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Impact of tetanus-diphtheria-acellular pertussis immunization during pregnancy on subsequent infant immunization seroresponses: follow-up from a large randomized placebo-controlled trial

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ABSTRACT

Background: Pertussis immunization during pregnancy results in high pertussis antibody concentrations in young infants but may interfere with infant immune responses to post-natal immunization.
Methods: This phase IV, multi-country, open-label study assessed the immunogenicity and safety of infant primary vaccination with DTaP-HepB-IPV/Hib and 13-valent pneumococcal conjugate vaccine

Abbreviations: AE, adverse event; ATP, according-to-protocol; CI, confidence interval; CPS, capsular polysaccharide; DTaP-HepB-IPV/Hib, diphtheria-tetanus-acellular pertussis-hepatitis B virus-inactivated poliovirus and *Haemophilus influenzae* type b vaccine; ECL, electrochemiluminescence; ELISA, enzyme-linked immunosorbent assay; FHA, filamentous hemagglutinin; HBs, hepatitis B surface antigen; GMC, geometric mean concentration; GMT, geometric mean titer; Hib, *Haemophilus influenzae* type b; LLoQ, lower limit of quantitation; PCV13, 13-valent pneumococcal conjugate vaccine; PRN, pertactin; PRP, polyribosylribitol phosphate; PT, pertussis toxoid; RCT, randomized controlled trial; SAE, serious adverse event; Tdap, diphtheria-tetanus-acellular pertussis vaccine; TVC, total vaccinated cohort.

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(PCV13). Enrolled infants (6–14 weeks old) were born to mothers who were randomized to receive reduced-antigen-content diphtheria-tetanus-three-component acellular pertussis vaccine (Tdap group) or placebo (control group) during pregnancy ($27^{0/7}$–$36^{6/7}$ weeks' gestation) with crossover immunization postpartum. All infants received 2 or 3 DTaP-HepB-IPV/Hib and PCV13 doses according to national schedules. Immunogenicity was assessed in infants pre- and 1 month post-primary vaccination. The primary objective was to assess seroprotection/vaccine response rates for DTaP-HepB-IPV/Hib antigens 1 month post-primary vaccination.

Results: 601 infants (Tdap group: 296; control group: 305) were vaccinated. One month post-priming, seroprotection rates were 100% (diphtheria; tetanus), ≥98.5% (hepatitis B), ≥95.9% (polio) and ≥94.5% (Hib) in both groups. Vaccine response rates for pertussis antigens were significantly lower in infants whose mothers received pregnancy Tdap (37.5–77.1%) versus placebo (90.0–99.2%). Solicited and unsolicited adverse event rates were similar between groups. Serious adverse events occurred in 2.4% (Tdap group) and 5.6% (control group) of infants, none were vaccination-related.

Conclusions: Pertussis antibodies transferred during pregnancy may decrease the risk of pertussis infection in the first months of life but interfere with the infant's ability to produce pertussis antibodies, the clinical significance of which remains unknown. Safety and reactogenicity results were consistent with previous experience.

Clinical Trial Registration: ClinicalTrials.gov: NCT02422264.

1. Introduction

Despite comprehensive global infant immunization programs, pertussis (*Bordetella pertussis*) continues to cause high morbidity and mortality among infants <2 months of age who are too young to be vaccinated [1,2]. Several strategies to optimize pertussis control and protection during this susceptible period were pursued [3]; vaccination of pregnant women is the most commonly implemented.

Randomized controlled trials (RCTs) and prospective cohort studies have provided evidence that adult-formulation diphtheria-tetanus-acellular pertussis (Tdap) immunization during the second or third trimester of pregnancy results in high levels of pertussis antibodies in cord blood [4–10].

This maternal immunization strategy has been observed to provide 69%–93% effectiveness against pertussis disease in the first 2 or 3 months of life [11–19]. However, several studies have raised concerns that transplacentally acquired antibodies could interfere with the infant's immune response to pertussis and other antigens in the primary infant series (i.e., immunological interference or blunting) [4–8,10,20–23]. We evaluated the immunogenicity and safety of a childhood hexavalent diphtheria-tetanus-three-compo nent acellular pertussis-hepatitis B virus-poliovirus and *Haemophilus influenzae* type b-tetanus toxoid conjugate vaccine (DTaP-HepB-IPV/Hib) co-administered with the 13-valent pneumococcal conjugate vaccine (PCV13) in infants born to mothers given reduced-antigen-content Tdap vaccine during pregnancy or postpartum.

2. Methods

2.1. Study design and participants

This phase IV, multi-center, open-label, non-randomized trial with two parallel groups was conducted between 22 January 2016 and 7 March 2018 in Australia, Canada, Czech Republic, Finland, Italy and Spain. The trial (ClinicalTrials.gov: NCT02422264) was performed according to the principles of Good Clinical Practice, the Declaration of Helsinki and applicable regulations. The centers' Institutional Review Boards and/or Ethics Committees (Supplementary material) approved the protocol, informed consent form and other study-related documents. An independent data monitoring committee oversaw the participants' safety.

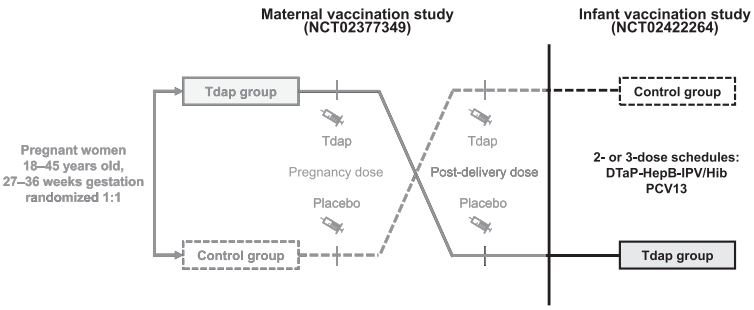
We enrolled healthy infants 6–14 weeks old whose mothers had participated in a phase IV, observer-blind, randomized, placebo-controlled maternal immunization trial (NCT02377349) in which they received reduced-antigen-content Tdap vaccine or placebo at $27^{0/7}$–$36^{6/7}$ weeks' gestation and crossover administration within 72 h postpartum [24]. Infants born prematurely (<37 weeks' gestation, but after 27 weeks' gestation) could be enrolled if they were medically stable. Exclusion criteria included a history of diphtheria, tetanus, pertussis, hepatitis B, polio, Hib or pneumococcal diseases; vaccination against any of these diseases since birth (except hepatitis B vaccination); administration of long-acting immune-modifying drugs, any chronic drug therapy or immunoglobulins and/or blood products; and immunosuppressive conditions. The Supplementary methods provide detailed inclusion and exclusion criteria. The parent(s) or legally acceptable representative(s) of each participant provided written informed consent before enrollment.

The infants' group allocation was determined by the intervention their mothers received during pregnancy (Tdap or placebo) in the maternal immunization trial [24]. The study had an open-label design because all infants received the same vaccines, but investigators and study staff involved in the infants' care and responsible for evaluating the study endpoints and laboratory testing remained blinded to the treatment allocation of the infants' mothers.

2.2. Procedures

Infants received 2 or 3 doses of DTaP-HepB-IPV/Hib (*Infanrix Hexa*, GSK) co-administered with PCV13 (*Prevnar 13*, Pfizer Inc.) at 2 and 4 months; or 3 and 5 months; or 2, 4 and 6 months; or 2, 3 and 4 months of age, according to the different countries' routine primary immunization schedules (Fig. 1). In some countries/regions with a 3-dose primary DTaP-HepB-IPV/Hib schedule, PCV13 was given as a 2-dose schedule at 2 and 4 months of age. DTaP-HepB-IPV/Hib and PCV13 were injected intramuscularly in opposite thighs. PCV13 contains capsular polysaccharides of 13 pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9 V, 14, 18C, 19A, 19F and 23), each conjugated to the diphtheria toxoid variant CRM_{197}. The composition of both vaccines is provided in the Supplementary methods.

Blood samples were collected before the first DTaP-HepB-IPV/Hib dose (2 mL, pre-primary) and 1 month (allowed interval: 21–48 days) after the last DTaP-HepB-IPV/Hib dose (5 mL, post-



2-dose schedules

Visit	Age (2-4 schedule)[a]	Age (3-5 schedule)[b]	Vaccination	Blood sampling
1	2 months	3 months	DTaP-HBV-IPV/Hib + PCV13	Pre-vaccination
2	4 months	5 months	DTaP-HBV-IPV/Hib + PCV13	
3	n/a[c]	n/a[c]		
4	5 months	6 months		Post-vaccination

3-dose schedules

Visit	Age (2-3-4 schedule)[d]	Age (2-4-6 schedule)[e]	Vaccination	Blood sampling
1	2 months	2 months	DTaP-HBV-IPV/Hib + PCV13	Pre-vaccination
2	3 months	4 months	DTaP-HBV-IPV/Hib + PCV13[f]	
3	4 months	6 months	DTaP-HBV-IPV/Hib + PCV13[f]	
4	5 months	7 months		Post-vaccination

Fig. 1. Study design. Abbreviations: DTaP-HepB-IPV/Hib, diphtheria-tetanus-acellular pertussis-hepatitis B virus-inactivated poliovirus and *Haemophilus influenzae* type b vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; Tdap, reduced-antigen-content diphtheria-tetanus-acellular pertussis vaccine. [a]2-dose DTaP-HepB-IPV/Hib primary schedule at 2 and 4 months of age administered in Spain. [b]2-dose DTaP-HepB-IPV/Hib primary schedule at 3 and 5 months of age administered in Finland and Italy. [c]Only for infants receiving a 3-dose DTaP-HepB-IPV/Hib primary schedule. Infants who received a 2-dose DTaP-HepB-IPV/Hib primary schedule did not attend visit 3. [d]3-dose DTaP-HepB-IPV/Hib primary schedule at 2, 3 and 4 months of age administered in Czechia. [e]3-dose DTaP-HepB-IPV/Hib primary schedule at 2, 4 and 6 months of age administered in Australia, Canada and Spain. [f]PCV13 was co-administered as a 2-dose or 3-dose primary vaccination schedule; in some countries, infants received a 3-dose DTaP-HepB-IPV/Hib schedule but a 2-dose PCV13 schedule (at 2 and 4 months of age).

primary) (Fig. 1). Antibodies against diphtheria, tetanus and the three pertussis antigens (filamentous hemagglutinin [FHA], pertactin [PRN] and pertussis toxoid [PT]) were measured using validated enzyme-linked immunosorbent assays (ELISAs), with assay cut-offs (lower limits of quantitation [LLoQs]) of 0.057 IU/mL (anti-diphtheria), 0.043 IU/mL (anti-tetanus), 2.046 IU/mL (anti-FHA), 2.187 IU/mL (anti-PRN) and 2.693 IU/mL (anti-PT) defining seropositivity. Seroprotection for diphtheria and tetanus was defined as an antibody concentration ≥0.1 IU/mL [25,26]. No correlate of protection has been established for pertussis [27]. Anti-hepatitis B surface antigen (HBs) antibodies were measured using a commercial chemiluminescence immunoassay (ADVIA Centaur anti-HBs2, Siemens Healthcare) with an assay cut-off of 6.2 mIU/mL and a concentration of 10 mIU/mL defining seroprotection [27–29]. Anti-poliovirus types 1, 2 and 3 antibodies were measured using a validated microneutralization test [30], with antibody titers ≥8 considered protective. Anti-Hib polyribosylribitol phosphate (PRP) antibodies were measured using a validated ELISA, with an assay cut-off of 0.066 μg/mL and concentrations of 0.15 μg/mL and 1.0 μg/mL indicative of short- and long-term protection, respectively [31,32]. Serotype-specific anti-pneumococcal capsular polysaccharide antibodies (for the PCV13 serotypes) were measured using validated multiplex electrochemiluminescence (ECL) assays [33]. A threshold of 0.35 μg/mL for the ECL assays was shown to be equivalent to the 0.35 μg/mL PCV licensure threshold established for the World Health Organization pneumococcal reference ELISA [33,34]. All assays were performed at GSK, Rixensart/Wavre, Belgium.

At each vaccination visit, the infants' parents or legally acceptable representatives (LARs) received diary cards to record solicited local (injection site pain, redness, swelling) and general (drowsiness, irritability, loss of appetite, fever) adverse events (AEs) within 4 days and unsolicited AEs within 31 days after each vaccination. Diary cards were returned at the next visit, during which the investigators asked the parents/LARs about any other possible AEs (or serious AEs [SAEs]) occurring since the previous visit. SAEs were collected from the first DTaP-HepB-IPV/Hib dose until study end. The total safety follow-up time depended on the vaccination schedule: approximately 5 months for infants receiving a 3-dose schedule at 2, 4 and 6 months of age and approximately 3 months for infants receiving the other schedules. The investigators assessed the intensity of all AEs and their causal relation to infant vaccination. Solicited local AEs were all considered vaccination-related.

2.3. Objectives

The primary objective was to assess the immune response to DTaP-HepB-IPV/Hib in terms of anti-diphtheria, anti-tetanus, anti-HBs, anti-poliovirus types 1–3 and anti-PRP seroprotection; and anti-FHA, anti-PRN and anti-PT vaccine responses 1 month post-primary vaccination. Vaccine response was defined as a post-vaccination antibody concentration at least as high as the LLoQ for infants with a pre-vaccination concentration <LLoQ; and a post-vaccination antibody concentration at least as high as the pre-vaccination concentration for infants with a pre-vaccination concentration ≥LLoQ. Because of the expected decline in maternally transferred pertussis antibodies between the pre- and post-vaccination time points, a post-vaccination concentration equal to the pre-vaccination concentration would correspond to at least a 2-fold increase in infant-induced antibodies.

Secondary immunogenicity objectives were to assess antibody concentrations or titers against all DTaP-HepB-IPV/Hib antigens, seropositivity for pertussis, and serotype-specific anti-pneumococcal antibody concentrations 1 month after the last DTaP-HepB-IPV/Hib primary dose; and the persistence of maternally transferred antibodies to all Tdap antigens in terms of concentrations and seroprotection/seropositivity before the first DTaP-HepB-IPV/Hib infant primary dose.

The reactogenicity and safety of DTaP-HepB-IPV/Hib and PCV13 primary vaccination were assessed as secondary objective in terms of the occurrence of solicited AEs, unsolicited AEs and SAEs.

2.4. Statistical analyses

The sample size was based on the number of mothers enrolled in the maternal immunization trial. The primary immunogenicity analyses were performed on the according-to-protocol (ATP) cohort for immunogenicity, including all eligible participants who received at least 1 dose of the study vaccines per protocol, complied with study procedures and intervals, were born full term (≥37 weeks' gestation) and had immunogenicity results available for at least one of the study vaccines' antigens. Seroprotection, seropositivity and vaccine response rates were calculated with exact 95% confidence intervals (CIs). Geometric mean antibody concentrations and titers (GMCs and GMTs) were calculated with 95% CIs by taking the anti-log of the mean of the log10 concentration or titer transformations. Antibody concentrations or titers

below the assay cut-offs were given arbitrary values of half the cut-offs for the GMC and GMT calculations.

We also performed exploratory subgroup analyses by dose schedule (2-dose vs 3-dose primary schedule).

The primary safety analyses were performed on the total vaccinated cohort (TVC), including all participants who received at least 1 study vaccine dose. Percentages of participants for whom solicited or unsolicited AEs were reported were calculated with exact 95% CIs. SAEs were described in detail.

Congenital anomalies which became apparent once the maternal immunization trial [24] ended were reported as pre-existing medical condition in the present study and analyzed post-hoc.

All endpoints were descriptive. Analyses were performed using SAS version 9.2.

3. Results

3.1. Study population

601 infants were enrolled and included in the TVC: 296 whose mothers were randomized to receive Tdap (Tdap group) and 305 whose mothers were randomized to receive placebo (control group) during pregnancy in the maternal immunization trial. 592 infants (98.5%) completed the study and 542 (90.2%) were included in the ATP cohort for immunogenicity (Fig. 2). Baseline characteristics were comparable between the two groups (Table 1). Most infants (~88%) received a 3-dose primary DTaP-HepB-IPV/Hib schedule (mainly at 2, 4 and 6 months of age); of those who received a 2-dose primary schedule, nearly all received their doses at 3 and 5 months of age (Table 1).

3.2. Immunogenicity

3.2.1. Response to the DTaP-HepB-IPV/Hib primary series

The percentages of infants who reached the seroprotective thresholds for anti-diphtheria, anti-tetanus, anti-HBs, anti-poliovirus types 1–3 and anti-PRP 1 month post-primary vaccination were similar between groups: 100% in both groups for anti-diphtheria and anti-tetanus, ≥98.5% for anti-HBs, ≥95.9% for anti-poliovirus and ≥94.5% for anti-PRP (Table 2). Vaccine response rates against the three pertussis antigens were lower in the Tdap than in the control group (39.6% vs 94.8% for anti-FHA, 37.5% vs 90.0% for anti-PRN and 77.1% vs 99.2% for anti-PT; Table 3). One month post-primary vaccination, all infants in the Tdap group were

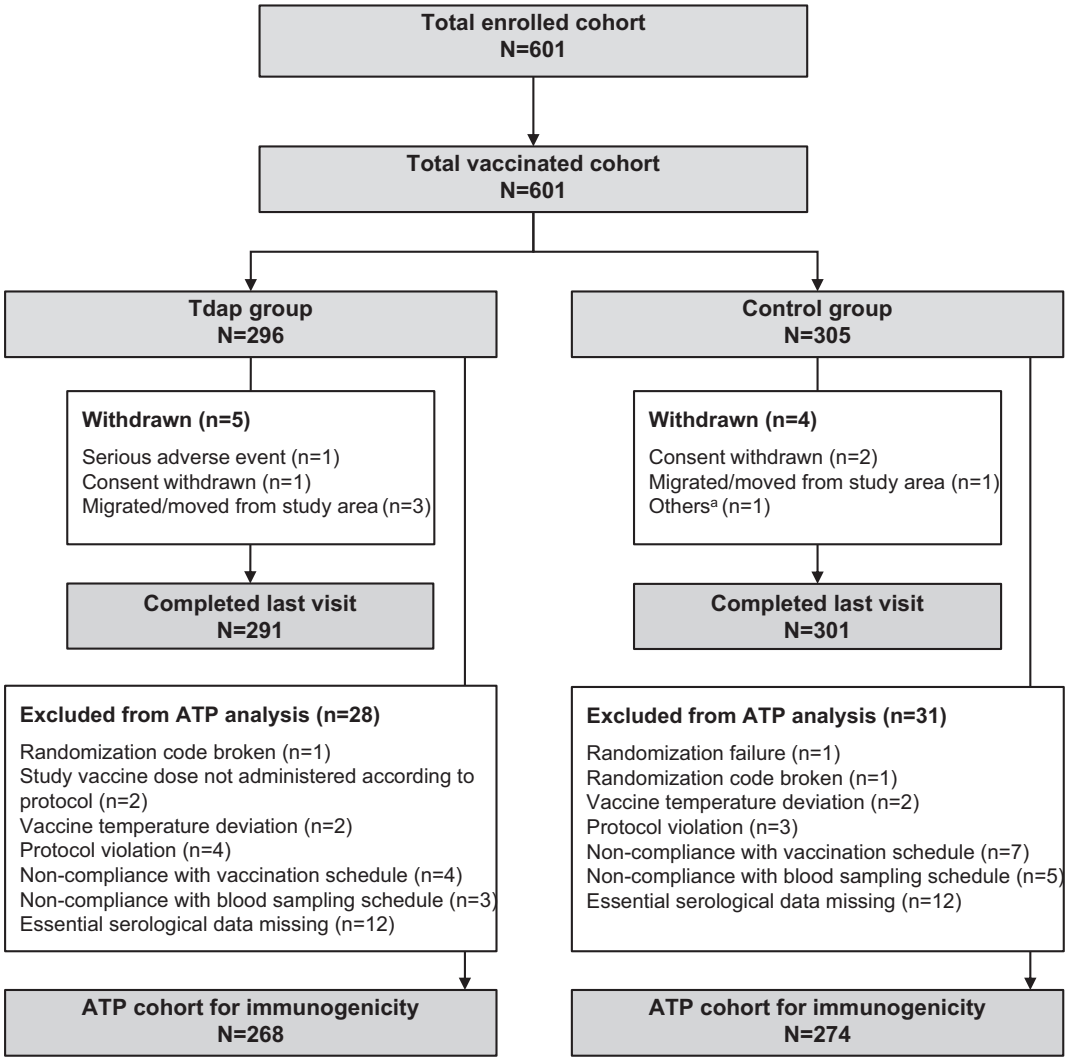


Fig. 2. Participant flow diagram. Abbreviations: ATP, according-to-protocol; N, number of infants per cohort/group; n, number of infants with the specified elimination code assigned (excluding those for whom a lower elimination code number was assigned; elimination codes from the maternal immunization trial carried forward into the follow-up infant immunization trial); Tdap, reduced-antigen-content diphtheria-tetanus-acellular pertussis vaccine. [a]This infant was withdrawn from the study at visit 2 as the infant was vaccinated outside of the study before migrating from the study site.

Table 1
Characteristics of participants in the total vaccinated cohort.

	Tdap group (N = 296)	Control group (N = 305)
Mean age ± SD at vaccination dose 1, weeks	8.7 ± 1.6	8.9 ± 1.8
Female sex, n (%)	141 (47.6)	144 (47.2)
Ethnic origin, n (%)		
White[a]	269 (90.8)	288 (94.4)
Asian	5 (1.7)	0 (0.0)
African/African American	4 (1.4)	9 (3.0)
Other	18 (6.1)	8 (2.6)
DTaP-HepB-IPV/Hib schedule, n (%)		
2-dose	32 (10.8)	41 (13.4)
3, 5 months	29 (9.8)	37 (12.1)
2, 4 months	3 (1.0)	4 (1.3)
3-dose	264 (89.2)	264 (86.6)
2, 4, 6 months	229 (77.4)	228 (74.8)
2, 3, 4 months	35 (11.8)	36 (11.8)
Mean weight ± SD, kg	5.3 ± 0.8	5.4 ± 0.8
Maternal age category at pregnancy dose, n (%)		
18–24 years	7 (2.4)	12 (3.9)
25–34 years	187 (63.2)	188 (61.6)
35–45 years	102 (34.5)	105 (34.4)
Gestational age category at pregnancy dose, n (%)		
27–32 weeks	174 (58.8)	179 (58.7)
33–36 weeks	121 (40.9)	126 (41.3)
>36 weeks	1 (0.3)	0 (0.0)

Abbreviations: 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, reduced-antigen-content diphtheria-tetanus-acellular pertussis vaccine.
[a] Includes White – Caucasian/European heritage (majority) and White – Arabic/North African heritage (1 in Tdap and 3 in control group)

seropositive for all pertussis antibodies but antibody GMCs were lower in the Tdap than in the control group (68.5 vs 103.5 IU/mL for anti-FHA, 60.5 vs 92.0 for anti-PRN, and 32.7 vs 54.7 for anti-PT; Table 3). Post-primary anti-diphtheria antibody GMCs were also lower in the Tdap than in the control group. For the other DTaP-HepB-IPV/Hib antigens, antibody GMCs were similar between the two groups (Table 2).

3.2.2. Response to the PCV13 primary series

One month post-primary vaccination, the percentages of infants with serotype-specific anti-pneumococcal antibody concentrations ≥0.35 μg/mL were similar in both groups for each PCV13 serotype (Table 4). Serotype-specific antibody GMCs were in the same range in both groups for most PCV13 serotypes; minimal differences in GMCs were observed for serotypes 4 and 19F (marginally lower in the Tdap group) (Table 4).

3.2.3. Persistence of maternally transferred antibodies to Tdap antigens

Before the first primary dose (i.e., at 2 or 3 months of age depending on the schedule the infant received), more infants in the Tdap group were seroprotected against diphtheria (82.6% in Tdap vs 43.7% in control); against tetanus (99.2% in Tdap vs 88.9% in control); and also more were seropositive for the three pertussis antigens (90.1%–100% in Tdap vs 34.8%–83.0% in control) (Table 5). Antibody GMCs at the pre-primary time point in the Tdap group were significantly higher than those in the control group for all Tdap antigens (Table 5).

3.2.4. Subgroup analysis by dose schedule

We evaluated the immune response to the DTaP-HepB-IPV/Hib and PCV13 primary series separately in infants who received a 2-dose schedule (nearly all at 3 and 5 months of age) and in infants who received a 3-dose schedule (predominantly at 2, 4 and 6 months of age). Generally, the trends we described for the overall population were observed for both schedules (Supplementary tables 1–3). However, interpretation of these results is limited by the small sample size of the 2-dose subgroup.

3.3. Reactogenicity and safety

Solicited AEs were reported at similar rates in both groups, with redness being the most commonly reported local AE at the DTaP-HepB-IPV/Hib and PCV13 injection sites (Table 6) and irritability the most commonly reported general AE (Table 6). Most solicited AEs in both groups were mild or moderate. Unsolicited AEs were reported for 54.4% and 56.7% of infants in the Tdap and control groups, respectively, most being mild or moderate (Table 6). Upper respiratory tract infection was the most common unsolicited AE (Tdap: 12.2%, 95% CI: 8.7–16.4; control: 10.8%, 7.6–14.9).

Table 2
Seroprotection rates and geometric mean concentrations or titers for diphtheria, tetanus, hepatitis B, poliovirus and Hib antibodies in infants 1 month after primary vaccination (ATP cohort for immunogenicity).

Antibody (Cut-off)	Tdap group			Control group		
	N	Seroprotection, % (95% CI)	GMC or GMT (95% CI)	N	Seroprotection, % (95% CI)	GMC or GMT (95% CI)
Anti-D (0.1 IU/mL)	264	100 (98.6–100)	1.75 (1.60–1.91)	271	100 (98.6–100)	2.75 (2.50–3.02)
Anti-T (0.1 IU/mL)	266	100 (98.6–100)	2.35 (2.14–2.58)	271	100 (98.6–100)	2.28 (2.07–2.51)
Anti-HBs (10 mIU/mL)	253	99.2 (97.2–99.9)	1322.8 (1116.7–1567.0)	263	98.5 (96.2–99.6)	1339.2 (1132.8–1583.3)
Anti-polio 1 (8 ED50)	237	98.3 (95.7–99.5)	432.1 (351.8–530.9)	244	99.2 (97.1–99.9)	489.9 (402.6–596.0)
Anti-polio 2 (8 ED50)	241	99.2 (97.0–99.9)	424.6 (342.7–526.2)	245	95.9 (92.6–98.0)	388.4 (306.3–492.6)
Anti-polio 3 (8 ED50)	230	99.1 (96.9–99.9)	730.6 (596.5–894.9)	237	99.6 (97.7–100)	775.6 (645.9–931.3)
Anti-PRP (0.15 μg/mL)	266	95.9 (92.7–97.9)	1.86 (1.55–2.23)	271	94.5 (91.0–96.9)	1.72 (1.43–2.06)
(1.0 μg/mL)		64.7 (58.6–70.4)			65.3 (59.3–71.0)	

Abbreviations: %, percentage of infants 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 infants with available results; PRP, Hib polyribosylribitol phosphate; T, tetanus; Tdap, reduced-antigen-content diphtheria-tetanus-acellular pertussis vaccine.

Table 3
Vaccine response rates, seropositivity rates and geometric mean concentrations for pertussis antibodies in infants 1 month after primary vaccination (ATP cohort for immunogenicity).

Antibody (LLoQ)	Tdap group					Control group				
	N	Vaccine response[a], % (95% CI)	N'	Sero-positivity, % (95% CI)	GMC, IU/mL (95% CI)	N	Vaccine response[a], % (95% CI)	N'	Sero-positivity, % (95% CI)	GMC, IU/mL (95% CI)
Anti-FHA (2.046 IU/mL)	240	39.6 (33.4–46.1)	266	100 (98.6–100)	68.5 (63.5–73.9)	251	94.8 (91.3–97.2)	271	100 (98.6–100)	103.5 (95.6–112.1)
Anti-PRN (2.187 IU/mL)	240	37.5 (31.4–44.0)	266	100 (98.6–100)	60.5 (54.2–67.6)	250	90.0 (85.6–93.4)	270	99.6 (98.0–100)	92.0 (81.6–103.6)
Anti-PT (2.693 IU/mL)	240	77.1 (71.2–82.2)	266	100 (98.6–100)	32.7 (30.2–35.3)	251	99.2 (97.2–99.9)	271	100 (98.6–100)	54.7 (51.0–58.6)

Abbreviations: %, percentage of infants who mounted a vaccine response or were seropositive (antibody concentration equal to or above the specified LLoQs); ATP, according-to-protocol; CI, confidence interval; FHA, filamentous hemagglutinin; GMC, geometric mean concentration; IU, international unit; LLoQ, lower limit of quantitation; N, number of infants with pre- and post-vaccination results available; N', number of infants with post-vaccination results available; PRN, pertactin; PT, pertussis toxoid; Tdap, reduced-antigen-content diphtheria-tetanus-acellular pertussis vaccine.

[a] Vaccine response to FHA, PRN and PT antigens is defined as a post-vaccination antibody concentration ≥LLoQ for infants with a pre-vaccination concentration <LLoQ; a post-vaccination concentration at least as high as the pre-vaccination concentration for infants with a pre-vaccination concentration ≥LLoQ.

Table 4
Percentages of infants with pneumococcal serotype-specific antibody concentrations ≥ 0.35 μg/mL and geometric mean concentrations 1 month after primary vaccination[a] (ATP cohort for immunogenicity).

Vaccine serotype	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	232	95.7 (92.2–97.9)	1.61 (1.43–1.80)	237	95.8 (92.4–98.0)	1.92 (1.73–2.14)
3	232	74.6 (68.5–80.0)	0.54 (0.49–0.60)	237	76.4 (70.4–81.6)	0.60 (0.55–0.67)
4	232	92.2 (88.0–95.3)	1.20 (1.07–1.35)	237	96.2 (92.9–98.2)	1.56 (1.40–1.75)
5	226	88.1 (83.1–92.0)	1.09 (0.96–1.24)	234	91.5 (87.1–94.7)	1.27 (1.13–1.43)
6A	232	95.3 (91.7–97.6)	2.16 (1.89–2.47)	237	95.4 (91.8–97.7)	2.59 (2.27–2.95)
6B	232	79.7 (74.0–84.7)	1.37 (1.12–1.68)	237	84.4 (79.1–88.8)	1.44 (1.20–1.73)
7F	232	98.7 (96.3–99.7)	2.39 (2.15–2.65)	237	99.6 (97.7–100)	2.67 (2.43–2.93)
9V	232	91.4 (87.0–94.7)	1.33 (1.19–1.50)	237	95.8 (92.4–98.0)	1.64 (1.47–1.83)
14	232	98.7 (96.3–99.7)	5.70 (4.99–6.52)	237	98.3 (95.7–99.5)	6.57 (5.71–7.56)
18C	232	94.8 (91.1–97.3)	1.61 (1.42–1.82)	237	94.1 (90.3–96.7)	1.79 (1.59–2.01)
19A	232	93.1 (89.0–96.0)	1.61 (1.43–1.82)	237	95.8 (92.4–98.0)	2.01 (1.78–2.27)
19F	232	99.1 (96.9–99.9)	2.57 (2.35–2.82)	237	98.3 (95.7–99.5)	3.24 (2.92–3.60)
23F	230	79.1 (73.3–84.2)	0.86 (0.74–0.99)	236	86.0 (80.9–90.2)	1.02 (0.88–1.17)

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

[a] 1 month after DTaP-HepB-IPV/Hib primary vaccination, which is 1 month after PCV13 primary vaccination for most infants, but 3 months after PCV13 primary vaccination for those who received a 3-dose DTaP-HepB-IPV/Hib schedule at 2, 4 and 6 months of age and a 2-dose PCV13 schedule at 2 and 4 months of age.

Table 5
Seropositivity or seroprotection rates and geometric mean concentrations for diphtheria, tetanus and pertussis antibodies in infants before primary vaccination (ATP cohort for immunogenicity).

Antibody (LLoQ or cut-off)	Tdap group			Control group		
	N	% ≥LLoQ or cut-off (95% CI)	GMC, IU/mL (95% CI)	N	% ≥LLoQ or cut-off (95% CI)	GMC, IU/mL (95% CI)
Anti-D (0.1 IU/mL)	242	82.6 (77.3–87.2)	0.423 (0.354–0.506)	252	43.7 (37.4–50.0)	0.089 (0.076–0.103)
Anti-T (0.1 IU/mL)	242	99.2 (97.0–99.9)	2.152 (1.925–2.406)	253	88.9 (84.4–92.5)	0.378 (0.330–0.434)
Anti-FHA (2.046 IU/mL)	242	100 (98.5–100)	88.3 (77.7–100.4)	253	83.0 (77.8–87.4)	6.6 (5.7–7.7)
Anti-PRN (2.187 IU/mL)	242	95.5 (92.0–97.7)	70.5 (56.1–88.5)	253	59.7 (53.4–65.8)	4.5 (3.7–5.4)
Anti-PT (2.693 IU/mL)	242	90.1 (85.6–93.5)	11.9 (10.3–13.6)	253	34.8 (28.9–41.0)	2.2 (2.0–2.5)

Abbreviations: %, percentage of infants with antibody concentrations greater than or equal to the specified seroprotection cut-offs (for diphtheria and tetanus) or LLoQs (for pertussis); ATP, according-to-protocol; CI, confidence interval; D, diphtheria; FHA, filamentous hemagglutinin; GMC, geometric mean concentration; IU, international unit; LLoQ, lower limit of quantitation; N, number of infants with available results; PRN, pertactin; PT, pertussis toxoid; T, tetanus; Tdap, reduced-antigen-content diphtheria-tetanus-acellular pertussis vaccine.

Nine SAEs were reported for seven (2.4%) infants in the Tdap group and 23 for 17 (5.6%) infants in the control group. The difference between the two groups mainly resulted from a greater number of respiratory tract and other infections reported as SAE in the control compared to the Tdap group (15 vs 2) (Supplementary table 4). One infant in the Tdap group was withdrawn due to SAEs (intestinal hemorrhage and milk allergy). No SAEs were deemed related to infant vaccination and no infants died during the study.

Congenital anomalies were reported for 24 (8.1%, 95% CI: 5.3–11.8) infants from Tdap-vaccinated mothers and 28 (9.2%, 6.2–13.0) infants from control mothers, atrial septal defect being most common (Tdap: 1.4%, 0.4–3.4; control: 2.6%, 1.1–5.1).

Table 6
Solicited and unsolicited adverse events after primary vaccination (total vaccinated cohort).

Adverse event	Tdap group (N = 294)		Control group (N = 303)	
	n	% (95% CI)	n	% (95% CI)
Solicited local AEs at the DTaP-HepB-IPV/Hib injection site (4 days post-vaccination)				
Pain				
Any	165	56.1 (50.2–61.9)	166	54.8 (49.0–60.5)
Grade 3	7	2.4 (1.0–4.8)	14	4.6 (2.5–7.6)
Redness				
Any	185	62.9 (57.1–68.5)	185	61.1 (55.3–66.6)
>20 mm	10	3.4 (1.6–6.2)	14	4.6 (2.5–7.6)
Swelling				
Any	137	46.6 (40.8–52.5)	138	45.5 (39.8–51.3)
>20 mm	4	1.4 (0.4–3.4)	12	4.0 (2.1–6.8)
Solicited local AEs at the PCV13 injection site (4 days post-vaccination)				
Pain				
Any	158	53.9 (48.0–59.7)	153	50.5 (44.7–56.3)
Grade 3	9	3.1 (1.4–5.8)	8	2.6 (1.1–5.1)
Redness				
Any	163	55.6 (49.7–61.4)	170	56.1 (50.3–61.8)
>20 mm	6	2.0 (0.8–4.4)	6	2.0 (0.7–4.3)
Swelling				
Any	114	38.9 (33.3–44.8)	128	42.2 (36.6–48.0)
>20 mm	8	2.7 (1.2–5.3)	11	3.6 (1.8–6.4)
Solicited general AEs (4 days post-vaccination)				
Drowsiness				
Any	216	73.5 (68.0–78.4)	232	76.6 (71.4–81.2)
Grade 3	14	4.8 (2.6–7.9)	16	5.3 (3.0–8.4)
Irritability				
Any	255	86.7 (82.3–90.4)	257	84.8 (80.3–88.7)
Grade 3	36	12.2 (8.7–16.5)	37	12.2 (8.7–16.4)
Loss of appetite				
Any	142	48.3 (42.5–54.2)	157	51.8 (46.0–57.6)
Grade 3	8	2.7 (1.2–5.3)	7	2.3 (0.9–4.7)
Fever				
Any	126	42.9 (37.1–48.7)	126	41.6 (36.0–47.4)
>39.0 °C	4	1.4 (0.4–3.4)	1	0.3 (0.0–1.8)
Unsolicited AEs (31 days post-vaccination)				
		(N = 296)		**(N = 305)**
Any	161	54.4 (48.5–60.2)	173	56.7 (51.0–62.4)
Grade 3	15	5.1 (2.9–8.2)	18	5.9 (3.5–9.2)
Related	11	3.7 (1.9–6.6)	13	4.3 (2.3–7.2)

Abbreviations: 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 infants with at least one documented dose (for solicited AEs) or at least one administered dose (for unsolicited AEs); n/%, number/percentage of infants for whom the specified AE was reported at least once during the follow-up periods after any of the doses; Tdap, reduced-antigen-content diphtheria-tetanus-acellular pertussis vaccine.
Grade 3 pain was defined as crying when the limb was moved or the limb being spontaneously painful; grade 3 irritability as crying that could not be comforted or irritability preventing normal activity; grade 3 drowsiness as drowsiness preventing normal activity; and grade 3 loss of appetite as not eating at all; unsolicited AEs were considered grade 3 if they prevented normal activity.

Supplementary Fig. 1 depicts a plain language summary outlining the findings and highlighting their clinical relevance.

4. Discussion

The key rationale for pertussis immunization during pregnancy is to protect infants too young to be vaccinated from pertussis disease and death by achieving persistent high levels of maternally transferred pertussis antibodies in infants between birth and the first primary immunization dose. Our study—the largest RCT to date to investigate this—showed that administration of the reduced-antigen-content Tdap vaccine during the third trimester of pregnancy resulted in high levels of maternally transferred pertussis antibodies in infants up to the pre-primary vaccination time point (at 2 or 3 months of age). Infants of Tdap-immunized mothers presented significantly higher antibody levels for all Tdap antigens before the primary series compared to infants of control mothers. However, immunological interference was evident for pertussis (in terms of GMCs and vaccine response rates) and to a lesser extent for diphtheria (in terms of GMC but not seroprotection rate) after the primary DTaP-HBV-IPV/Hib series in infants born to Tdap mothers.

There was no evidence of maternally derived antibodies interfering with the infant immune response to tetanus, hepatitis B, poliovirus or Hib PRP in terms of seroprotection rates and GMCs after the primary DTaP-HBV-IPV/Hib series. Likewise, we observed no interference with the response to PCV13 in terms of percentages of infants achieving the 0.35 μg/mL threshold (shown to be equivalent to the threshold used for PCV licensure [33,34]). The minimal GMC differences observed for serotypes 4 and 19F between the two groups may be explained by the absence of statistical adjustments for multiple testing.

The concept of immune interference or blunting, where maternal antibodies reduce antibody generation to the infant primary series [35,36], resulting in lower post-primary antibody concentrations, is not new. Previous studies assessing the administration of

Tdap vaccines (three- or five-component pertussis vaccines) during pregnancy have reported significantly lower antibody responses to one or more pertussis antigens in infants born to Tdap-vaccinated mothers following completion of their primary series compared to infants whose mothers had not been Tdap-vaccinated [4–8,10,20–22]. However, this effect was not consistently observed [4–6,10,21,22]. Similarly, several studies have shown that maternally transferred diphtheria antibodies can interfere with diphtheria and diphtheria-derived CRM-conjugated pneumococcal vaccine responses to the infant primary series following Tdap immunization during pregnancy [4,5,10,21,22,37]. In contrast to other studies [4,5,21,22] we did not see an enhancement of the immune response to tetanus post-primary series, despite higher pre-primary anti-tetanus GMCs in infants of Tdap-vaccinated mothers than in controls.

Maternal antibody interference with the immune response to infant vaccination has also been described as a natural phenomenon in the absence of maternal immunization in a recent meta-analysis of 32 clinical trials [38]. This meta-analysis showed that pre-existing antibodies inhibit infant immune responses to primary immunization for 20 of 21 measured antigens, and this in the absence of maternal vaccination [38]. Two-fold higher maternal antibody levels were estimated to result in 11% lower post-vaccination antibody levels for PT and FHA, 22% lower for PRN, 13% lower for tetanus and 24% lower for diphtheria [38]. Given that maternal antibodies decline rapidly over the first months of life, mathematical modeling indicated that the inhibitory effect of a 2-fold to 5-fold increase in maternal antibody concentrations in infants can be offset by a delay of 2.2–5.0 weeks in starting primary vaccination [38]. A recent RCT in the Netherlands investigated the effect of maternal Tdap immunization on pertussis antibody responses of infants starting primary vaccination at 3 months (instead of 2 months) and still found significant blunting [8]. In our subgroup analysis by dose schedule, nearly all infants in the 2-dose subgroup started their primary series at 3 months of age; interference with the pertussis response seemed to occur in this subgroup as well; however, the small sample size of this subgroup precludes any sound conclusions.

Importantly, the clinical relevance of the lower post-primary antibody levels in infants from Tdap-vaccinated mothers remains unknown, since there is no established correlate of protection for pertussis. To date, there is no evidence that the observed immunological interference is of clinical significance as there has been no increase in pertussis disease in infants born to Tdap-vaccinated mothers following primary series vaccinations in the first year of life [13,14,39]. However, this requires ongoing monitoring.

This study provides further evidence of the tolerability and safety of DTaP-HepB-IPV/Hib and PCV13 in infants whose mothers received Tdap vaccine during pregnancy. Rates of solicited and unsolicited AEs were similar between groups. SAEs were not reported more frequently in the Tdap group compared to the control group. Hence, the safety profile of DTaP-HepB-IPV/Hib and PCV13 in infants did not change depending on whether their mothers received Tdap during pregnancy.

The current study has several potential limitations. It was conducted in six high-income countries, and participants of the original study (NCT02377349) were mainly white Caucasian women and women with low-risk pregnancies. Hence, the results may not be generalizable to low- and middle- income countries, to infants of other ethnic groups or infants born from high-risk pregnancies. In addition, analyses were descriptive with no adjustment for multiplicity. We also did not assess the effect of breastfeeding on antibody levels before and after the infant primary series. Because mothers in the control group received Tdap postpartum, antibodies to Tdap antigens might have been transferred through breast milk to infants in the control group.

A follow-up study is ongoing in the same infant cohort to investigate the effect of maternal Tdap immunization on the persistence of antibodies induced by the primary DTaP-HepB-IPV/Hib and PCV13 series up to 11–18 months of age and the effect on the immune response to booster DTaP-HepB-IPV/Hib and PCV13 vaccination in the child's second year of life (NCT02853929).

5. Conclusion

Our study is the largest RCT to date providing evidence that immunization against pertussis during pregnancy leads to high levels of pertussis antibodies in young infants that persist until the start of the primary immunization series. These high levels of maternal pertussis antibodies can help to further close the susceptibly gap to severe pertussis disease and death in young infants but appear to interfere with the infant's immune response to the primary pertussis immunization series. The clinical significance of this interference remains unknown in the absence of a correlate of protection. Ongoing epidemiological surveillance of the maternal immunization strategy is required to further understand if there is a clinical impact. We found no evidence of maternally derived antibodies to Tdap antigens interfering with the infant immune response to tetanus, hepatitis B, poliovirus or Hib PRP and only minimal potential interference with the response to diphtheria and two pneumococcal serotypes. The reactogenicity and safety of DTaP-HepB-IPV/Hib and PCV13 in infants did not seem impacted by whether their mothers received Tdap or placebo during pregnancy.

6. Contributors

ACM, BC, FOT, JTRA, KPP, NMes, OGV, SAH, SOK and TN were involved in study conception and design.

BAN, FMT, FOT, GVZ, JGS, JMMA, JTRA, LK, MAC, MB, MJCO, MMV, MV, NMes, NMey, OGV, PGM, PM, SAH, TN and ZS performed the study and participated in data collection.

BC, FMT, JMMA, LK, MAC, MV, NMes, NMey, PGM, SAH, SOK, TN and ZS were involved in data analysis and interpretation.

KPP wrote the manuscript and all authors have revised and approved the manuscript.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [BAN reports grant from the GSK group of companies (GSK) and personal fees from Pfizer, MSD and Sanofi Pasteur. BC, MAC, NMes, NMey and SOK are employees of GSK, and BC and NMes own GSK restricted shares. FMT, KPP, OGV, SAH and TN's institutions received grants from GSK during the conduct of the study. FMT's institution received financial support from GSK during the conduct of the study, as well as financial and non-financial support outside the submitted work; he also received personal fees from Pfizer, Novavax, MSD and Sanofi Pasteur; his institution also received financial support as trial fees from Ablynx, Jansen, Regeneron, Medimmune, Pfizer, MSD, Sanofi Pasteur, Novavax and Novartis, as well as non-financial support from Pfizer and MSD and grants from MSD and AstraZeneca. JMMA reports receiving fees and non-financial

support from GSK during the conduct of the study, as well as fees from GSK, Pfizer and MSD outside the submitted work. LK is working as consultant for GSK. SAH is member of ad-hoc advisory committees for GSK and Sanofi Pasteur and he has a patent for novel triple adjuvant issued. ACM, FOT, GVZ, JGS, JTRA, MB, MJCO, MMV, MV, PGM, PM and ZS declare no conflicts of interest.].

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Data sharing

The study protocol is available at https://www.gsk-studyregister.com/study/5349. Anonymized individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.10.104.

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