Immunogenicity and Safety of a Booster Dose of the 10-valent Pneumococcal *Haemophilus Influenzae* Protein D Conjugate Vaccine Coadministered With the Tetravalent Meningococcal Serogroups A, C, W-135 and Y Tetanus Toxoid Conjugate Vaccine in Toddlers: A Randomized Trial

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Background: This open, randomized clinical trial (NCT00758264) evaluated the coadministration of a booster dose of the 10-valent pneumococcal conjugate vaccine (PHiD-CV) and a single dose of the tetravalent meningococcal conjugate vaccine (MenACWY-TT) in Taiwanese and Mexican toddlers.

Methods: Healthy toddlers aged 12–23 months (N = 363) were randomized (2:1:1) to receive either both vaccines at first visit, MenACWY-TT at first visit and 1 month later PHiD-CV, or PHiD-CV at first visit and 1 month later MenACWY-TT. Immune responses were measured 1 month after MenACWY-TT vaccination by meningococcal serum bactericidal activity (rSBA) assay and 1 month after PHiD-CV vaccination by pneumococcal 22F-inhibition enzyme-linked immunosorbent assay and functional opsonophagocytic activity assay. Solicited and unsolicited symptoms were recorded for days 4 and 31 postvaccination, respectively. Serious adverse events were recorded throughout the study.

Results: The prespecified criteria for noninferiority of coadministration versus individual administrations were met for all meningococcal serogroups (in terms of percentages of toddlers with rSBA titer ≥8) and all vaccine pneumococcal serotypes (in terms of antibody geometric mean concentration ratios), except pneumococcal serotype 18C. For each meningococcal serogroup, ≥97.5% of toddlers across the 3 groups had rSBA titers ≥128 at 1 month after MenACWY-TT vaccination. For each pneumococcal serotype, at 1 month after PHiD-CV vaccination, ≥96.0% and ≥92.9% of toddlers across the 3 groups had antibody concentrations ≥0.2 μg/mL and opsonophagocytic activity titers ≥8, respectively. The safety profiles of both vaccines when coadministered were clinically acceptable.

Conclusions: This study supports the coadministration of PHiD-CV and MenACWY-TT in toddlers.

Key Words: *Streptococcus pneumoniae*, *Neisseria meningitidis*, coadministration, conjugate vaccines, toddlers

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Neisseria meningitidis and *Streptococcus pneumoniae* are 2 major causes of invasive bacterial infections and subsequent mortality in young children worldwide.[1–3] In Taiwan, the annual incidence of meningococcal diseases reached 0.2 per 100,000 inhabitants in 2001[4] and that of pneumococcal diseases 15.6 per 100,000 children aged 2–4 years in 2007.[5] In Mexico, an increase of meningococcal diseases incidence, which is reportedly low, has recently been noted in a surveillance study conducted at the border with the United States.[6] Moreover, a retrospective study conducted in a hospital in Mexico City showed that, between 1997 and 2004, invasive pneumococcal diseases were a significant cause of morbidity and mortality in children, especially in those with previous underlying disease.[7] Vaccination with polysaccharide-protein conjugate vaccines remains the best strategy to prevent meningococcal and pneumococcal diseases in infants and toddlers, who are at high risk for bacterial infections caused by these pathogens.[8]

A new tetravalent meningococcal serogroups A, C, W-135 and Y conjugate vaccine using tetanus toxoid (TT) as carrier protein (MenACWY-TT, Nimenrix, GlaxoSmithKline [GSK], Rixensart, Belgium) has been recently approved by the European Medicines Agency for use in individuals aged 12 months and above. In previous clinical trials, MenACWY-TT was shown to be immunogenic and well-tolerated in toddlers, children, adolescents and young adults.[9–15] A 10-valent pneumococcal nontypeable *Haemophilus influenzae* (NTHi) protein D conjugate vaccine (Synflorix, GSK; hereafter referred to as PHiD-CV) has been licensed progressively since end 2008 onwards in more than 110 countries across the world for the vaccination of infants and toddlers.[16] The composition of PHiD-CV has been described previously,[17] and this vaccine was

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shown to be immunogenic and well-tolerated when administered for primary immunization in infancy with a booster dose in the second year of life or for catch-up immunization in children younger than 5 years.[17–27]

Because both of these vaccines are intended for immunization of toddlers, investigation of the safety and immunogenicity of their coadministration are important. Such coadministration would facilitate the inclusion of the MenACWY-TT vaccine into the increasingly crowded pediatric vaccination schedules and could reduce the number of vaccination visits required and the associated costs and increase vaccine uptake and schedule compliance. However, the interaction between 2 simultaneously administered vaccines is unpredictable, and potential immune interferences or enhancements of antibody responses must be evaluated empirically.[8,28]

Therefore, the present study was conducted to evaluate if the coadministration of the booster dose of PHiD-CV and a single dose of the MenACWY-TT vaccine in the second year of life impacts the immunogenicity, reactogenicity or safety of either of the vaccines.

MATERIALS AND METHODS

Study Design

This phase IIIb, open, randomized, controlled, booster vaccination study was conducted in 2 centers in Mexico and 3 centers in Taiwan between October 2008 and November 2009. This study was a continuation of 2 primary vaccination studies in which infants were vaccinated with PHiD-CV and with a combined hexavalent diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated poliovirus and Hib conjugate vaccine (DTPa-HBV-IPV/Hib; Infanrix hexa™, GSK) at 1.5, 3 and 6 months of age in Taiwan (NCT00533507)[29] or at 2, 4 and 6 months of age in Mexico (NCT00489554).[30] In addition, a live, attenuated rotavirus vaccine was coadministered with the first 2 doses in both primary vaccination studies. Here, the toddlers in each country willing to participate in a booster vaccination study were randomized (2:1:1, block size of 4) in 3 parallel groups to receive (1) MenACWY-TT and PHiD-CV coadministered at the same vaccination visit (Coad group); (2) MenACWY-TT at first visit and 1 month later PHiD-CV (MenACWY-TT group); or (3) PHiD-CV at first visit followed by MenACWY-TT 1 month later (PHiD-CV group).

A randomization list was generated at GSK using a standard Statistical Analysis System (SAS; SAS Institute Inc., Cary, NC) program to number the vaccines. The treatment allocation at the investigator site was performed using a central internet randomization system. The randomization algorithm used a minimization procedure accounting for center to ensure a balanced distribution of the population in each group. The vaccines were administered in an open manner due to the differing vaccination schedules among the groups and the differing container and presentation of the 2 study vaccines. Laboratory personnel responsible for immunogenicity testing were blinded to the treatment group.

The study was conducted in accordance with the Good Clinical Practice Guidelines and the Declaration of Helsinki, and the protocol and associated documents were reviewed and approved by local ethics committees. Written informed consent was obtained from the parents/guardians of the toddlers before study entry. This study has been registered at http://clinicaltrials.gov/ct2/show/NCT00758264. A summary of the protocol is available at http://www.gsk-clinicalstudyregister.com (GSK study ID 111393).

Study Objectives

The coprimary objectives were to demonstrate the immunologic noninferiority of the coadministration of a single dose of MenACWY-TT and a booster dose of PHiD-CV in the second year of life over separate administration of the 2 vaccines. Noninferiority of MenACWY-TT coadministered with PHiD-CV versus MenACWY-TT given alone in terms of functional antibody titers measured by a serum bactericidal activity assay using baby rabbit complement as the exogenous complement source (rSBA) was demonstrated if the lower limit (LL) of the 2-sided standardized asymptotic 95% confidence interval (CI) for the group difference (Coad minus MenACWY-TT) in percentage of toddlers with rSBA titer ≥8 was greater than or equal to −10% for each vaccine meningococcal serogroup. The noninferiority of PHiD-CV coadministered with MenACWY-TT over PHiD-CV given alone was demonstrated if the LL of the 95% CI of the ratio of antipneumococcal geometric mean antibody concentration (GMC) adjusted for country and prevaccination concentrations between the 2 groups (Coad over PHiD-CV) was above 0.5 for each vaccine pneumococcal serotype.

The secondary objectives were to evaluate the immunogenicity, reactogenicity and safety of both vaccines in the 3 study groups, to assess the persistence of antipneumococcal antibodies and opsonophagocytic activity (OPA) before booster vaccination and to assess preexisting rSBA titers against meningococcal serogroups A, C, W-135 and Y.

Study Participants

Study participants were healthy boys or girls between 12 and 23 months of age at the time of the first vaccination visit in this booster study, who received 3 doses of PHiD-CV in the primary vaccination study in Mexico or Taiwan. Toddlers were excluded from participation if they were immunosuppressed for any cause, had previously been vaccinated with a meningococcal vaccine at any time or with a TT-containing vaccine within the month before study enrollment, had previous administration of a fourth dose of a pneumococcal vaccine, had history of meningococcal or pneumococcal invasive disease, had received immunoglobulins or blood products within 3 months before the study, had received other investigational products or other vaccines within 30 days before the study, had allergic disease likely to be exacerbated by any component of the vaccine, had major congenital defects or a serious chronic illness or had acute disease at the time of enrollment.

Vaccines

One dose of the MenACWY-TT vaccine (Nimenrix, GSK) contained 5 µg of capsular polysaccharide for each meningococcal serogroup (A, C, W-135 and Y) conjugated to approximately 44 µg of TT. One dose of PHiD-CV (Synflorix, GSK) contained 1 µg of each capsular polysaccharide for pneumococcal serotypes 1, 5, 6B, 7F, 9V, 14 and 23F and 3 µg of capsular polysaccharide for serotype 4 conjugated to 9–16 µg of NTHi protein D, 3 µg of capsular polysaccharide for serotype 18C conjugated to 5–10 µg of TT and 3 µg of capsular polysaccharide for serotype 19F conjugated to 3–6 µg of DT. MenACWY-TT is a lyophilized vaccine requiring reconstitution with a saline diluent, whereas PHiD-CV is a liquid vaccine. Both vaccines were administered intramuscularly, the MenACWY-TT vaccine into the right thigh and PHiD-CV into the left thigh.

Immunogenicity Assessment

Blood samples were collected from all the toddlers before and at 1 month after each vaccination. The blood samples were stored at −20°C until analyses were conducted at GSK laboratory. All methods and equipment were validated as required, and the laboratories had an established Quality System, established

Quality Control procedures and were audited regularly for quality assessment.

The cutoff of the rSBA assay, which was used to measure functional antibodies against each meningococcal serogroup-specific polysaccharide, was a serum dilution of 8 and this cutoff is considered indicative of seroprotection for rSBA-MenC.[31,32] Antibody titers ≥8 were also considered indicative of seroprotection for the other serogroups in this study.[33] In addition, data were also analyzed using a threshold rSBA titer of 128.

GSK's 22F-inhibition enzyme-linked immunosorbent assay was used to measure antipneumococcal serotype-specific antibody concentrations (with assay cutoff concentration of 0.05 µg/mL).[34,35] The percentages of toddlers with antibody concentrations ≥0.2 µg/mL were evaluated for each vaccine pneumococcal serotype. It was previously established that an antibody concentration of 0.2 µg/mL as measured by the GSK's 22F-inhibition enzyme-linked immunosorbent assay is equivalent to an antibody concentration of 0.35 µg/mL as measured by ELISA without 22F inhibition, which is the threshold used by the World Health Organization for the comparison of immune responses induced by different pneumococcal conjugate vaccines at 1 month after the third primary dose.[35,36] In addition, OPA was measured for each vaccine pneumococcal serotype by a killing assay using an HL60 cell line.[37] The results were presented as the reciprocal dilution of serum (OPA titer) able to sustain 50% killing of live pneumococci under the assay conditions, with a cutoff OPA titer of 8. The antibody concentrations and OPA titers for cross-reactive pneumococcal serotypes 6A and 19A were also determined.

An in-house ELISA assay was used to measure antibody concentrations against NTHi protein D with an assay cutoff of 100 EL.U/mL[25] and against TT with an assay cutoff of 0.1 IU/mL.[38]

Safety and Reactogenicity Assessment

Reactogenicity and safety were evaluated using diary cards that were completed by parents/guardians. Solicited local symptoms at the injection sites (pain, redness and swelling) and solicited general symptoms (drowsiness, irritability, loss of appetite and fever [rectal temperature ≥38.0°C]) were recorded during a 4-day follow-up period after each vaccination. Unsolicited adverse events (AEs) were recorded for a period of 31 days after each vaccination. The intensity of each symptom was graded on a 1–3 scale. All solicited local symptoms were defined in the protocol to be considered as causally related to vaccination. Using their clinical judgment, the investigators assessed the presence or absence of a possible causal relationship to vaccination for all other AEs.

In addition, the occurrence of new onset of chronic illnesses and serious AEs (SAEs) was reported throughout the study including an extended safety follow-up period (up to 6 months after last vaccination). SAEs were defined as any medical event resulting in death, any life-threatening event or any event causing disability or requiring hospitalization or prolongation of hospitalization.

Statistical Analyses

With 300 evaluable toddlers, the global power to meet the 2 coprimary objectives was at least 82.1%. Assuming that up to 15% of the toddlers enrolled might be excluded from the according to protocol cohort for immunogenicity, 348 toddlers were planned to be enrolled.

The total vaccinated cohort, on which the primary safety analyses were performed, included all toddlers with at least 1 vaccine administration documented. The immunogenicity analyses were conducted on the according to protocol cohort for immunogenicity, which included toddlers meeting all eligibility criteria, who had received at least 1 dose of study vaccine, complying with procedures defined in the protocol, with no elimination criteria during the study and for whom assay results were available for antibodies against at least 1 vaccine antigen at 1 month after the first vaccination.

For each antigen, the antibody GMCs or geometric mean titers (GMTs) and the percentages of toddlers with titers or concentrations above the prespecified thresholds were calculated with their exact 95% CIs. Comparability of the immune response to each antigen was evaluated through the computation of the asymptotic standardized 95% CI on the group difference in the percentage of toddlers with titers or concentrations above the proposed cutoffs/thresholds and through the computation of the 95% CI of the adjusted GMT or GMC ratios among groups (exploratory analyses). This was performed using an analysis of covariance model on the $\log_{10}$ transformation of the titers or concentrations using the prevaccination $\log_{10}$ transformation of the titers or concentrations, the country and the vaccine groups as covariates.

The incidence and intensity of each solicited and unsolicited AE were calculated with exact 95% CI for each group, and new onset of chronic illnesses and SAEs were described in detail. The statistical analyses were performed using the SAS software version 9.2 (SAS Institute Inc.) and Proc StatXact 8.1 (Cytel Inc., Cambridge, MA).

RESULTS

Study Subjects

A total of 363 toddlers were enrolled and vaccinated in this study (183 in Mexico and 180 in Taiwan); 357 of them completed the active phase of the study, and none withdrew due to an AE (Fig. 1). The according to protocol cohort for immunogenicity included 175 toddlers in the Coad group, 81 toddlers in the MenACWY-TT group and 81 toddlers in the PHiD-CV group.

The 3 groups were comparable in terms of demographic characteristics, except for the MenACWY-TT group, in which more females than males were enrolled (61.5% versus 38.5%; Table 1). All the toddlers who participated in the study were Hispanic in Mexico and East Asian in Taiwan.

Meningococcal Bactericidal Antibodies

At 1 month postvaccination with MenACWY-TT, the LL of the 95% CI for the group differences (Coad minus MenACWY-TT) in percentages of toddlers with postvaccination rSBA titer ≥8 were −1.09, −2.11, −0.94 and −2.18 for serogroups A, C, W-135 and Y, respectively; hence, the prespecified criterion for noninferiority of MenACWY-TT coadministered with PHiD-CV over MenACWY-TT given alone was met.

For each of the meningococcal serogroup, the prevaccination seropositivity rates (rSBA titer ≥8) ranged from 10.5% to 65.4% in the 3 groups (Table 2). One month after vaccination with MenACWY-TT, 97.5–100% of the toddlers had rSBA titers ≥128 for each serogroup, and GMTs increased between prevaccination and postvaccination (range 75.4-fold to 439.1-fold).

Antipneumococcal Antibodies

The LL of the 95% CI for the adjusted antibody GMC ratios between the Coad and the PHiD-CV groups at 1 month after the booster vaccination with PHiD-CV ranged between 0.66 and 0.84 for all vaccine pneumococcal serotypes, except serotype 18C for which the LL was 0.41; hence, the prespecified statistical criterion for noninferiority of PHiD-CV coadministered with MenACWY-TT over PHiD-CV given alone was met for all vaccine pneumococcal serotypes, except serotype 18C.

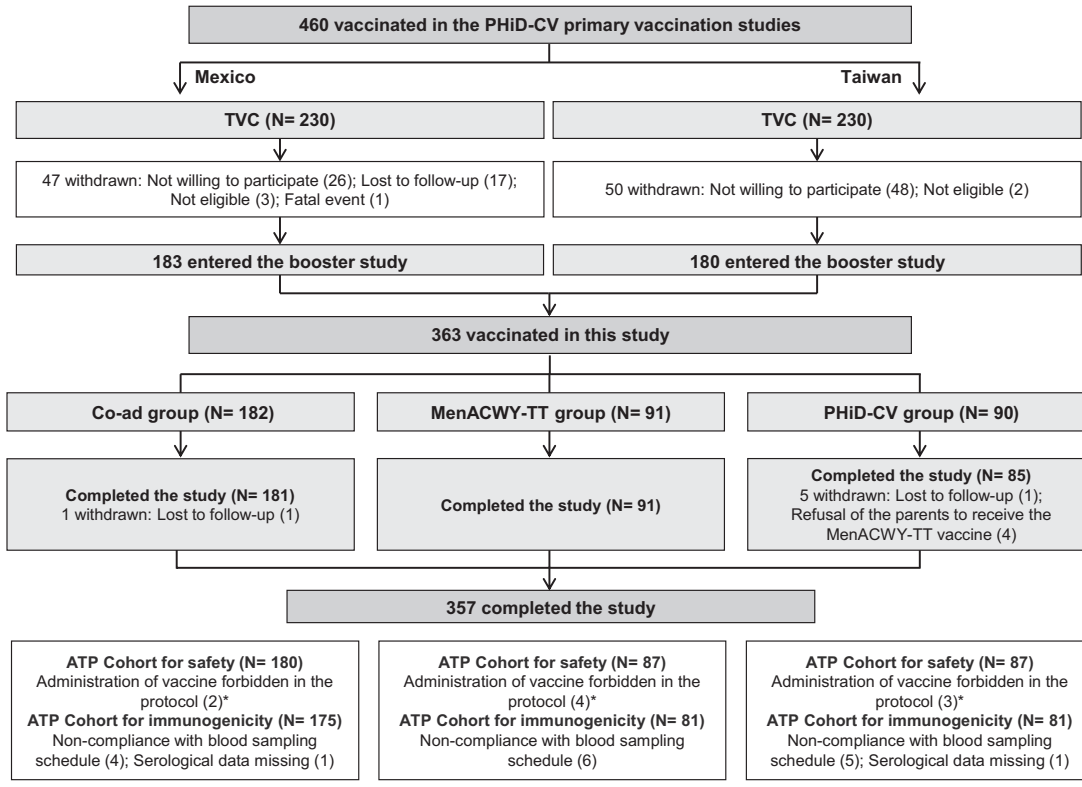

FIGURE 1. Participant flow through the study. TVC indicates total vaccinated cohort; N, number of toddlers ; ATP, according-to-protocol. *Forbidden vaccines included combined vaccines against diphtheria, tetanus, pertussis, poliomyelitis and *Haemophilus influenza*e type b diseases, oral polio vaccine, hepatitis B virus and Bacillus Calmette-Guérin vaccines.

Before the booster vaccination in the present study, for each of the vaccine pneumococcal serotype, at least 75.0% of toddlers in the 3 groups had antibody concentrations 0.2 µg/mL, except for serotype 1 (51.3%; Table 3). One month after booster vaccination with PHiD-CV, at least 96.0% of toddlers in the 3 groups reached antibody concentrations ≥0.2 µg/mL for each serotype, and robust increases in antibody GMCs (range 5.1-fold to 22.4-fold) were observed from prebooster to postbooster vaccination. At the prebooster stage, for serotypes 1, 4, 5 and 7F, the observed percentages of toddlers with antibody concentrations ≥0.2 µg/mL tended to be lower in the Mexican toddlers than in the Taiwanese toddlers, although no statistical comparisons have been made to support this statement (Table, Supplemental Digital Content 1, http://links.lww.com/INF/B378). In contrast, at 1 month after the booster vaccination, for each vaccine pneumococcal serotype, high percentages of toddlers had antibody concentrations ≥0.2 µg/mL in both Mexico and Taiwan.

TABLE 1. Summary of Demographic Characteristics (Total Vaccinated Cohort)

Characteristic	Coad	MenACWY-TT	PHiD-CV
N	182	91	90
Age (mo)			
Mean ± SD	17.2 ± 1.97	17.2 ± 1.88	17.4 ± 1.86
Range	13–21	14–22	14–21
Sex			
Female, n (%)	83 (45.6)	56 (61.5)	47 (52.2)
Male, n (%)	99 (54.4)	35 (38.5)	43 (47.8)
Race			
East Asian, n (%)	91 (50.0)	45 (49.5)	44 (48.9)
Hispanic, n (%)	91 (50.0)	46 (50.5)	46 (51.1)

N indicates number of toddlers; n (%), number (percentage) of toddlers with the specified characteristic; SD, standard deviation.

For each vaccine pneumococcal serotype, at least 53.6% of toddlers in the 3 groups had OPA titer ≥8 before booster vaccination, except for serotypes 1, 4 and 18C (Table 4). One month after booster vaccination with PHiD-CV, for each vaccine pneumococcal serotype, at least 92.9% of toddlers in each group reached OPA titers ≥8. For serotype 18C, 98.2% of toddlers in the Coad group and all the toddlers in the PHiD-CV and MenACWY-TT groups had OPA titers ≥8 at 1 month after PHiD-CV booster vaccination.

For each vaccine pneumococcal serotype, robust increases in OPA GMTs (range 2.6-fold to 425.5-fold) were observed from prebooster to postbooster vaccination in all the groups.

For cross-reactive serotypes 6A and 19A, at least 85.5% and 90.8% of toddlers in each group reached antibody concentrations ≥0.2 µg/mL at 1 month after vaccination with PHiD-CV (Table 3) and at least 82.4% and 70.2% of toddlers reached OPA titers ≥8 (Table 4).

Antitetanus Antibodies

Seroprotective concentrations (≥0.1 IU/mL) of anti-TT antibodies before vaccination were observed in 94.9–100% of toddlers across the 3 vaccine groups. One month after the MenACWY-TT vaccination, all the toddlers in the 3 groups were seroprotected, and an increase in anti-TT antibody GMCs (range 13.773 to 18.446 IU/mL) as compared with those measured before vaccination (range 0.499 to 0.566 IU/mL) were observed in all study groups (data not shown).

TABLE 2. Percentage of Toddlers With Meningococcal Serum Bactericidal Antibody (rSBA) Titers Equal to or Above the Cutoff Values of 8 and 128 and GMTs (ATP Cohort for Immunogenicity)

	Coad				PHiD-CV				MenACWY-TT			
Timing	N	% ≥8 (95% CI)	% ≥128 (95% CI)	GMT (95% CI)	N	% ≥8 (95% CI)	% ≥128 (95% CI)	GMT (95% CI)	N	% ≥8 (95% CI)	% ≥128 (95% CI)	GMT (95% CI)
MenA												
M0	148	29.7 (22.5–37.8)	26.4 (19.5–34.2)	15.2 (10.8–21.4)	69	49.3 (37.0–61.6)	46.4 (34.3–58.8)	39.4 (22.1–70.5)	69	30.4 (19.9–42.7)	26.1 (16.3–38.1)	15.3 (9.3–25.2)
M1	174	99.4 (96.8–100)	99.4 (96.8–100)	6673.7 (5680.7–7840.4)	63	36.5 (24.7–49.6)	34.9 (23.3–48.0)	23.5 (12.7–43.5)	80	97.5 (91.3–99.7)	97.5 (91.3–99.7)	5016.6 (3594.8–7000.8)
M2	—	—	—	—	78	100 (95.4–100)	100 (95.4–100)	5288.0 (4498.0–6216.9)	80	98.8 (93.2–100)	98.8 (93.2–100)	4746.8 (3568.4–6314.3)
MenC												
M0	172	10.5 (6.3–16.0)	5.2 (2.4–9.7)	5.8 (4.9–6.9)	78	19.2 (11.2–29.7)	10.3 (4.5–19.2)	7.7 (5.6–10.7)	81	11.1 (5.2–20.0)	7.4 (2.8–15.4)	6.1 (4.7–8.0)
M1	174	99.4 (96.8–100)	98.9 (95.9–99.9)	2496.6 (2096.5–2973.0)	76	11.8 (5.6–21.3)	7.9 (3.0–16.4)	6.4 (4.7–8.7)	80	98.8 (93.2–100)	98.8 (93.2–100)	2044.0 (1522.8–2743.6)
M2	—	—	—	—	78	100 (95.4–100)	100 (95.4–100)	1579.9 (1218.9–2048.0)	81	98.8 (93.3–100)	97.5 (91.4–99.7)	1834.6 (1408.1–2390.4)
MenW-135												
M0	165	60.0 (52.1–67.5)	48.5 (40.6–56.4)	46.4 (33.7–63.7)	73	54.8 (42.7–66.5)	46.6 (34.8–58.6)	39.6 (23.9–65.4)	75	50.7 (38.9–62.4)	41.3 (30.1–53.3)	32.7 (19.8–53.9)
M1	174	100 (97.9–100)	100 (97.9–100)	11731.0 (10270.7–13398.9)	74	54.1 (42.1–65.7)	41.9 (30.5–53.9)	35.8 (21.5–59.6)	80	98.8 (93.2–100)	97.5 (91.3–99.7)	8407.7 (6225.4–11355.0)
M2	—	—	—	—	78	100 (95.4–100)	100 (95.4–100)	7666.8 (6502.8–9039.0)	81	98.8 (93.3–100)	97.5 (91.4–99.7)	5817.6 (4390.8–7708.0)
MenY												
M0	171	62.0 (54.3–69.3)	54.4 (46.6–62.0)	66.0 (46.6–93.6)	79	63.3 (51.7–73.9)	59.5 (47.9–70.4)	77.0 (45.5–130.4)	78	65.4 (53.8–75.8)	57.7 (46.0–68.8)	74.4 (44.7–123.9)
M1	173	100 (97.9–100)	100 (97.9–100)	6797.8 (6014.4–7683.4)	79	60.8 (49.1–71.6)	53.2 (41.6–64.5)	68.4 (39.9–117.2)	80	100 (95.5–100)	100 (95.5–100)	5606.2 (4484.5–7008.3)
M2	—	—	—	—	78	100 (95.4–100)	100 (95.4–100)	6461.1 (5329.3–7833.2)	81	100 (95.5–100)	100 (95.5–100)	4669.5 (3735.6–5836.8)

M0 indicates time of the first vaccination; M1, 1 month after the first vaccination, M2, 1 month after the second vaccination; N, number of toddlers with available results.

TABLE 3. Percentage of Toddlers With 22F-ELISA Antibody Concentrations ≥0.2 μg/mL and Antibody GMCs Against the Vaccine Pneumococcal and Cross-reactive Serotypes (ATP Cohort for Immunogenicity)

	Coad			PHiD-CV			MenACWY-TT		
Timing	N	% ≥0.2 μg/mL (95% CI)	GMC (μg/mL) (95% CI)	N	% ≥0.2 μg/mL (95% CI)	GMC (μg/mL) (95% CI)	N	% ≥0.2 μg/mL (95% CI)	GMC (μg/mL) (95% CI)
Vaccine serotypes									
Anti-1									
M0	167	67.7 (60.0–74.7)	0.31 (0.26–0.36)	76	51.3 (39.6–63.0)	0.24 (0.19–0.30)	76	71.1 (59.5–80.9)	0.33 (0.27–0.40)
M1	175	100 (97.9–100)	3.43 (3.06–3.85)	81	100 (95.5–100)	3.60 (2.96–4.38)	76	72.4 (60.9–82.0)	0.33 (0.27–0.41)
M2	—	—	—	76	100 (95.3–100)	2.32 (1.88–2.87)	79	100 (95.4–100)	4.19 (3.31–5.31)
Anti-4									
M0	165	77.6 (70.4–83.7)	0.45 (0.38–0.53)	75	82.7 (72.2–90.4)	0.49 (0.39–0.61)	76	86.8 (77.1–93.5)	0.57 (0.45–0.72)
M1	172	100 (97.9–100)	6.86 (6.06–7.76)	79	100 (95.4–100)	6.98 (5.82–8.37)	75	82.7 (72.2–90.4)	0.52 (0.41–0.65)
M2	—	—	—	77	100 (95.3–100)	4.05 (3.30–4.97)	81	100 (95.5–100)	7.80 (6.32–9.61)
Anti-5									
M0	166	85.5 (79.3–90.5)	0.61 (0.53–0.71)	78	82.1 (71.7–89.8)	0.48 (0.39–0.59)	78	85.9 (76.2–92.7)	0.55 (0.45–0.67)
M1	175	100 (97.9–100)	4.97 (4.40–5.62)	81	100 (95.5–100)	5.07 (4.34–5.93)	78	87.2 (77.7–93.7)	0.54 (0.43–0.67)
M2	—	—	—	77	100 (95.3–100)	3.22 (2.75–3.78)	81	100 (95.5–100)	4.65 (3.86–5.62)
Anti-6B									
M0	163	77.9 (70.8–84.0)	0.57 (0.46–0.70)	76	75.0 (63.7–84.2)	0.48 (0.36–0.64)	75	81.3 (70.7–89.4)	0.53 (0.40–0.69)
M1	175	96.0 (91.9–98.4)	2.91 (2.48–3.41)	80	98.8 (93.2–100)	3.14 (2.55–3.85)	76	82.9 (72.5–90.6)	0.53 (0.41–0.68)
M2	—	—	—	76	98.7 (92.9–100)	2.18 (1.77–2.69)	78	96.2 (89.2–99.2)	2.95 (2.23–3.91)
Anti-7F									
M0	161	94.4 (89.7–97.4)	0.79 (0.69–0.90)	75	93.3 (85.1–97.8)	0.71 (0.58–0.87)	73	98.6 (92.6–100)	0.95 (0.80–1.14)
M1	175	100 (97.9–100)	5.32 (4.75–5.95)	79	100 (95.4–100)	5.10 (4.26–6.10)	78	100 (95.4–100)	0.88 (0.74–1.04)
M2	—	—	—	77	100 (95.3–100)	3.85 (3.18–4.66)	81	100 (95.5–100)	6.49 (5.50–7.65)
Anti-9V									
M0	164	93.3 (88.3–96.6)	0.79 (0.67–0.92)	75	90.7 (81.7–96.2)	0.75 (0.61–0.93)	76	96.1 (88.9–99.2)	0.85 (0.68–1.06)
M1	175	100 (97.9–100)	4.70 (4.15–5.32)	80	100 (95.5–100)	4.74 (3.96–5.67)	80	95.0 (87.7–98.6)	0.79 (0.64–0.98)
M2	—	—	—	77	100 (95.3–100)	3.07 (2.53–3.73)	81	100 (95.5–100)	5.31 (4.28–6.59)
Anti-14									
M0	168	94.0 (89.3–97.1)	1.30 (1.09–1.56)	76	96.1 (88.9–99.2)	1.29 (0.97–1.72)	78	94.9 (87.4–98.6)	1.37 (1.06–1.77)
M1	175	100 (97.9–100)	8.89 (7.65–10.33)	80	100 (95.5–100)	9.00 (7.40–10.95)	76	92.1 (83.6–97.0)	1.16 (0.88–1.51)
M2	—	—	—	77	100 (95.3–100)	6.79 (5.51–8.36)	81	100 (95.5–100)	10.60 (8.63–13.01)
Anti-18C									
M0	168	94.6 (90.1–97.5)	0.79 (0.67–0.94)	78	89.7 (80.8–95.5)	0.87 (0.68–1.11)	78	87.2 (77.7–93.7)	0.82 (0.61–1.10)
M1	175	100 (97.9–100)	9.81 (8.40–11.45)	78	100 (95.4–100)	19.48 (15.20–24.97)	77	88.3 (79.0–94.5)	0.73 (0.56–0.94)
M2	—	—	—	77	100 (95.3–100)	14.14 (11.08–18.04)	80	100 (95.5–100)	7.78 (6.21–9.74)
Anti-19F									
M0	166	95.8 (91.5–98.3)	1.36 (1.10–1.68)	76	93.4 (85.3–97.8)	1.08 (0.78–1.50)	75	90.7 (81.7–96.2)	1.35 (0.97–1.88)
M1	175	100 (97.9–100)	19.16 (16.60–22.12)	80	100 (95.5–100)	20.79 (17.67–24.45)	78	93.6 (85.7–97.9)	1.39 (1.00–1.92)
M2	—	—	—	77	100 (95.3–100)	12.35 (10.23–14.91)	78	100 (95.4–100)	16.90 (13.83–20.65)
Anti-23F									
M0	165	84.2 (77.8–89.4)	0.62 (0.51–0.76)	77	80.5 (69.9–88.7)	0.53 (0.39–0.70)	77	84.4 (74.4–91.7)	0.67 (0.48–0.92)
M1	174	98.9 (95.9–99.9)	4.46 (3.80–5.25)	81	97.5 (91.4–99.7)	3.79 (2.94–4.88)	81	79.0 (68.5–87.3)	0.59 (0.43–0.81)
M2	—	—	—	77	98.7 (93.0–100)	2.72 (2.15–3.43)	81	100 (95.5–100)	5.25 (4.06–6.79)
Cross-reactive serotypes									
Anti-6A									
M0	166	53.6 (45.7–61.4)	0.25 (0.20–0.31)	77	51.9 (40.3–63.5)	0.26 (0.20–0.35)	76	51.3 (39.6–63.0)	0.25 (0.19–0.35)
M1	173	85.5 (79.4–90.4)	1.54 (1.22–1.96)	81	96.3 (89.6–99.2)	2.07 (1.56–2.76)	75	57.3 (45.4–68.7)	0.28 (0.20–0.39)
M2	—	—	—	77	94.8 (87.2 98.6)	1.30 (0.98–1.71)	81	87.7 (78.5–93.9)	1.59 (1.10–2.31)
Anti-19A									
M0	166	39.8 (32.3–47.6)	0.17 (0.14–0.21)	76	36.8 (26.1–48.7)	0.16 (0.12–0.21)	75	48.0 (36.3–59.8)	0.18 (0.13–0.25)
M1	174	90.8 (85.5–94.7)	2.00 (1.58–2.52)	79	94.9 (87.5–98.6)	2.50 (1.89–3.30)	77	53.2 (41.5–64.7)	0.22 (0.16–0.30)
M2	—	—	—	76	93.4 (85.3–97.8)	1.51 (1.12–2.04)	79	92.4 (84.2–97.2)	2.21 (1.58–3.09)

M0 indicates time of the first vaccination; M1, 1 month after the first vaccination; M2, 1 month after the second vaccination; N, number of toddlers with available results.

TABLE 4. Percentage of Toddlers With OPA Titers ≥8 and GMTs Against the Vaccine Pneumococcal and Cross-reactive Serotypes (ATP Cohort for Immunogenicity)

	Coad			PHiD-CV			MenACWY-TT		
Timing	N	% ≥8 (95% CI)	GMT (95% CI)	N	% ≥8 (95% CI)	GMT (95% CI)	N	% ≥8 (95% CI)	GMT (95% CI)
Vaccine serotypes									
OPA-1									
M0	68	19.1 (10.6–30.5)	6.6 (5.1–8.7)	27	14.8 (4.2–33.7)	6.2 (4.0–9.9)	28	14.3 (4.0–32.7)	5.8 (4.0–8.4)
M1	56	92.9 (82.7–98.0)	395.3 (244.4–639.6)	26	96.2 (80.4–99.9)	438.6 (224.4–857.2)	20	25.0 (8.7–49.1)	8.2 (4.4–15.3)
M2	—	—	—	26	96.2 (80.4–99.9)	238.0 (117.5–482.0)	15	93.3 (68.1–99.8)	200.8 (86.1–468.2)
OPA-4									
M0	54	31.5 (19.5–45.6)	13.7 (7.6–24.5)	22	50.0 (28.2–71.8)	24.9 (9.0–68.6)	23	43.5 (23.2–65.5)	14.9 (6.7–32.9)
M1	58	100 (93.8–100)	2638.7 (1938.8–3591.1)	26	100 (86.8–100)	3621.3 (2488.8–5269.1)	16	50.0 (24.7–75.3)	10.3 (5.3–19.8)
M2	—	—	—	25	96.0 (79.6–99.9)	1427.7 (683.8–2980.6)	15	100 (78.2–100)	2536.5 (1485.3–4331.9)
OPA-5									
M0	67	55.2 (42.6–67.4)	12.9 (9.6–17.3)	26	61.5 (40.6–79.8)	12.7 (7.7–20.9)	28	53.6 (33.9–72.5)	10.8 (7.1–16.4)
M1	59	98.3 (90.9–100)	362.1 (261.6–501.2)	26	100 (86.8–100)	332.7 (227.2–487.1)	19	42.1 (20.3–66.5)	11.0 (5.9–20.6)
M2	—	—	—	24	100 (85.8–100)	308.9 (188.2–506.9)	15	100 (78.2–100)	233.3 (132.0–412.2)
OPA-6B									
M0	59	61.0 (47.4–73.5)	37.4 (20.4–68.5)	20	55.0 (31.5–76.9)	42.7 (12.5–146.5)	23	60.9 (38.5–80.3)	26.1 (11.0–61.9)
M1	57	93.0 (83.0–98.1)	678.9 (422.1–1091.9)	23	95.7 (78.1–99.9)	1158.7 (539.3–2489.4)	16	75.0 (47.6–92.7)	56.1 (16.9–186.8)
M2	—	—	—	25	88.0 (68.8–97.5)	560.6 (223.6–1405.3)	15	93.3 (68.1–99.8)	1112.1 (402.1–3075.8)
OPA-7F									
M0	62	100 (94.2–100)	1795.6 (1416.5–2276.1)	23	100 (85.2–100)	1843.1 (1385.7–2451.6)	24	100 (85.8–100)	1588.3 (1103.6–2286.0)
M1	55	100 (93.5–100)	5485.4 (4608.2–6529.5)	23	100 (85.2–100)	5421.5 (3948.8–7443.5)	18	100 (81.5–100)	2051.2 (1304.0–3226.5)
M2	—	—	—	22	100 (84.6–100)	4444.3 (2918.5–6768.0)	15	100 (78.2–100)	5235.8 (2619.9–10463.7)
OPA-9V									
M0	60	100 (94.0–100)	728.3 (553.7–957.9)	24	100 (85.8–100)	1269.8 (798.4–2019.4)	25	100 (86.3–100)	627.7 (427.3–921.9)
M1	57	100 (93.7–100)	2697.7 (2191.1–3321.4)	24	100 (85.8–100)	4862.0 (3345.5–7065.9)	20	95.0 (75.1–99.9)	445.2 (218.9–905.5)
M2	—	—	—	24	100 (85.8–100)	3392.6 (2236.5–5146.4)	15	100 (78.2–100)	2709.7 (1635.5–4489.5)
OPA-14									
M0	59	94.9 (85.9–98.9)	329.5 (228.7–474.9)	22	90.9 (70.8–98.9)	320.5 (155.6–660.3)	25	92.0 (74.0–99.0)	324.7 (161.5–625.9)
M1	58	100 (93.8–100)	3178.4 (2423.1–4169.1)	26	100 (86.8–100)	3914.0 (2506.7–6111.5)	18	77.8 (52.4–93.6)	214.3 (69.3–663.1)
M2	—	—	—	24	100 (85.8–100)	2149.0 (1381.5–3342.8)	15	100 (78.2–100)	2563.7 (1531.3–4292.1)
OPA-18C									
M0	58	36.2 (24.0–49.9)	13.9 (8.1–24.0)	26	46.2 (26.6–66.6)	13.2 (6.6–26.2)	22	40.9 (20.7–63.6)	10.5 (5.1–21.6)
M1	55	98.2 (90.3–100)	1969.9 (1340.0–2895.9)	23	100 (85.2–100)	5616.8 (3209.6–9829.3)	17	35.3 (14.2–61.7)	8.2 (3.7–18.2)
M2	—	—	—	22	100 (84.6–100)	4190.1 (2326.8–7545.6)	15	100 (78.2–100)	1229.9 (668.5–2263.0)
OPA-19F									
M0	67	73.1 (60.9–83.2)	33.5 (21.2–52.9)	27	70.4 (49.8–86.2)	32.9 (14.7–73.4)	28	71.4 (51.3–86.8)	34.2 (15.5–75.1)
M1	57	100 (93.7–100)	2074.7 (1456.3–2955.6)	24	100 (85.8–100)	2534.2 (1794.5–3578.8)	19	84.2 (60.4–96.6)	57.3 (19.0–172.5)
M2	—	—	—	22	100 (84.6–100)	1260.4 (788.8–2013.8)	15	100 (78.2–100)	2110.4 (1192.5–3734.8)
OPA-23F									
M0	60	73.3 (60.3–83.9)	262.3 (128.9–533.7)	23	78.3 (56.3–92.5)	460.2 (139.7–1515.3)	23	60.9 (38.5–80.3)	98.1 (27.6–348.2)
M1	58	98.3 (90.8–100)	3808.8 (2708.9–5355.4)	26	100 (86.8–100)	3794.6 (2392.0–6019.9)	15	66.7 (38.4–88.2)	199.0 (33.9–1168.7)
M2	—	—	—	26	100 (86.8–100)	3284.2 (2184.6–4937.3)	15	100 (78.2–100)	3432.4 (2187.3–5386.3)
Cross-reactive serotypes									
OPA-6A									
M0	58	58.6 (44.9–71.4)	55.8 (30.1–103.5)	19	78.9 (54.4–93.9)	127.6 (48.9–333.0)	26	69.2 (48.2–85.7)	73.1 (30.6–174.7)
M1	51	82.4 (69.1–91.6)	355.2 (188.7–668.8)	22	95.5 (77.2–99.9)	696.6 (359.9–1348.2)	16	62.5 (35.4–84.8)	59.4 (17.4–202.7)
M2	—	—	—	19	84.2 (60.4–96.6)	284.1 (105.4–765.5)	15	93.3 (68.1–99.8)	566.0 (210.4–1522.8)
OPA-19A									
M0	68	8.8 (3.3–18.2)	5.3 (4.1–6.9)	27	14.8 (4.2–33.7)	7.0 (3.8–13.2)	27	11.1 (2.4–29.2)	5.7 (3.6–9.0)
M1	57	70.2 (56.6–81.6)	100.2 (50.3–199.5)	24	87.5 (67.6–97.3)	214.5 (75.3–611.0)	20	15.0 (3.2–37.9)	6.3 (3.5–11.3)
M2	—	—	—	23	73.9 (51.6–89.8)	86.8 (28.2–267.2)	15	86.7 (59.5–98.3)	203.2 (57.6–716.6)

M0 indicates time of the first vaccination; M1, 1 month after the first vaccination; M2, 1 month after the second vaccination; N, number of toddlers with available results.

Anti-protein D Antibodies

One month after vaccination with PHiD-CV, seropositivity rates for anti-protein D antibodies reached 97.7–100% of the toddlers, and an increase in anti-protein D antibody GMCs (range 2340.3 to 2921.6 EL.U/mL) as compared with those measured before vaccination (range 514.6 to 633.4 EL.U/mL) were observed in all study groups (data not shown).

Safety

Pain at the injection site was the most frequently reported solicited local symptom after each vaccination in the 3 groups (Fig., Supplementary Digital Content 2, http://links.lww.com/INF/B380). Pain with grade 3 intensity was reported in 6.8–8.8% of toddlers at the PHiD-CV injection site and in 2.4–7.8% of toddlers at the MenACWY-TT injection site.

The most common solicited general symptom in all groups was irritability (Fig., Supplemental Digital Content 3, http://links.lww.com/INF/B381). Less than 1.2% of toddlers in the 3 groups reported solicited general symptoms with grade 3 intensity. The incidence of fever ranged from 11.0% to 19.8% after each vaccination with 1 toddler in the MenACWY-TT group reporting grade 3 fever (rectal temperature ≥40.0°C) after the PHiD-CV vaccination, which was considered by the investigator as causally related to vaccination.

The percentage of toddlers reporting unsolicited AEs during the 31-day follow-up period after the first vaccination was within the same ranges in each group (42.9–46.7%). After the second vaccination, unsolicited AEs were reported in 44.0% and 34.4% of the toddlers in the MenACWY-TT and PHiD-CV groups, respectively. After the first vaccination, grade 3 unsolicited symptoms were reported in 11 toddlers (6.0%) in the Coad group and in 2 toddlers (2.2%) in both the MenACWY-TT and the PHiD-CV groups. After the second vaccination, 6 toddlers (6.6%) in the MenACWY-TT group and 3 toddlers (3.3%) in the PHiD-CV group reported a grade 3 unsolicited symptom. No grade 3 unsolicited AEs were considered causally related to vaccination.

During the active phase of the study (up to 1 month after the last vaccination), 1 or more SAEs were reported in 2 toddlers in the Coad group (bronchopneumonia and bronchiolitis and infantile asthma), 3 toddlers in the MenACWY-TT group (bronchopneumonia, head injury and bronchopneumonia and acute otitis media) and 1 toddler in the PHiD-CV group (gastroenteritis). Overall, 13 toddlers experienced 1 or more SAEs throughout the study period; 6 toddlers (3.3%) in the Coad group, 3 toddlers (3.3%) in the MenACWY-TT group and 4 toddlers (4.4%) in the PHiD-CV group. No SAEs were considered by the investigator as related to vaccination, and all SAEs resolved without sequelae. In addition, 1 toddler in the MenACWY-TT group reported a new onset of chronic illness (allergic rhinitis) during the 6-month follow-up period.

DISCUSSION

The aim of the present study was to investigate the coadministration of a single dose of MenACWY-TT and the booster dose of PHiD-CV in the second year of life. The predefined statistical criterion of noninferiority of MenACWY-TT coadministered with PHiD-CV over MenACWY-TT administered alone was met for the 4 vaccine meningococcal serogroups. The MenACWY-TT vaccine elicited high bactericidal titers against serogroups A, C, W-135 and Y when given alone or when coadministered with or given 1 month after PHiD-CV. The immune responses observed in the present study were in line with previous studies, in which the immunogenicity of the MenACWY-TT vaccine was assessed when it was given alone or coadministered with other childhood vaccines in toddlers.[11,12,15]

The predefined statistical criterion of noninferiority of PHiD-CV coadministered with MenACWY-TT over PHiD-CV given alone was met for all vaccine pneumococcal serotypes, except serotype 18C. The present study showed that no negative interferences between MenACWY-TT and PHiD-CV were observed for any of the serotypes conjugated to NTHi protein D, a carrier protein that was chosen in part to avoid carrier-mediated immune suppression, and for serotype 19F, which was conjugated to DT, a carrier protein chosen to increase the immunogenicity to this serotype.[8,39] For each vaccine pneumococcal serotype and for cross-reactive serotypes 6A and 19A, the booster dose of PHiD-CV induced a robust increase in antibody concentrations and OPA titers when the vaccine was given alone or when it was coadministered with or administered 1 month after MenACWY-TT. The immune response to PHiD-CV was, in general, in line with that observed in previous studies evaluating the immune response to the booster dose of this vaccine.[17,22,25] Of note, although the baseline antibody levels were observed to be higher in Taiwanese toddlers than in Mexican toddlers in this booster study, no apparent differences were observed between the 2 countries at 1 month after the booster dose administration. These results suggest that the persistence of antipneumococcal antibodies may differ from one country to another, but the reasons for this observation are not understood.

The lower immune response to serotype 18C, which was observed when both vaccines were coadministered compared with the separate administration of PHiD-CV, may be related to the choice of TT as carrier for both serotype 18C in PHiD-CV and all 4 meningococcal serogroups in MenACWY-TT. It is well-known that TT can influence the response of coadministered conjugate vaccines and the negative effect of high TT dosages on the immune responses was hypothesized to be related to competition for T-helper cells.[8,40,41] Nonetheless, the lower immune response to pneumococcal serotype 18C observed in the present study in the Coad group, may be of limited clinical relevance considering that, for this serotype, there was a robust increase in antibody concentration and OPA titer levels after the booster dose of PHiD-CV. Although comparisons among studies using different vaccines should be made cautiously, a higher response against serotype 18C was observed in a previous study in subjects who received PHiD-CV coadministered with MenC-TT than in subjects who received PHiD-CV coadministered with MenC-CRM_{197}.[17] The reasons for the discrepancies in observations are not fully understood, but these findings illustrate the unpredictability of the immune responses observed when vaccines using the same carrier protein are coadministered.[41,42] Other studies have suggested that pneumococcal and meningococcal conjugate vaccines using common carrier proteins (TT, DT, CRM_{197}) may interfere when they are coadministered.[8,28] Among others, a recent study has shown that the immune response induced by the licensed 7-valent pneumococcal vaccine using mutated diphtheria toxoid protein (CRM_{197}) as carrier protein (Prevnar, Wyeth, New York, NY) coadministered with a licensed tetravalent meningococcal conjugate vaccine using DT as carrier protein (MenACWY-DT; Menactra, Sanofi Pasteur, Swiftwater, PA) was lower than the immune response induced by 7-valent pneumococcal vaccine administered alone in children aged 12 months.[43,44] These findings indicate that as vaccines increase in complexity and in number of coadministered antigens, the specific vaccination schedules need to be carefully considered.

The immune responses to the TT carrier protein, which were observed in the 3 groups, could increase protection against tetanus,

although functionality of the anti-TT antibodies remains currently unknown. Immune responses to NTHi protein D were also measured in the 3 groups and could lead to protection against acute otitis media caused by NTHi as previously shown in an efficacy trial with a predecessor 11-valent pneumococcal protein D conjugate vaccine.[45]

The results of this study support the comparability of the safety profile of coadministered vaccines and of either vaccine administered alone. Solicited symptoms of grade 3 intensity were infrequent and were reported in at most 8.8% of toddlers across all vaccine groups and for both doses. Additionally, no SAEs considered as possibly related to vaccination were reported. This indicates that coadministration of MenACWY-TT and PHiD-CV is clinically acceptable and did not alter the safety profile of either vaccine.[11,12,15,16,23,39]

A potential limitation of this study was its open design due to the differing vaccination schedules in the groups, which is unlikely to have influenced the immunogenicity assessments but may have biased the safety assessment toward increased reporting of AEs in the toddlers who received the PHiD-CV vaccine coadministered with MenACWY-TT. However, the observed AEs reporting rate was balanced among the groups. The interpretation of the results has also been limited by the absence of a known immune correlate of protection for the pneumococcal serotypes, although it is generally accepted that for pneumococcal conjugate vaccines, OPA titers demonstrate the functionality of the elicited antibodies and correlate better with protection than the antibody concentrations.[46–48] A further limitation was that we did not collect history of DTPa-HBV-IPV/Hib booster dose administration, whether it was given before or after the series of study vaccination, and no information could be retrieved to determine whether it was balanced among groups. Unbalanced distribution of DTPa-HBV-IPV/Hib booster dose may have contributed to the study findings, particularly in children who received the booster vaccination close to the time of study enrollment. A longer follow-up period would be needed to evaluate the long-term effect of pneumococcal and meningococcal vaccination when both vaccines were coadministered.

In conclusion, this study showed that coadministration of the booster dose of PHiD-CV and a single dose of MenACWY-TT in the second year of life did not adversely impact the immunogenicity, safety and reactogenicity of either of the vaccines, except for the immune response against pneumococcal serotype 18C. This observation, with unknown but likely limited clinical relevance because all toddlers had a robust response for all the vaccine pneumococcal serotypes, shed some new light on potential interferences among different vaccines sharing the same carrier protein. The data from this study indicate that the coadministration of MenACWY-TT and PHiD-CV can be undertaken to increase the uptake of both vaccines in routine pediatric vaccination schedules.

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