FMT's institution received financial and non-financial support from Ablynx, Astra Zeneca, GSK, Jansen, MedImmune, MSD, Novavax, Pfizer, Regeneron, Roche, Sanofi Pasteur and Seqirus outside the submitted work. SAH received honoraria for participation in ad-hoc advisory committees for GSK and Sanofi Pasteur. TN received personal fees from GSK for serving as chair of advisory committees outside the submitted work. BT's institution received grants from GSK during the conduct of the study; research grants for clinical trials were also given to his institution by Pfizer, MSD and Sanofi Pasteur. KPP received a fellowship from National Health and Medical Research Council for conducting the present work, and her institution received financial support from DBV Technologies, MedImmune, Novavax and Pfizer for conducting trials outside the submitted work. ISC has received grants and/or honoraria as a consultant/advisor or to attend conferences and practical courses from GSK, Sanofi Pasteur, MSD and Pfizer outside the submitted work. JMMA reports receiving fees and non-financial support from GSK, as well

as fees from Pfizer and MSD outside the submitted work. LK is working as consultant for GSK. MB, JB, MJCO, EEP, JJ, MMV, PK, PGM, JTRA, SR, ZS, MV, OGV and GVZ declare no conflicts of interest.

## Acknowledgements

The authors would like to thank all mothers and infants for participating in the study, the members of the independent data monitoring committee and all the research staff at participating centers, in particular, Dr. L Redondo-Collazo, Dr. C Rodriguez-Tenreiro, Dr. M Macías-Cortiñas, and Dr. I Rivero-Calle from Hospital Clínico Universitario de Santiago; Dr. S Natalini from Hospital Universitario Madrid Sanchinarro; Dr. I Coro, from Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan; Dr. M Kacerovsky from University Hospital Hradec Kralove; and L Campora and A Kuznetsova from the safety team at GSK, Wavre.

The authors also thank Modis for editorial assistance and manuscript coordination, on behalf of GSK: Natalie Denef provided medical writing support and Quentin Deraedt coordinated the manuscript development and provided editorial support.

## Trademarks

Boostrix and Infanrix Hexa are trademarks of the GSK group of companies. Prevnar 13 is a trademark of Pfizer Inc.

## Data sharing

The study protocol is available at https://www.gsk-studyregister.com/study/5350. Anonymized individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

All authors attest they meet the ICMJE criteria for authorship.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2021.02.001.

## References

[1] Munoz FM. Current Challenges and Achievements in Maternal Immunization Research. Front Immunol 2018;9:436. https://doi.org/10.3389/fimmu.2018.00436.
[2] World Health Organization SAGE pertussis working group. Background paper; SAGE April 2014, http://www.who.int/immunization/sage/meetings/2014/april/1_Pertussis_background_FINAL4_web.pdf [accessed 27 February 2020].
[3] Masseria C, Martin CK, Krishnarajah G, Becker LK, Buikema A, Tan TQ. Incidence and Burden of Pertussis Among Infants Less Than 1 Year of Age. Pediatr Infect Dis J 2017;36:e54–61. https://doi.org/10.1097/inf.0000000000001440.
[4] Kandeil W, van den Ende C, Bunge EM, Jenkins VA, Ceregido MA, Guignard A. A systematic review of the burden of pertussis disease in infants and the effectiveness of maternal immunization against pertussis. Expert Rev Vaccines 2020;19:621–38. https://doi.org/10.1080/14760584.2020.1791092.
[5] Centers for Disease Control and Prevention (CDC). Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine (Tdap) in pregnant women and persons who have or anticipate having close contact with an infant aged <12 months — Advisory Committee on Immunization Practices (ACIP), 2011. MMWR Morb Mortal Wkly Rep 2011;60:1424-6.
[6] Department of Health and Social Care. Whooping Cough Vaccination Programme for Pregnant Women, https://www.gov.uk/government/publications/whooping-cough-vaccination-programme-for-pregnant-women. 2012 [accessed 27 February 2020].
[7] Gkentzi D, Katsakiori P, Marangos M, Hsia Y, Amirthalingam G, Heath PT, et al. Maternal vaccination against pertussis: a systematic review of the recent literature. Arch Dis Child Fetal Neonatal Ed 2017;102:F456–63. https://doi.org/10.1136/archdischild-2016-312341.
[8] World Health Organization. Pertussis vaccines: WHO position paper - September 2015. Wkly Epidemiol Rec 2015;90:433–58.
[9] Vygen-Bonnet S, Hellenbrand W, Garbe E, von Kries R, Bogdan C, Heininger U, et al. Safety and effectiveness of acellular pertussis vaccination during pregnancy: a systematic review. BMC Infect Dis 2020;20:136. https://doi.org/10.1186/s12879-020-4824-3.
[10] Donegan K, King B, Bryan P. Safety of pertussis vaccination in pregnant women in UK: observational study. BMJ 2014;349:g4219.
[11] Kharbanda EO, Vazquez-Benitez G, Lipkind HS, Klein NP, Cheetham TC, Naleway A, et al. Evaluation of the association of maternal pertussis vaccination with obstetric events and birth outcomes. JAMA 2014;312:1897–904. https://doi.org/10.1001/jama.2014.14825.
[12] Amirthalingam G, Andrews N, Campbell H, Ribeiro S, Kara E, Donegan K, et al. Effectiveness of maternal pertussis vaccination in England: an observational study. Lancet 2014;384:1521–8. https://doi.org/10.1016/s0140-6736(14)60686-3.
[13] Amirthalingam G, Campbell H, Ribeiro S, Fry NK, Ramsay M, Miller E, et al. Sustained Effectiveness of the Maternal Pertussis Immunization Program in England 3 Years Following Introduction. Clin Infect Dis 2016;63:S236–43. https://doi.org/10.1093/cid/ciw559.
[14] Baxter R, Bartlett J, Fireman B, Lewis E, Klein NP. Effectiveness of Vaccination During Pregnancy to Prevent Infant Pertussis. Pediatrics 2017;139:. https://doi.org/10.1542/peds.2016-4091e20164091.
[15] Bellido-Blasco J, Guiral-Rodrigo S, Miguez-Santiyan A, Salazar-Cifre A, Gonzalez-Moran F. A case-control study to assess the effectiveness of pertussis vaccination during pregnancy on newborns, Valencian community, Spain, 1 March 2015 to 29 February 2016. Euro Surveill 2017;22:30545. https://doi.org/10.2807/1560-7917.es.2017.22.22.30545.
[16] Dabrera G, Amirthalingam G, Andrews N, Campbell H, Ribeiro S, Kara E, et al. A case-control study to estimate the effectiveness of maternal pertussis vaccination in protecting newborn infants in England and Wales, 2012–2013. Clin Infect Dis 2015;60:333–7. https://doi.org/10.1093/cid/ciu821.
[17] Saul N, Wang K, Bag S, Baldwin H, Alexander K, Chandra M, et al. Effectiveness of maternal pertussis vaccination in preventing infection and disease in infants: The NSW Public Health Network case-control study. Vaccine 2018;36:1887–92. https://doi.org/10.1016/j.vaccine.2018.02.047.
[18] Romanin V, Acosta AM, Juarez MDV, Briere E, Sanchez SM, Cordoba BL, et al. Maternal Vaccination in Argentina: Tetanus, Diphtheria, and Acellular Pertussis Vaccine Effectiveness During Pregnancy in Preventing Pertussis in Infants <2 Months of Age. Clin Infect Dis 2020;70:380–7. https://doi.org/10.1093/cid/ciz217.
[19] Fernandes EG, Sato APS, Vaz-de-Lima LRA, Rodrigues M, Leite D, de Brito CA, et al. The effectiveness of maternal pertussis vaccination in protecting newborn infants in Brazil: A case-control study. Vaccine 2019;37:5481–4. https://doi.org/10.1016/j.vaccine.2019.03.049.
[20] Perrett KP, Halperin SA, Nolan T, Martínez Pancorbo C, Tapiero B, Martinón-Torres F, et al. Immunogenicity, transplacental transfer of pertussis antibodies and safety following pertussis immunization during pregnancy: Evidence from a randomized, placebo-controlled trial. Vaccine 2020;38:2095–104. https://doi.org/10.1016/j.vaccine.2019.10.105.
[21] Perrett KP, Halperin SA, Nolan T, Carmona Martínez A, Martinón-Torres F, García-Sicilia J, et al. Impact of tetanus-diphtheria-acellular pertussis immunization during pregnancy on subsequent infant immunization seroresponses: follow-up from a large randomized placebo-controlled trial. Vaccine 2020;38:2105–14. https://doi.org/10.1016/j.vaccine.2019.10.104.
[22] Barug D, Pronk I, van Houten MA, Versteegh FGA, Knol MJ, van de Kassteele J, et al. Maternal pertussis vaccination and its effects on the immune response of infants aged up to 12 months in the Netherlands: an open-label, parallel, randomised controlled trial. Lancet Infect Dis 2019;19:392–401. https://doi.org/10.1016/s1473-3099(18)30717-5.
[23] Halperin SA, Langley JM, Ye L, MacKinnon-Cameron D, Elsherif M, Allen VM, et al. A Randomized Controlled Trial of the Safety and Immunogenicity of Tetanus, Diphtheria, and Acellular Pertussis Vaccine Immunization During Pregnancy and Subsequent Infant Immune Response. Clin Infect Dis 2018;67:1063–71. https://doi.org/10.1093/cid/ciy244.
[24] Hardy-Fairbanks AJ, Pan SJ, Decker MD, Johnson DR, Greenberg DP, Kirkland KB, et al. Immune responses in infants whose mothers received Tdap vaccine during pregnancy. Pediatr Infect Dis J 2013;32:1257–60. https://doi.org/10.1097/INF.0b013e3182a09b6a.
[25] Hoang HT, Leuridan E, Maertens K, Nguyen TD, Hens N, Vu NH, et al. Pertussis vaccination during pregnancy in Vietnam: Results of a randomized controlled trial Pertussis vaccination during pregnancy 151–159. Vaccine 2016;34. https://doi.org/10.1016/j.vaccine.2015.10.098.
[26] Ladhani SN, Andrews NJ, Southern J, Jones CE, Amirthalingam G, Waight PA, et al. Antibody responses after primary immunization in infants born to women receiving a pertussis-containing vaccine during pregnancy: single arm observational study with a historical comparator. Clin Infect Dis 2015;61:1637–44. https://doi.org/10.1093/cid/civ695.
[27] Maertens K, Cabore RN, Huygen K, Hens N, Van Damme P, Leuridan E. Pertussis vaccination during pregnancy in Belgium: Results of a prospective controlled cohort study. Vaccine 2016;34:142–50. https://doi.org/10.1016/j.vaccine.2015.10.100.
[28] Maertens K, Hoang TT, Nguyen TD, Cabore RN, Duong TH, Huygen K, et al. The Effect of Maternal Pertussis Immunization on Infant Vaccine Responses to a Booster Pertussis-Containing Vaccine in Vietnam. Clin Infect Dis 2016;63:S197–204. https://doi.org/10.1093/cid/ciw551.
[29] Munoz FM, Bond NH, Maccato M, Pinell P, Hammill HA, Swamy GK, et al. Safety and immunogenicity of tetanus diphtheria and acellular pertussis

(Tdap) immunization during pregnancy in mothers and infants: a randomized clinical trial. JAMA 2014;311:1760–9. https://doi.org/10.1001/jama.2014.3633.
[30] Villarreal Perez JZ, Ramirez Aranda JM, de la O Cavazos M, Zamudio Osuna MJ, Perales Davila J, Ballesteros Elizondo MR, et al. Randomized clinical trial of the safety and immunogenicity of the Tdap vaccine in pregnant Mexican women. Hum Vaccin Immunother 2017;13:128–35. 10.1080/21645515.2016.1232786.
[31] Camargo ME, Silveira L, Furuta JA, Oliveira EP, Germek OA. Immunoenzymatic assay of anti-diphtheric toxin antibodies in human serum. J Clin Microbiol 1984;20:772–4.
[32] Melville-Smith ME, Seagroatt VA, Watkins JT. A comparison of enzyme-linked immunosorbent assay (ELISA) with the toxin neutralization test in mice as a method for the estimation of tetanus antitoxin in human sera. J Biol Stand 1983;11:137–44.
[33] Anderson P. The protective level of serum antibodies to the capsular polysaccharide of Haemophilus influenzae type b. J Infect Dis 1984;149:1034–5.
[34] Kayhty H, Peltola H, Karanko V, Makela PH. The protective level of serum antibodies to the capsular polysaccharide of Haemophilus influenzae type b. J Infect Dis 1983;147:1100.
[35] Plotkin SA. Correlates of protection induced by vaccination. Clin Vaccine Immunol 2010;17:1055–65. https://doi.org/10.1128/cvi.00131-10.
[36] Centers for Disease Control and Prevention (CDC). Hepatitis B virus: a comprehensive strategy for eliminating transmission in the United States through universal childhood vaccination. Recommendations of the Immunization Practices Advisory Committee (ACIP). MMWR Recomm Rep 1991;40:1–25.
[37] Jack AD, Hall AJ, Maine N, Mendy M, Whittle HC. What level of hepatitis B antibody is protective?. J Infect Dis 1999;179:489–92. https://doi.org/10.1086/314578.
[38] World Health Organization Expanded Programme on Immunization. Guidelines for WHO/EPI collaborative studies on poliomyelitis: standard procedure for determining immunity to poliovirus using the microneutralization test, http://www.who.int/iris/handle/10665/70486. 1993 [accessed 2 March 2020].
[39] Feyssaguet M, Bellanger A, Nozay F, Friel D, Merck E, Verlant V, et al. Comparison between a new multiplex electrochemiluminescence assay and the WHO reference enzyme-linked immunosorbent assay to measure serum antibodies against pneumococcal serotype-specific polysaccharides. Vaccine 2019;37:2208–15. https://doi.org/10.1016/j.vaccine.2019.03.011.
[40] 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;25:3816–26. https://doi.org/10.1016/j.vaccine.2007.01.119.
[41] Squires J, Bricker D. Ages & Stages Questionnaires®, Third Edition (ASQ- 3™). A parent-completed child-monitoring system. Baltimore: Paul H. Brookes Publishing Co.; 2009.
[42] Bayley N. Manual for the Bayley Scales of Infant and Toddler Development. San Antonio, TX: NCS Pearson; 2006.
[43] Becker-Dreps S, Butler AM, McGrath LJ, Boggess KA, Weber DJ, Li D, et al. Effectiveness of Prenatal Tetanus, Diphtheria, Acellular Pertussis Vaccination in the Prevention of Infant Pertussis in the U.S. Am J Prev Med 2018;55:159–66. https://doi.org/10.1016/j.amepre.2018.04.013.
[44] Public Health England. Pertussis: laboratory confirmed cases reported in England 2018 - Laboratory confirmed cases of pertussis (England): annual report for 2018, https://www.gov.uk/government/publications/pertussis-laboratory-confirmed-cases-reported-in-england-2018#history. 2018 [accessed 2 March 2020].
[45] Bento AI, Rohani P. Forecasting Epidemiological Consequences of Maternal Immunization. Clin Infect Dis 2016;63:S205–12. https://doi.org/10.1093/cid/ciw557.
[46] Albrecht M, Arck PC. Vertically Transferred Immunity in Neonates: Mothers, Mechanisms and Mediators. Front Immunol 2020;11:555. https://doi.org/10.3389/fimmu.2020.00555.
[47] Maertens K, Cabore RN, Huygen K, Vermeiren S, Hens N, Van Damme P, et al. Pertussis vaccination during pregnancy in Belgium: Follow-up of infants until 1 month after the fourth infant pertussis vaccination at 15 months of age. Vaccine 2016;34:3613–9. https://doi.org/10.1016/j.vaccine.2016.04.066.
[48] Kandeil W, Savic M, Ceregido MA, Guignard A, Kuznetsova A, Mukherjee P. Immune interference (blunting) in the context of maternal immunization with Tdap-containing vaccines: is it a class effect?. Expert Rev Vaccines 2020;19:341–52. https://doi.org/10.1080/14760584.2020.1749597.
[49] Barug D, Berbers GAM, van Houten MA, Kuijer M, Pronk I, Knol MJ, et al. Infant antibody levels following 10-valent pneumococcal-protein D conjugate and DTaP-Hib vaccinations in the first year of life after maternal Tdap vaccination: An open-label, parallel, randomised controlled trial. Vaccine 2020;38:4632–9. https://doi.org/10.1016/j.vaccine.2020.04.001.
[50] 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;13:21–30. https://doi.org/10.1016/j.eclinm.2019.06.010.
[51] Maertens K, Burbidge P, Van Damme P, Goldblatt D, Leuridan E. Pneumococcal Immune Response in Infants Whose Mothers Received Tetanus, Diphtheria and Acellular Pertussis Vaccination During Pregnancy. Pediatr Infect Dis J 2017;36:1186–92. https://doi.org/10.1097/inf.0000000000001601.